Biofabrication and its \textit{in vitro} toxicity mechanism of silver nanoparticles using \textit{Bruguiera cylindrica} leaf extract

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Abstract

Silver nanoparticles (AgNPs) were synthesized using \textit{Bruguiera cylindrica} (\textit{B. cylindrica}) leaf extract, the extract was acted as a reducing and stabilizing agent. The formation of AgNPs was observed by UV–vis spectroscopy and surface plasmon resonance (SPR) occurred at 450 nm. The X-ray diffraction (XRD) analysis shows that the particles are face centered cubic (fcc) structure and average size of AgNPs was found at 16 nm. Fourier transmission infrared spectroscopy (FTIR) suggests that the presence of thirteen functional groups in capping with AgNPs. Transmission electron microscopy (TEM) image shows the well dispersed particles with size of 9–24 nm. Furthermore, the biosynthesized AgNPs were exhibited an excellent cytotoxic effect against MCF-7 breast cancer cell lines.

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Keywords: Green synthesis; \textit{Bruguiera cylindrica}; Surface plasmon resonance; Cell line culture

1. Introduction

Nanotechnology is the engineering of functional systems at the nano scale level with multi-disciplinary areas of applied science and engineering. It deals with the design, manufacture and characterization of extremely small components and systems \cite{1–4}. Nanometals are more reactive because of their small size and greater surface area \cite{5}. Synthesis of nanoparticles is done by various methods. But they are having some limitations in their synthesis and application aspects \cite{6,7}. Bio synthesis of nanoparticle provides the advisable applications like eco-friendly, cost effective and the minimum time consuming method \cite{8–12}. Abou El-nour et al. \cite{13}, reported the various phytochemicals present in plant will act as a reducing and capping agent for synthesis of AgNPs. \textit{Bruguiera cylindrica} is an important medicinal mangrove tree, belongs Rhizophoraceae with diverse of phyto-constituents. It is in medicinal practice against to malignant ulcers, hepatitis, diarrhea and malaria \cite{14,15}. Bio-synthesized AgNPs was performed to analyze the cytotoxic effect against MCF-7 breast cancer cell lines \cite{16–18}. The present study has aimed to synthesis,
characterization and cytotoxic effect of green AgNPs using *B. cylindrica* leaf extract.

2. Materials and methods

2.1. Collection of plant materials

Matured fresh leaves of *B. cylindrica* were collected from Pichavaram Mangrove forest, Tamil Nadu, India. *B. cylindrica* was confirmed by Department of Botany, Annamalai University, Annamalai Nagar, Tamilnadu, India.

2.2. Preparation of leaf extract

The collected leaves were washed in running water followed by rinsed with double distilled water and shade-dried at room temperature for 10 days. The fully dried leaves were powdered with a sterile electric blender. The powdered samples were preserved in an airtight container and away from the sunlight for further use. Two grams of powder leaf was mixed with 100 ml of de-ionized sterile water, heat up to 100 °C for 30 min and filtered by Whatman No.1 filter paper, finally the residue was re-extracted using vacuum pumps.

2.3. Phytochemical screening

Qualitative phytochemical screening of *B. cylindrica* aqueous leaf extracts was performed [13].

2.4. Biosynthesis of AgNPs

Silver Nitrate (AgNO₃) stock solution was prepared by dissolving 6.299 g of AgNO₃ in 100 ml of de-ionized water. 2 ml of *B. cylindrica* leaf extract was added to 20 ml of AgNO₃ solution and kept at room temperature [17].

