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Green synthesis and characterization of silver nanoparticles using alcoholic flower extract of *Nyctanthes arbortristis* and in vitro investigation of their antibacterial and cytotoxic activities

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ABSTRACT

Here we report the synthesis of silver nanoparticles using ethanolic flower extract of *Nyctanthes arbortristis*, UVvisible spectra and TEM indicated the successful formation of silver nanoparticles. Crystalline nature of the silver nanoparticles was confirmed by X-ray diffraction. Fourier Transform Infra-Red Spectroscopy analysis established the capping of the synthesized silver nanoparticles with phytochemicals naturally occurring in the ethanolic flower extract of *N. arbortristis*. The synthesized silver nanoparticles showed antibacterial activity against the pathogenic strain of *Escherichia coli* MTCC 443. Furthermore, cytotoxicity of the silver nanoparticles was tested on mouse fibroblastic cell line (L929) and found to be non-toxic, which thus proved their biocompatibility. Antibacterial activity and cytotoxicity assay carried out in this study open up an important perspective of the synthesized silver nanoparticles.

1. Introduction

Silver nanoparticles (AgNPs) have been the focus of increasing research interest in the past decade. In the recent times, AgNPs serve as an excellent candidate for most of the therapeutic purposes [1]. Studies on AgNPs have confirmed their potential application in many fields of science [2]. AgNPs have shown positive results in wound healing, retinal therapies, DNA sequencing and pharmaceuticals along with other conventional uses like in electronics, optics, catalysis and Raman scattering [3–11]. The fundamental uses of AgNPs have been highly recognized in water treatment process. Contemporary AgNP filters are effective and utilized against contamination in water treatment and filtration processes [12,13]. The efficiency of such filters is strongly attributed to the antimicrobial properties acquired by AgNPs [14].

Green synthesis of metallic nanoparticles has been gaining importance since the time people realized the possible toxicity of chemically synthesized nanoparticles [15–18]. "Environmentally benign" biological sources are nowadays successfully employed to produce ecofriendly AgNPs [19]. Biologically mediated nanoparticle synthesis provides capping or stabilizing agents on the surface of the nanoparticles and prevents the particles from agglomeration. Many phytochemicals of plant origin brings about reduction of metals in ionic form to metallic nanoparticle and can help in overcoming the deleterious consequences of chemically synthesized AgNPs [20–22].

N. arbortristis commonly known as "Parijat, Sewali" or "Harsingar" in India owes great importance in the traditional Indian medicinal system [23]. The flowers are edible, antimicrobial, antimalarial, antispasmodic, antihelminthic, antidepressant and comprise of other important phytochemicals such as anti-oxidants, phenolic compounds and flavonoids [24–30]. Alcoholic extract of *N. arbortristis* flowers has been reported to possess strong reducing power [31]. Ethanolic flower extract (EFE) of *N. arbortristis* has already been reported to reduce gold (Au^{+}) ions to metallic gold nanoparticles [32].

2. Materials and methods

2.1. Materials

Mouse fibroblastic cells (L929) were purchased from the National Centre for Cell Science (NCCS), Pune, India. $AgNO_3$ of analytical grade

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was purchased from Merck (Mumbai, India) and ethanol was obtained from Helix India (Guwahati, India). *N. arbortristis* flowers were collected from a locality near IIT Guwahati and Majuli River Island. Flowers were shade dried at room temperature for about one month. Dried flowers were grinded and sieved to obtain fine powder. One gram of the powder was extracted in 10 mL of ethanol under incubation cum mild shaking condition at room temperature (25 °C). After 72 h, the ethanolic flower extract (EFE) was purified by double filtration (Whatman filter paper). The filtered EFE obtained was directly used for the synthesis of AgNPs as well as stored at 4 °C for further analyses.

2.2. Green synthesis of AgNPs

For synthesis of AgNPs, EFE concentration of 5% (v/v) was treated with 1 mM aqueous solution of $AgNO_3$ with a reaction volume of 500 μl and final volume made up to 2 mL with double distilled water. The reaction mixture was subjected to mild stirring (C-MAG-HS7, IKA®) of 200 rpm at 80 °C and observed for color change. Reaction parameters were optimized by varying the volume of EFE (1–10%), (v/v) against 1 mM $AgNO_3$, molar concentration kept fixed at 80 °C.

2.3. Characterization of the AgNPs

2.3.1. UV–visible spectroscopy

All UV–visible (UV–vis) spectroscopic studies were carried out on Cary 100 BIO UV–vis spectrophotometer (Varian, Palo Alto, CA, USA), to find out the surface plasmonic resonance (SPR) of the AgNPs.

2.3.2. Transmission electron microscope (TEM)

Sample preparation for TEM analysis includes centrifugation of synthesized AgNP colloidal solution (5 mL) twice at 20,000 rpm for 20 min to remove the non-covalently bounded molecules on their surfaces. The resulting pellet was redispersed in 1 mL of distilled water, a few drops were placed over a carbon-coated copper grid and the water was evaporated in a hot air oven (Daihan Labtech Co. Ltd. model LDO-150F, New Delhi, India) at 60 °C for 4 h. Transmission electron microscope (TEM) measurements were performed on a TEM instrument (JEOL model 2100, JEOL Ltd., Tokyo, Japan) operated at 190 V of 200 kV.

