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Green synthesis of gold nanoparticles and their enhanced synergistic antitumor activity using HepG2 and MCF7 cells and its antibacterial effects

Thangavelu Muthukumar[a,d], Sudhakumari[b], Balaji Sambandam[c], Adithan Aravinthan[d], Thotapalli Parvathaleswara Sastry[a,*], Jong-Hoon Kim[d,**]

[a] Bio-Products Laboratory, CSIR-Central Leather Research Institute, Adyar, Chennai 600020 Tamil Nadu, India
[b] Biomedical Instrumentation Science Loyola College, Chennai, 600034 Tamil Nadu, India
[c] Department of Chemistry, Indian Institute of Technology/Madras, Chennai 600 036, India
[d] Department of Physiology, College of Veterinary Medicine, Chonbuk National University, 79 Gobong-ro, Iksan-city, Jeollabuk-Do 570-752 Republic of Korea

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ABSTRACT

One pot green synthesize of gold nanoparticles (AuNPs) were achieved using the leaf extracts of *Carica papaya* (CP) and *Catharanthus roseus* (CR) and the combination of these two extracts (CPCRM). The synthesized AuNPs were characterized for their size, morphology, crystallinity and their effects on bacterial and cancer cell lines were investigated. The electron microscopy results showed that AuNPs were mostly spherical, however, triangle and hexagonal shaped morphologies were also observed. The infrared spectroscopy and microscopies studies revealed that alkaloids, flavonoids and proteins were present along with AuNPs which are responsible for their stabilization, non-agglomeration and biologically active characteristics including antibacterial and anticancer activity. Among the AuNPs prepared in this study, CPCRM extract showed enhanced activity due to the synergistic effects of both plant extracts.

1. Introduction

Today, especially gold nanoparticles (AuNPs) have drawn the attention of scientists because of their wide application in the development of new technologies in the areas of chemistry, electronics, medicine, and biotechnology at the nanoscale [1–3]. The surface plasmon resonance (SPR) or their larger total surface area per unit volume makes it more advantageous in biological field application [4,5]. These nanoparticles are thermal scalpels which behave as tiny, precise and powerful heaters after entering into a cell which kills cancer cells [6,7] due to its non-toxic and non-immunogenic features [8].

Nanoparticle synthesized using physical and chemical processes are extremely expensive and use toxic and hazardous chemicals, which may create serious environmental problems [9,10]. Biological methods are considered to be eco-friendly, and less expensive that involves the use of different microorganism [11,12], enzymes [13], fungus [14], and plants or plant extracts [15,16]. Green synthesis of AuNPs is preferred as cost-effective, environmental friendly, rapid, and a single step method [17]. Plant extracts contribute as both reducing and stabilizing agents and also add its own medicinal values to the particles, which enhances its antibacterial and anticancer activity [18]. Presence of biomolecules such as amino acid, proteins, and salts in AuNPs would make it more biocompatible and effective in in vivo model, which also cause irreversible agglomeration due to chemical similarity among the cellular growth components required for cell growth and proliferation [19]. AuNPs could be effectively used in cancer targeting [20–22], thermal ablation of tumors [23,24], imaging [25], delivery of therapeutics [26] and gene targeting [27].

Catharanthus roseus (CR) and *Carica papaya* (CP) are the perennial plants in which CR is one of the important medicinal herbs, due to the presence of two crucial anti-cancer drugs, i.e., vincristine and vinblastine. It also contains more than 70 alkaloids, flavonoids and polyphenols. These all ingredients make it as perfect anticancer compounds by inhibiting or denaturing the function of angiogenesis inducer proteins. It has been also proven active against the treatment of various kinds of cancers: Lymphomas, Hodgkin's disease, breast cancer, acute Lymphocytic, Leukemia, soft tissue sarcomas, multiple Myeloma, Neuroblastoma [28]. CP

* Corresponding author. Fax: +91 44 24911589.
** Corresponding author. Fax: +82 270 3780.
E-mail addresses: sastrytp@hotmail.com, sastrytp56@gmail.com (T.P. Sastry), jhkim1@chonbuk.ac.kr (J.-H. Kim).

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belongs to the family of Caricaceae, where several species of this family have been used as therapy against several diseases [29]. CP has been reported to contain many active components, such as papain, saponins, chymopapain, ∝-tocopherol, ascorbic acid, cystatin, flavonoids and phenolic compounds that have been associated with the prevention of cancer [30].

Hence the aim of the present study is to develop a novel approach for the green synthesis of AuNPs using herbal plants CR, CP and blend of both plant leaves to see its synergistic effect on in vitro antibacterial and anticancer activity against cancer cell lines.

2. Materials and methods

2.1. Chemicals

CP and CR were identified using taxonomic keys. The healthy and matured leaves were collected from the campus of Loyola College, Chennai, India. The chloroauric acid ($HAuCl_4$) was purchased from Sigma–Aldrich (Bangalore, India). All chemicals and microbiological and cell culture media were purchased from Sigma, and HiMedia, India, respectively.

2.2. Preparation of aqueous leaf extract

Mature and healthy leaves of CP and CR were collected and washed thoroughly with double distilled water to remove dust materials and shade-dried in dust-free condition at room temperature and coarsely powdered with the help of domestic mixer. Dried papaya leaves powder weighing 20 g was subjected to extraction (Soxhlet apparatus) using 150 mL sterile distilled water and then extraction process continued until the powder was free of extracts. The leaves extracted were stored at 4 °C and used within a week. The same process was repeated for combination [CPCRM] of both leaves.

