Journal of Photochemistry & Photobiology, B: Biology 159 (2016) 8–13

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

In vitro evaluation of silver nanoparticles cytotoxicity on Hepatic cancer (Hep-G2) cell line and their antioxidant activity: Green approach for fabrication and application

Brajesh Kumar *, Kumari Smita, Rachid Seqqat, Karen Benalcazar, Marcelo Grijalva, Luis Cumbal

Centro de Nanociencia y Nanotecnologia, Universidad de las Fuerzas Armadas ESPE, Av. Gral. Rumiñahui s/n, Sangolqui, P.O. BOX 171-5-231B, Ecuador

ARTICLE INFO

Article history:
Received 14 March 2015
Received in revised form 7 March 2016
Accepted 9 March 2016
Available online 10 March 2016

Keywords:
Green synthesis
Silver nanoparticles
FTIR
TEM
Hep-G2 cells
Antioxidant

ABSTRACT

In this article, biosynthesis of silver nanoparticles (AgNPs) using Andean Mora (*Rubus glaucus* Benth.) leaf has been reported. Different analytical techniques including UV–vis spectroscopy, dynamic light scattering (DLS), transmission electron microscopy (TEM) and X-ray diffraction (XRD) were used for the characterization of AgNPs. The initial appearance of color change with the intense surface plasmon resonance (SPR) bands around 440–455 in UV–visible spectra revealing the formation of AgNPs. The TEM image showed the AgNPs to be anisotropic, quasi-spherical in shape with sizes in the range of 12–50 nm. On the other hand, XRD studies revealed the formation of face-centered cubic structure for AgNPs. The surface modified AgNPs showed no cytotoxicity at the concentration ranging from 0.01 μM to 1.0 μM on the Hepatic cancer (Hep-G2) cell line and observed antioxidant efficacy > 70% at the concentration 0.05 mM/0.20 mL against 1, 1-diphenyl-2-picrylhydrazyl. From the results obtained it is suggested that AgNPs could be used effectively in future drug delivery systems and other biomedical concerns.

1. Introduction

Many research works have focused on the fabrication and application of metallic nanomaterials in biomedical, industrial and electronic fields. Nanoparticles of noble metals (gold, silver, platinum and palladium) are of great interest due to their unusual optical, chemical and electronic properties [1]. Nowadays, biocompatible and surface functionalized silver nanoparticles (AgNPs) open new scope in the consumer products. It is widely used in ointment to prevent infection of burns and open wounds [2], polymers, sporting equipment [3], clothing, cleaning agents [4,5] *etc.* Presently there is an urgent need to develop an eco-friendly technique that does not use noxious and highly volatile organic solvents in the nanoparticle synthesis procedures [6,7]. Eco-friendly and green, synthetic procedures include amino acids [8], vitamins [9], starch [10], chitosan [11], irradiation [12], oil of *Plukenetia volubilis* L. [13], flower extract of *Lantana camara* [14], fruit extract of *Piper longum* and *Genipa americana* [15,16], bark extract of *Cinnamon zeylanicum* [17], natural dyes of *Dactylopius coccus* [18], leaf extract of *Alternanthera sessilis* [19] and peel extract of *Nephelium lappaceum* [20] have been reported. It may act both as reducing and capping agent; also provides uniform nucleation and growth conditions for the synthesis of nanoparticles. Using plant sources can scale up for large-scale synthesis of nanoparticles. However, there is still need for a more economical, commercially viable, and environmentally green synthesis route to synthesize AgNPs.

Rubus species, commonly referred to as the blackberry has been used in traditional medicine for their many medicinal properties [21]. The Andean blackberry (*Rubus glaucus* Benth.) or Mora is native to the high-altitude areas of South and Central America, mainly Ecuador and Colombia. The blackberry leaves have been used for their astringent, antidiarrheals, hypoglycemic activities and as an anti-inflammatory agent for the mucous membrane of the oral cavity and throat [22]. It contains a notable amount of flavonoids, tannins and ellagic acid [23]. The berry fruit is appreciated for its attractive dark-red color, juiciness, and flavor, in comparison to most cultivated blackberries [24]. It is consumed fresh or processed into products such as frozen pulp, juice, jam, and, to a minor extent, wines. The phenolic composition, mainly with regard to ellagitannins and anthocyanins [25,26].

