Advanced Powder Technology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Advanced Powder Technology

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

Original Research Paper

Nigella arvensis leaf extract mediated green synthesis of silver nanoparticles: Their characteristic properties and biological efficacy

Azam Chahardoli [a,b], Naser Karimi [a,*], Ali Fattahi [b,c,*]

[a] Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran
[b] Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
[c] Pharmaceutical Sciences Research Center, School of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran

ARTICLE INFO

Article history:
Received 2 February 2017
Received in revised form 14 September 2017
Accepted 2 November 2017
Available online xxxx

Keywords:
Silver nanoparticles
Black seed
Biological activities
Green synthesis

ABSTRACT

In the present study, the potential of aqueous leaf extract of *Nigella arevensis* for biosynthesis of silver nanoparticles (AgNPs) was evaluated. The formation of AgNPs was confirmed by color changes and UV–visible spectroscopy, which showed absorbance maxima peak at 416 nm. The transmission electron microscope (TEM) image showed the AgNPs to be anisotropic and mostly spherical with sizes in the range of 5–100 nm. Fourier transform infrared (FTIR) analysis indicated that the flavonoids, alkaloids and phenolic groups present in leaf extract were involved in the reduction and capping of phytogenic AgNPs. These nanoparticles showed the cytotoxic effects against H1229 and MCF-7 cancer cell lines with an IC_{50} value of 10 μg/mL. AgNPs showed insignificant antioxidant properties compared to the crude extract, and it was effective against clinical isolated bacterial strains. Furthermore, the bioderived AgNPs displayed significant catalytic activity against methylene blue. These results confirmed the advantages and applications of these phytogenic AgNPs using the green method in various fields.

1. Introduction

Synthesis of metallic nanoparticles was performed by a variety of physical and chemical methods [1]. However, these methods may use toxic chemicals and are harmful to the environment [2]. Recently, biological or green synthesis of nanoparticles (NPs) received enormous attention over the physical and chemical synthesis, as it is a clean, non-toxic and an eco-friendly approach which includes design and development of syntheses using renewable materials, benign reaction media and non-hazardous as well as non-toxic solvents [3,4].

Currently, plant extract has been used widely as a reducing and capping agent for the synthesis of AgNPs due to it containing various phytochemicals compounds such as phenols, flavonoids, terpenoids, and also, plant enzymes like hydrogenases, reductases, and quinones which act as reductants in the presence of metal salts [5,6]. Comparing to the bacterial and fungal synthesis of metal NPs, using plant materials does not need any elaborate processes such as intracellular synthesis, compound purification steps and the maintenance of microbial and fungal cell cultures [7]

Among the various types of metallic nanomaterials, silver nanoparticles (AgNPs) have received considerable attention because of their wide range of applications like antibacterial, antiviral, anticancer, catalytic, biosensing, medicinal and optoelectronic [8]. Green synthesis of AgNPs using various herbs such as *Prosopis juliflora* [9], *Citrus sinensis* [10], *Alstonia scholaris* [11], *Syzygium alternifolium* [12], and *Atrocarpus altilis* [13] has been reported. Our previous report showed synthesis of the AgNPs by seed exudate of *N. arvensis* [14]. However, we hypothesized that other (edible) parts (like leave) exudates of *N. arvensis* can provide the vital biocomponents and chemical stability needed for the formation of biocompatible metallic nanoparticles.

Herein, we reported the phytosynthesis of AgNPs from aqueous leaf extract of *Nigella arvensis* and their biological properties. *Nigella arvensis,* belonging to the family Ranculaceae, is a black seed which contains several active compounds such as flavonoids, alkaloids, terpenoids and proteins [15]. Black seeds have been used in traditional medicine as therapeutic substances for the treatment of many diseases. It has been utilized for the treatment of worm infestation, allergic, viral & inflammatory [16]. Flavonoids and protein presented in the plant extract may act as a stabilizing and reducing agent in the synthesis of silver nanoparticles [17].

* Corresponding authors at: Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran (A. Fattahi); Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran (N. Karimi).
E-mail addresses: nkarimi@razi.ac.ir (N. Karimi), alifattahi@kums.ac.ir (A. Fattahi).

https://doi.org/10.1016/j.apt.2017.11.003
0921-8831/

2. Material and methods

2.1. Materials

Silver nitrate ($AgNO_3$), 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), trypsin, DMSO, 1,1-Diphenyl-2- picrylhydrazyl (DPPH), Mueller Hinton agar and broth, Methylene blue (MB), Methanol, Folin-Ciocalteu reagent, aluminum chloride ($AlCl_3$), gallic acid and quercetin were purchased from Sigma–Aldrich, USA. The leaves of the tested plant *N. arvensis* were collected freshly and healthily from research greenhouse of Razi University campus, Kermanshah, Iran. Six Pathogenic strains of bacteria namely *Staphylococcus epidermidis* (ATCC 12228), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 43300), *Escherichia coli* (ATCC 25922), *Serratia marcescens* (ATCC 13880), and *Pseudomonas aeruginosa* (ATCC 27253) were supplied from Microbiology laboratory of Biology department, Faculty of Science, Razi University, Kermanshah, Iran.

2.2. Extraction procedure

Aerial parts of collected *N. arvensis* were dried and powdered in the experimental condition. Then 10 g of the powder was added to 200 mL double distilled water in 250 mL Erlenmeyer flask and was boiled for 15 min. After cooling down to room temperature, the preparation of *N. arvensis* leaf extract was done by centrifuging at 8000 rpm for 15 min and filtration through Whatman No. 1 filter paper. The supernatants were used for the green synthesis of AgNPs and determining antioxidant, total phenol and flavonoid content.