2.3.3. XRD, TGA, DTA and FT-IR analyses

To obtain the X-ray diffraction (XRD) pattern, AgNP solution was placed on a microscope glass slide and allowed to dry in a hot air oven at 50 °C, and the process was repeated to form a layer on the glass slide. The dried samples were analyzed with the help of an XRD instrument (Bruker Advance D8 XRD machine, Bruker, Madison, WI, USA) with a Cu source at 1.5406 Å wavelengths in thin film mode.

For FTIR analysis, AgNP colloidal solution (50 mL) was synthesized with optimum parameters (5% of EFE, 1 mM $AgNO_3$) and centrifuged at 20,000 rpm for 20 min. The resulting pellet was resuspended in 5 mL of distilled water and lyophilized (Christ Gefriertrocknungsanlagen GmbH Model 1–4, Osterode, Germany) for 16 h. Infrared spectra were recorded using a Fourier transform infrared (FT-IR) spectroscope (Spectrum One, Perkin Elmer, Waltham, MA, USA) from 4000/cm to 450/cm, with a resolution of 2 cm and five scans/sample by using 1 mg of finely powdered AgNPs prepared with 200 mg of KBr. 5 mg of lyophilized AgNPs was used for thermogravimetric (TGA) and differential thermal analysis (DTA) analyses.

2.4. Antioxidant assay

Antioxidant activity of the plant material was determined by DPPH scavenging assay with ascorbic acid as a standard followed by experimental analysis for presence of total phenolic compounds with gallic acid as a standard was performed according to Sánchez-Moreno et al. and Chang et al. respectively [33,34].

2.5. Antimicrobial assay

In vitro antibacterial activity was evaluated by using agar well diffusion assay with Mueller Hinton Agar (MHA) as growth media and determination of zone of inhibition measurement in millimeters [35]. Antibacterial activity was evaluated against gram negative bacteria *Escherichia coli* MTCC 443 at different concentrations of AgNPs synthesized from the N. *arbortristis* EFE. Fresh overnight cultures as inoculums of *E. coli* MTCC 443 (100 μl) were seeded on MHA plates using sterile cotton swabs. Agar media were bored with a sterile gel borer to create wells of 5 mm in diameter. 100 μl of different concentrations of AgNPs (50, 150, 250 and 500 μg/mL) were poured into separate wells and plates were incubated at 37 °C for 24 h. The diameters of inhibition zones were used to determine the antimicrobial activity and the average of 3 replicas was calculated. Growth curve of AgNP treated *E. coli* MTCC

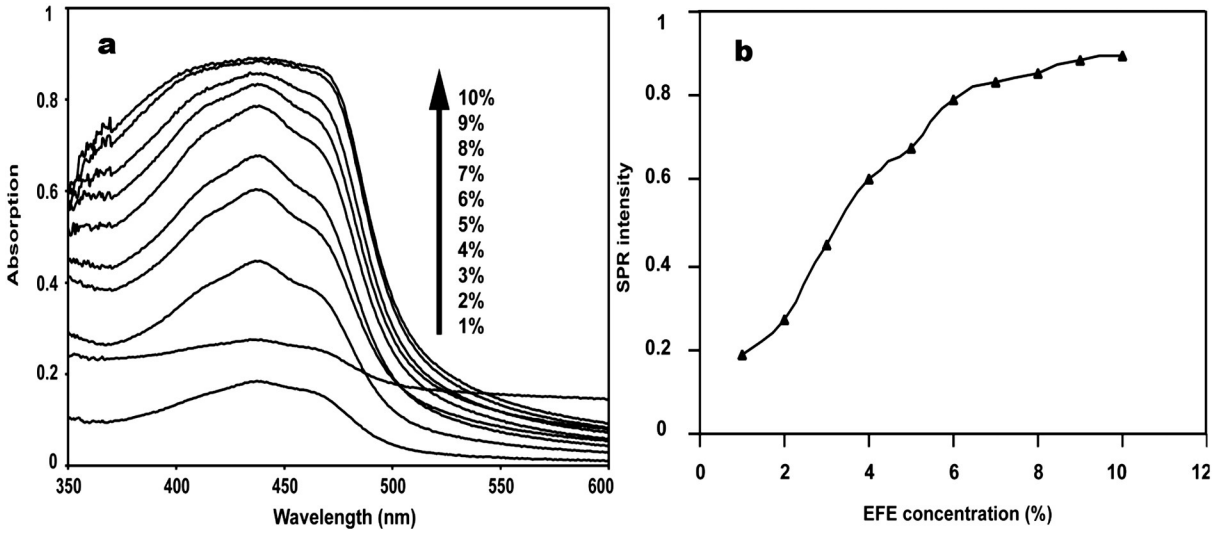

Fig. 1. (1a) UV–visible absorption spectra of AgNPs synthesized with different concentrations of EFE (1–10%) against 1 mM $AgNO_3$ at 80 °C. (1b) The SPR peak intensities against different concentrations of EFE (1–10%).