The aims of this study were to investigate the biofabrication of AgNPs using Mora leaf (ML) and evaluate as prepared ML-AgNPs for; (a) *in vitro* cytotoxicity on Hepatic cancer cell line (Hep-G2) and (b) antioxidant efficacy against 1, 1-diphenyl-2-picrylhydrazyl (DPPH). The results would help to utilize as synthesized ML-AgNPs effectively in future biomedical concerns.

2. Experimental

2.1. Synthesis of AgNPs

All chemicals used were of analytical grade and used without any purification. Silver nitrate ($AgNO_3$, 99.0%) was purchased from

* Corresponding author.
E-mail address: krmbraj@gmail.com (B. Kumar).

http://dx.doi.org/10.1016/j.jphotobiol.2016.03.011
1011-1344/

Spectrum, USA and fresh Andean blackberry/Mora leaf were collected from the local garden of Playa Chica 1, near Universidad de las Fuerzas Armadas, Sangolqui, Ecuador. DPPH (>99.5%) was purchased from Sigma Aldrich, USA. Milli-Q water was used in all experiments. The collected fresh Andean blackberry leaves (5 g) were washed thoroughly with Milli-Q water; heated (65–70 °C) in 50 mL of deionized water for 60 min. After cooling, the yellowish color extract was filtered using Whatman No. 1 paper. The filtrate was collected in 50 mL Erlenmeyer flask and stored at 4 °C for further use. For green synthesis, 1 mL of filtrate was mixed with 10 mL of 1 mM $AgNO_3$ solution and observed at different time interval in the presence of visible light (200–250 Cd/m^2) at room temperature incubation. The synthesized ML-AgNPs were further subjected for characterization studies.

2.2. Characterization of AgNPs

UV–vis spectra were measured using a spectrophotometer (Thermo Spectronic, GENESYS™ 8, England, Quartz Cell, path length 10 mm and graph plotted on the Origin 6.1 program). The particle size distributions of nanoparticles were determined using the dynamic light scattering (DLS) instrument (LB-550, Horiba, Japan). Size and selective area electron diffraction (SAED) pattern of nanoparticles are studied on transmission electron microscopy, TEM (FEI, TECNAI, G2 spirit twin, Holland). X-ray diffraction (XRD) studies on thin films of the nanoparticle were carried out using a PANalytical brand θ–2θ configuration (generator–detector) X-ray tube copper λ = 1.54 Å and EMPYREAN diffractometer. Fourier transform infrared-attenuated total reflection (FTIR-ATR) spectra were recorded on a Spectrum two IR spectrophotometer (Perkin Elmer, USA) to detect the different functional groups of extract, involved in AgNPs synthesis. For FTIR and XRD analysis of the ML-AgNPs and ML extract, a thin film of the sample was prepared on a glass slide by dropping 2000 μL (500 μL × 4 times) of the sample on the slide. After which, the glass slide was dried at 60–65 °C for 25–30 min for the complete evaporation of the H_2O/solvent. Then the thin film of the samples was scratched and performed FTIR-ATR analysis [16].

2.3. In Vitro Anticancer Studies of Synthesized AgNPs

2.3.1. Evaluation of Cytotoxicity

Reagents for cell culturing were obtained from Gibco, Invitrogen. Hep-G2 cells, from a 15 year old Caucasian man were kindly provided by Javier Camacho's laboratory at CINVESTAV, Mexico. The cells were cultivated in Dulbecco's modified Eagle Medium Advanced (D-MEM Advanced), supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic mixture (penicillin, streptomycin with added glutamine), in a 5% CO_2 atmosphere at 37 °C, grown to 85% confluence. Exposure to different concentrations of ML-AgNPs was done on subconfluent cells. Cell confluences were assessed by inverted microscopy visualization (Lomo NXE0228, St. Petersburgh).

2.3.2. Exposure Conditions

Cells were seeded at 6-well culture plates at the density of 1×10^6 in complete medium. After 24 h of cell attachment, plates were washed with 1 mL/well of phosphate buffered saline (PBS). Cells were then exposed to suspensions of 2 mL of ML-AgNPs at concentrations of 0.01; 0.1; 0.2; 0.5 and 1.0 μM for 2 h. No fetal bovine serum was used in these preparations in order to avoid interaction with nanoparticles.