2.3. Phyto-synthesis and characterization of N. arvensis leaf extract mediated silver nanoparticles

Phyto-reduction of $AgNO_3$ to AgNPs was carried out by mixing of 10 mL of *N. arvensis* leaf extract with 90 mL of aqueous solution of $AgNO_3$ (1 mM) and exposed to sunlight in ambient environment temperature. The AgNPs formation was confirmed by UV–Vis spectrophotometry method. Then, the phytogenic AgNPs were separated by repeated centrifugation at 10,000 rpm for 15 min and washed thrice with double-distilled water and re-dispersed. The obtained pellet used for future analyses.

The optical properties of purified AgNPs were monitored with UV–visible spectroscopy (Shimadzu UV-2400, Japan) at a resolution of 1 nm. The spectra were recorded at the different intervals (5–100 min), and the distilled water was used as a baseline. The shape and size of the phytogenic nanoparticles were determined using transmission electron microscopy (TEM) by LEO 906 at 80 kV. For TEM analysis, phytogenic AgNPs were dispersed by sonication of the sample and dropping it on carbon-coated copper grids. Crystalline metallic silver was tested by X-ray diffraction (XRD). The XRD pattern was recorded using APD 2000- Italian structures X-ray generator operated at a voltage of 40 kV and a current of 30 mA with Cu K^{-1} radiation. Fourier transform infrared (FTIR) spectra of AgNPs and the extract to identify the bioactive compounds are recorded using an IR prestige-21 Shimadzu spectrometer by the KBr pellet in the range of 4000–400 cm^{-1}.

2.4. In vitro biological activity of phytogenic AgNPs

2.4.1. Antioxidant capacity

Antioxidant capacities of phytogenic AgNPs and crude extract were determined using DPPH and total phenolic and flavonoid content assays as described below.

2.4.1.1. Determination of radical scavenging capacity by DPPH assay. DPPH free radical scavenging potential of samples were determined by the method explained by Choi et al. with minor modification [18]. Different concentrations (100, 200, 300, 400 and 500 μg/mL) of plant extract and phytogenic AgNPs were taken in different test tubes. Then, one mL of 0.1 mM DPPH fresh solution (in methanol) was added and vortexed thoroughly. The reaction mixture incubated in the dark for 30 min and after it, the absorbance was recorded at 517 nm against a blank. DPPH in methanol was used as a control. The percentage of inhibition of free radicals was determined by the following formula:

$$\text{Percentage of inhibition } (\%) = [(\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{sample}}) / \text{Absorbance}_{\text{Control}}] \times 100$$

2.4.1.2. Quantitative determination of total phenolic and flavonoid content. The total phenolic content in plant extract and phytogenic AgNPs samples were determined using the Folin – Ciocalteu method [19]. Sample solutions (0.5 mL) at varying concentrations (100, 200, 300, 400 and 500 μg/mL) and freshly prepared 0.2 N Folin-Ciocalteu reagent (2.5 mL) were taken in the test. Afterward, 2 mL of the 75 g/L sodium carbonate (Na_2CO_3) was added into the test tubes and incubated for 2 h at room temperature. The absorbance of the reaction mixture was measured at 760 nm. Phenolic content was measured by using gallic acid calibration curve and was expressed as gallic acid equivalents (μg GAE).

Total flavonoid content was measured by described method of Quettier – Deleu (2000) with slight modification. Aluminum chloride solution prepared in 2% methanol was mixed with different concentrations (100, 200, 300, 400 and 500 μg/mL) of experimental samples. Absorbance was measured at 415 nm, and the total flavonoid content was determined using a standard curve of quercetin and was expressed as quercetin equivalents (μg QE).

2.4.2. Tests for Antimicrobial assay

The antibacterial activity was tested by standard methods of well diffusion and Minimum Inhibitory Concentration (MIC). The AgNPs synthesized by *N. arvensis* leaf extract were tested against the six pathogenic strains of bacteria which introduced in material part. The bacterial suspension (5×10^5 CFU/mL) was swabbed onto Mueller Hinton Agar (MHA) plates using a sterile cotton swab. Then, 100 μL of silver nanoparticle prepared from *N. arvensis* was added into the wells with 6 mm diameter. The plates were incubated at 37 °C for 24 h. Positive test results were scored when a zone of inhibition was observed around the well after the incubation period. The minimal inhibitory concentrations (MICs) of the phytogenic silver nanoparticles were examined using the standard broth dilution method (CLSI-M07-A8). Live cells of experimented pathogenic strains at final concentrations of 5×10^5 CFU/mL were inoculated into 96 well plates followed by 50 μL of the phytogenic silver nanoparticles. After incubation for 24 h at 37 °C, their concentrations were recorded. The pure medium and medium containing bacteria were served as the negative and positive controls. The MIC was calculated based on the lowest concentration of extract or phytogenic nanoparticles that inhibits the bacterial growth.

2.4.3. Cytotoxicity potential

The cell growth inhibitory effects of the aqueous leaf extract of *N. arvensis* and phytogenic AgNPs on human breast cancer (MCF-7) and human non-small cell lung carcinoma (H1299) cell lines was assayed by MTT method. Briefly, cells were seeded as triplicates at a concentration of 5×10^4 cells/mL on 96-well plates for 24 h. Subsequently, 20 μL of freshly prepared phytogenic AgNPs suspension in distilled water or the extracts at different concentrations

Please cite this article in press as: A. Chahardoli et al., *Nigella arvensis* leaf extract mediated green synthesis of silver nanoparticles: Their characteristic properties and biological efficacy, Advanced Powder Technology (2017), https://doi.org/10.1016/j.apt.2017.11.003