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Research paper

Green synthesis of silver nanoparticles using *Lippia nodiflora* aerial extract and evaluation of their antioxidant, antibacterial and cytotoxic effects

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ABSTRACT

Silver nanoparticles biosynthesis has received increasing attention in the field of nanotechnology due to their antimicrobial and biomedical applications. Green synthesis of metal nanoparticles is anticipated as a cost effective and eco-friendly alternative in the current research scenario. With this aim, the aqueous extracts made from the aerial parts of *Lippia nodiflora* were used as the reducing agents to synthesize silver nanoparticles (AgNPs) and their antioxidant, antibacterial and cytotoxic properties have also been evaluated. The biosynthesized AgNPs were characterized by UV–Visible Spectroscopy, Fourier-Transform Infrared Spectroscopy (FTIR) and X-Ray Diffraction (XRD) analysis. The AgNPs were found to be stable at −25.2 mV through zeta potential study. The morphology and size of synthesized silver nanoparticles were confirmed by Scanning Electron Microscope with energy dispersive spectra (SEM-EDX) and Transmission Electron Microscopy (TEM) analysis with size range from 30 to 60 nm. Biosynthesized AgNPs exhibited strong antioxidant activity as well as showed potent antibacterial activity against human pathogenic bacteria. The cytotoxicity study of AgNPs was also revealed against MCF-7 breast cancer cell lines in a dose-dependent manner. The recognized bioactivity confirmed by the synthesized AgNPs directs towards the clinical use as an antioxidant, antibacterial and cytotoxic agent.

1. Introduction

Nanotechnology is one of the most interesting areas of research in this era, which is budding day by day, with an impact in diverse aspects of human life [1]. For the past few years, nanometallic particles are gaining much attention owing to their applicability in the field of medicines, biology, material sciences and electronics at the nanoscale level [2]. Even though several routes are accessible, the synthesis of nanoparticles with a biological approach turns out to be crucial [3]. In the present scenario, the biosynthesis of nanoparticles using plant extracts are much simpler than the chemical synthesis method and may be employed for several therapeutic applications [4,5]. Moreover, the synthesis of nanoparticles using the plant extract as a reducing and capping agent was more beneficial than microbial synthesis. The effects of diverse plants like *Ficus carica* [6], *Piper longum* [7], *Rosmarinus officinalis* [8], *Lantana camara* [9], *Prunus yedoensis* [10], *Zingiber officinale* [11] *Azadirachta indica* [12], *Erythrina indica* [13], beet root [14], etc., were also reported for the synthesis of various metal nanoparticles.

Antioxidants are very essential to protect cells and biological macromolecules from degenerative reactions produced by free radicals and reactive oxygen species [15]. The antioxidant property of various plant products, such as polyphenolic substances (*e.g.*, flavonoids and tannins) derived from various plants and herbal extracts have been reported [16–18]. In recent studies, the oxygen-based free radicals have been proved to be scavenged effectively by inorganic nanoparticles [19,20]. Moreover, silver has long been documented as a valuable antimicrobial agent that reveals low toxicity in humans and comprises various *in vitro* and *in vivo* applications among the other metals [21]. The highly reactive metal oxide nanoparticles are well known to demonstrate tremendous bactericidal activity against Gram-positive and Gram-negative bacteria [22]. Recently, AgNPs exhibit lot of scope in the field of high sensitivity biomolecular detection, catalysis, biosensors and medicine along with the anti-fungal, anti-inflammatory and anti-angiogenesis activities [23,24]. In addition to antioxidant and antimicrobial activity, the cytotoxicity studies also comprise promi-

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nent significance and numerous studies are underway to elucidate these aspects.

In Indian traditional medicine, herbal extracts have long been used for treating varied pathological processes including respiratory, neurodegenerative and cardiovascular ailments [25,26]. Thus, nanoparticles synthesized by using such herbal extract may perhaps serve together as bactericidal and antioxidants. In the present study, we have explored the biosynthesis of AgNPs using the aqueous extract of aerial parts of *Lippia nodiflora. L. nodiflora* (Verbenaceae), a crawling recurrent herb, grow up in maritime areas near rivers and found throughout India. The plant exhibits anodyne, cardiotonic, antibacterial, diuretic, parasiticide and refrigerant properties [27]. Several studies regarding pharmacological properties of aerial parts of *L. nodiflora* were reported. However, till date, there is no report that portrays its prospective in nanobiotechnology to synthesize nanoparticles and thereby assessing its biomedical efficacy. Hence the present study was carried out to synthesize and characterize silver nanoparticles and to examine their antioxidant and antibacterial activities. In addition, the cytotoxic effect of green synthesized AgNPs against MCF-7 breast cancer cell line was also determined.

2. Materials and methods

2.1. Reagents

The reagents such as silver nitrate ($AgNO_3$), 2-Deoxy-D-ribose, butylated hydroxyl toluene (BHT), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), phenazine methosulphate (PMS), nitroblue tetrazolium (NBT), MTT [3-(4, 5-dimethylthiazol- 2-yl)-2, 5-diphenyltetrazolium bromide] were obtained from (Sigma Chemicals St. Louis, MO, USA). 2, 4,6- tripyridyl- S- triazine (TPTZ), thiobarbituric acid (TBA), trichloroacetic acid (TCA) ethylene diaminetetraacetic acid (EDTA), ferric chloride ($FeCl_3$), hydrogen peroxide (H_2O_2), and nicotinamide adenine dinucleotide-reduced (NADH), were obtained from M s^{-1} (Sisco Research laboratories, Mumbai, India). Nutrient agar and broth for antimicrobial activity were purchased from Hi-Media, Mumbai, India. All other chemicals and reagents used in this study were of analytical grade.