Three repeats of the above treatment were done, using one negative control per plate, without neither FBS nor ML-AgNPs.

2.3.3. Cytotoxicity Assay

After 2 h of exposition, control and treated cells were accounted using a Neubauer chamber, to assess the cytotoxic effect of ML-AgNPs on Hepatic cancer cells (Hep-G2) by cellular density measurement.

2.4. Evaluation of Antioxidant Activity

The scavenging activity of the AgNPs was measured by using DPPH as a free radical model and a method adapted from Kumar et al., 2015 [14,20]. An aliquot (1.0–0.2 mL) of ML-AgNPs/ML extract or control and (1.0–1.8 mL) of H_2O was mixed with 2.0 mL of 0.2 mM (DPPH) in absolute methanol. The mixture was vortexed vigorously and allowed to stand at room temperature for 30 min in the dark. Absorbance of the mixture was measured spectrophotometrically at 517 nm, and the free radical scavenging activity was calculated using Eq. (1):

$$\text{Scavenging effect}\ (\%) = [1-\{\text{absorbance of sample}/\text{absorbance of control}\}] \times 100. \quad (1)$$

The scavenging percentage of all samples were plotted. The final result was expressed as % of DPPH free radical scavenging activity (mL).

3. Results and Discussion

3.1. Visual and UV–Vis Spectra Analysis

It is already reported that AgNPs exhibit reddish brown color in aqueous solution due to excitation of surface plasmon resonance (SPR) in AgNPs [15,27]. Reduction of the Ag^+ to AgNPs using ML extracts could be followed by color change as shown in the Fig. 1 and the absorption of ML-AgNPs was shown in Fig. 2. It should be noted that the position of single SPR peak for the ML-AgNPs was slightly shifted during the reaction from 440 to 455 nm, this is indicative of the spherical and poly dispersed nature of the ML-AgNPs formed. The peak intensity increases steadily as a function of reaction time due to more production of ML-AgNPs [28]. The maximum absorbance occurs

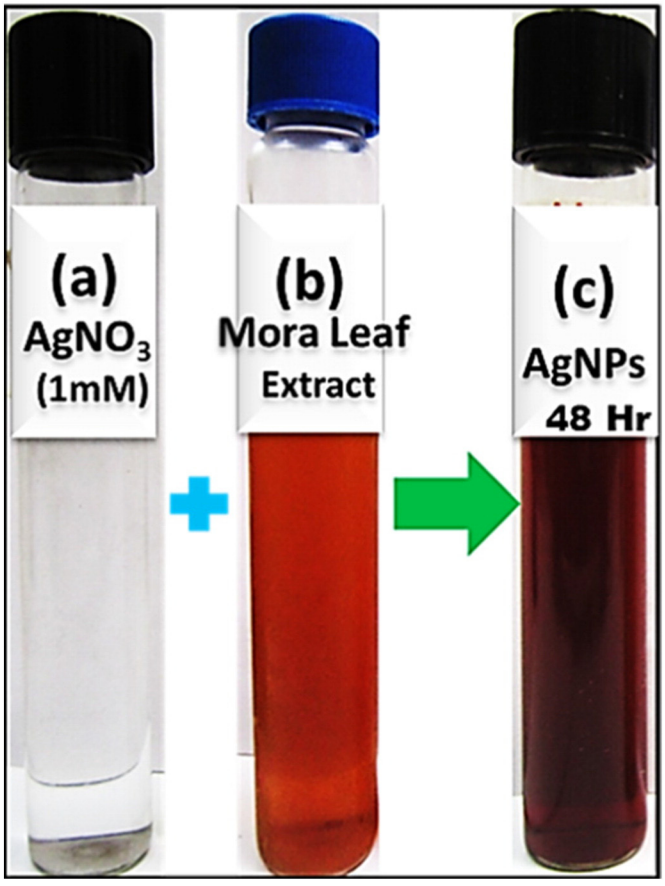

Fig. 1. (a) 1 mM $AgNO_3$, (b) Andean ML extract and (c) ML-AgNP solution after addition of extract to $AgNO_3$ solution.