2.3. Synthesis of gold nanoparticles

Green synthesis of AuNPs were done by reducing 1 mM chloroauric acid ($HAuCl_4$) using different concentration of extracts (CP, CR and CPCRM) ranging from 5, 10, 25, 50, 75, 100, 125, 150, 175 and 200 μL. In brief, 0.1 M $HAuCl_4$ was prepared as a stock solution. From the stock solution, made 200 mL of 1 mM $HAuCl_4$ (using sterile distilled water) and this stock solution was equally added in 10 different tubes and then different concentration of leaf extract (ranging from 5–200 μL) was added in the respective tubes of different concentration and mixed well by gentle tapping and covered with aluminum foil. The tubes were incubated in water bath at 60 °C for 5 min, changes in color were observed and optical spectrum was measured from 200–800 nm. The sample was then centrifuged at 14,000 rpm for 30 min. The supernatant was discarded and pellet was again washed in sterile deionized water twice. After final wash pellet was freeze-dried to obtain dried powder.

2.4. Characterization

Optical absorption measurements were carried out by UV–vis spectrophotometer (JASCO UV–vis spectrophotometer model: V570 UV–vis–NIR) between 200 and 800 nm. FTIR spectroscopy measurements were carried out using Perkin–Elmer FT-IR spectrometer (Jasco FTIR-410). Morphology of the materials was analyzed by HITACHI (S3400N) SEM instrument. HRTEM technique (HRTEM, JEOL 3010) with operating voltage of 300 KV is used for analyzing the particle size and morphology. Powder X-ray diffraction measurements were done with the help of X-ray diffractometer (Bruker D8 ADVANCE).

2.5. Bacterial culture, antimicrobial activity and minimum inhibitory concentration

Antimicrobial activity of AuNPs was evaluated against gram positive bacterial strains; *S. aureus* (NCIM 5021), *Bacillus subtilis* (NCIM 2718) and gram negative bacterial strains; *Escherichia coli* (NCIM 293), *Proteus vulgaris* (NCIM 2813) using disc diffusion and 96 well plate serial dilution method. All the bacterial strains were maintained on nutrient agar medium at room temperature (30 ± 2 °C) for further experiments and subcultures were freshly prepared before use. Discs were prepared by using Whatman No.1 filter paper 6 mm diameter. The discs were placed on agar plates and 50 μg of synthesized AuNPs dissolved in DMSO were loaded on the disc with the help of micropipette and evaluation was done using the Kirby–Bauer disk diffusion method [31]. Muller–Hinton agar were sterilized and poured on petri plates and allowed to solidify under laminar airflow. The bacterial cells 100 μL [$\sim 1 \times 10^8$ CFU (colony forming units)] of each bacterial culture was spread on the agar surface (Müller–Hinton agars) using sterile glass spreader. The AuNPs coated discs were placed on the plate. A sterile filter paper disc without the nanoparticles was used as a control. Antibiotic namely Tetracycline were used as positive controls. Then the plates were incubated for 24 h at 35 ± 2 °C. The antibacterial activity was evaluated by measuring the diameter of zone of inhibition against the test organism. All tests were carried out in triplicate.

2.6. Minimum inhibitory concentration using resazurin assay

MIC of the AuNPs against bacterial cultures were evaluated using 96 well plate method with the use of resazurin dye test [32]. Resazurin dye has been used an indicator for detecting microbial growth in extremely small volumes of solution in 96 well plates without the use of spectrophotometer. Briefly, the assay was performed in 96 well plates, through serial dilution. Muller–Hinton broth was used to grow the bacterial strains; bacterial strains were grown and suspended in Muller–Hinton broth. One mg/100 μL of the each test compound i.e., CP, CR and CPCRM was pipetted into the wells of the first row aseptically, which contain double strength Mueller–Hinton broth, while the second row wells were filled with 100 μL of single strength Mueller–Hinton broth. Serial dilution of the test compound was done using multichannel pipette. All the wells are added with 20 μL of bacterial culture to obtain a final concentration of 5×10^6 cells. A well devoiding the bacterial culture served as a control. The plates were prepared in triplicate and incubated for 24 h at 37 °C after that 10 μL of 0.01% resazurin solution was added and incubated for 2 h then the color change was assessed visually with the highest dilution remain blue indicating minimum inhibitory concentration. The average of three values was calculated and taken as MIC for the test compound and bacterial strain.

2.7. In vitro cytotoxicity study (MTT assay)

The MCF7 and HepG2 cells were cultured to carryout in vitro anticancer activity. The cells were grown in folate-free Dulbecco Modified Eagles Medium (FFDMEM) containing 10% fetal bovine serum (FBS), 100 units/mL penicillin and 100 μg/mL streptomycin and 4 mM L-glutamine, incubated at 37 °C in a 5% CO_2. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was carried out to determine the viability of cells based on the reductive cleavage of MTT (a yellow salt) to formazan (a purple compound) by mitochondrial dehydrogenase of living cells. Cytotoxicity of samples on MCF7 and HepG2 (cell line