2.2. Preparation of L. nodiflora aerial extract

The fresh aerial parts of *L. nodiflora* were collected from Karaikudi, Sivagangai District, Tamilnadu and its identification was confirmed by a taxonomist, as reported earlier [28]. The freshly collected plant material was washed thoroughly with tap water, shade dried for 2 weeks at room temperature and then powdered with kitchen blender. The aerial parts of *L. nodiflora* exhibit highest polyphenolic content (98.31 ± 0.004 mg GAE/g), determined using the standard curve of gallic acid. In the present study, 10 g of powder was taken and mixed with 100 ml of sterilized double distilled water and boiled in a water bath at 60 °C for 10 min. After cooling, the mixture was filtered with Whatman No.1 filter paper and the aqueous filtrate was used for further study.

2.3. Biosynthesis of AgNPs

For the biosynthesis of AgNPs, 10 ml of aqueous extract was added to 190 ml of 1 mM aqueous silver nitrate solution and the synthesis was carried out using the conical flask. The mixture was gradually boiled in a water bath at varying temperatures ranging from 30 to 95 °C for 10 min, in order to study the temperature effects on the synthesis rate of AgNPs. Reduction of silver nanoparticles was observed by change in their color of the reaction mixture during temperature treatment. A control setup was also maintained without *L. nodiflora* extract. The AgNPs solution were subsequently purified by repeated centrifugation at 10,000 rpm for 20 min at 4 °C and the pellet obtained was suspended in sterilized double distilled water and freeze dried using lyophilizer.

2.4. Characterization of AgNPs

An aliquot of synthesized nanoparticles was initially characterized by UV–visible spectrophotometer in the wavelength range of 300–700 nm with Shimadzu spectrophotometer (Model UV-1800, Shimadzu, Kyoto, Japan) operating at 1 nm resolution. Fourier transform infrared spectroscopy (FTIR) analysis was carried out on Bruker Tensor 27 instrument (Germany) and the spectra were recorded in the range of wavelength between 4000 cm^{-1} and 400 cm^{-1}. For comparison, UV–visible and FTIR spectral analysis was also performed to the extract before addition of the silver nitrate solution. For XRD studies, dried nanoparticles were coated on XRD grid and the spectra was recorded using Cu Kα radiation ($\lambda = 1.54060$ Å) with nickel monochromator in the range of 2θ from 10° to 80°. The average crystalline size of the synthesized AgNPs was calculated using Scherrer's formula ($D = 0.9\lambda/\beta\cos\theta$). A small portion of AgNPs suspension was placed on glass slide to make thin film, dried over hot air oven and then the thin film was used for the SEM analysis equipped with EDX (SEM FEI QUANTA 250). The particle size distribution of AgNPs was evaluated using dynamic light scattering (DLS) measurements and zeta potential analysis was conducted with a Malvern Zetasizer Nanoseries compact scattering spectrometer (Malvern Instruments Ltd., Malvern, UK). The AgNPs were dissolved in physiological saline (0.9% w/v of NaCl) for zeta potential analysis. Data obtained were analyzed using Zetasizer software. The shape and size of the particles were measured with transmission electron microscopy (TEM) using Tecnai 10 instrument at 120 kV.

2.5. Determination of antioxidant activities

2.5.1. DPPH radical scavenging assay

The DPPH radical scavenging activity of silver nanoparticles was determined, as previously described [29]. Different concentrations (25–500 µg/ml) of AgNPs were prepared in de-ionized water and 0.1 ml of AgNPs solution was added to 1 ml of 0.1 mM of freshly prepared DPPH solution in ethanol. The reaction mixtures were shaken vigorously and absorbance at 517 nm was determined after 20 min at room temperature. Control sample was prepared without silver nanoparticles. BHT was used as positive control in all the assays. The radical scavenging activity was measured as a decrease in the absorbance of $DPPH^{\bullet}$ and calculated as follows:

$$\text{Scavenging effect}\ (\%) = [(A_c - A_s)/A_c] \times 100$$

where, A_c is the absorbance of the control, and A_s is the absorbance of the sample or standard.

2.5.2. Superoxide anion radical-scavenging assay

The superoxide anion radical-scavenging effect was determined by the method of Nishikimi et al. [30]. The purple formazan formed by nitroblue tetrazolium (NBT) by reacting with the superoxide radicals produced from phenazine methosulfate–nicotinamide adenine dinucleotide (PMS/NADH) non-enzymatic system was measured spectrophotometrically. In this assay, the reaction mixture consists of NBT (1 mM) in phosphate buffer (0.1 M, pH 7.4), NADH (1 mM), PMS (0.1 mM) and different concentrations (25–500 µg/ml) of AgNPs was incubated at room temperature for 5 min and the absorbance was recorded at 560 nm. The inhibition percentage was calculated against a control without the sample. The scavenging ability was calculated using the equation as described for DPPH assay.

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