2.5. Characterization of AgNPs

The absorption spectrum of the reaction mixture was recorded at room temperature using UV–vis spectrophotometer (Hitachi-U-2001) from 300 to 800 nm at 1 nm resolution for detection of AgNPs formation. The residue containing silver nanoparticles solution was dispersed in sterile de-ionized water to remove the biological impurities. The pure residue was dried in oven at 70 °C overnight. Fourier Transform Infrared (FTIR) studies, dry powder of the nanoparticles was prepared by centrifuge at 10,000 rpm for 15 min by Perkin–Elmer spectrum instrument resolution at 4 cm⁻¹ in the transmission mode of 4000–400 cm⁻¹ in KBR pellets. XRD measurement, the silver nanoparticles solution was drop-coated on glass on XPERT-PRO, D-8, with 30kv, 40 mA with Cu kα radians at 20 angle. X-ray powder diffraction is a rapid analysis for phase identification of a crystalline material and can provide information on unit-cell dimensions. The crystallite size was calculated by width of the XRD peaks using the Scherrer’s formula, $D = \frac{K\lambda}{β\cosθ}$. The size distribution and shape of NPs were estimated on the basis of TEM micrographs. The sample was placed over a carbon tape and dried. A pinch of dried sample was coated with a thin layer of platinum in an auto fine coater. The transmission electron microscopy (TEM) was performed the synthesized AgNPs using JEM 1011, JEOL, Japan.

2.6. Cytotoxicity assay

MCF-7 cells were collected from National Centre for Cell Science (NCCS), Pune, India. The cell viability was estimated using the MTT calorimetric technique. Cells were cultured in 96 well plate for 6 h at 37 °C and kept in CO₂ incubator. 100 μL of MTT solution (3-(4,5-dimethlthiazoyl-2)-2,5-diphenyltetrazolium bromide, a yellow tetrazole) without phenol red (5 mg/mL) in phosphate buffer solution was added to each well and incubated for 5 h at 37 °C, for the reduction of MTT by metabolically active cells, in part by the action of dehydrogenase enzymes to generate reducing equivalents. Purple color formazone crystals formed and then dissolved with 100 μL of isopropanol or dimethyl sulphoxide (DMSO). After the treatment, these crystals were observed at 540 nm absorbance which is directly proportional to the number of living cells in culture. The percentage of viability was calculated by using the following formula,

\[
\text{Percentage of viability} = \frac{\text{Sample absorbance (AgNPs)}}{\text{Control absorbance (untreated)}} \times 100
\]

3. Results and discussion

Phytochemical screening of *B. cylindrica* was observed that the aqueous fraction contains alkaloids, saponins, phenols and flavonoids, while the tannins, glycosides and cardiac glycosides were absent in the extract [19,20]. The colorless silver nitrate solution color was turned into yellow in color when it was mixed with *B. cylindrica* leaf extract and after some
incubation period the reaction mixture was turned into dark brown in color, which designates the formation of silver nanoparticles [21–23]. Thus, the nanoparticle was obtained by reduction of Ag\(^+\) ions using *B. cylindrica* leaf extract [24,25]. Fig. 1 shows the UV–vis spectrum of AgNPs synthesis with increased time intervals at 0 to 5, 10, 15 and 20 min and absorption peaks of SPR observed at 450 nm without any shifting of peak and similar results were accepted by *Arbutus unedo* and *Cynodon dactylon* [26,27]. The XRD spectrum revealed the occurrence of four major intense peaks by leaf extract at 2\(\theta\) values of 35.89\(^\circ\), 40.30\(^\circ\), 64.52\(^\circ\) and 76.09\(^\circ\) which are corresponding to (111), (200), (220) and (311) (Fig. 2). Based on the intense peaks diffraction pattern shows the face centered cubic (fcc) crystal structure. The intense peak at 64.52\(^\circ\) indicates high degree of crystallinity and the similar patterns also been reported by Sanjenbam et al. and Shameli et al. [28,29]. The average size of AgNPs was calculated by Debye-Scherrer’s formula,
D = \frac{K \lambda}{\beta \cos \theta}

where D is the particle diameter size, K is a constant equal to 1, \( \lambda \) is X-ray wavelength (0.1541 nm), \( \beta \) is full width half maximum (FWHM) and \( \theta \) is the diffraction angle. The result from the Scherrer's formula the average diameters of the AgNPs according to the strong diffraction peak was around 12 nm [30]. In FTIR, the stabilized AgNPs showed their vibration spectrum (Fig. 3) at 3361.01 cm\(^{-1}\) (alcohols group of O–H stretching), 2281.91 cm\(^{-1}\) (alkynes–C–C– stretching), 1575.12 cm\(^{-1}\) (carbonyl group of amides N–H stretching), 1034.79 cm\(^{-1}\) (aliphatic amines C–N stretching), 821.96 cm\(^{-1}\) (alkyl halides C–Cl stretching), 717.07 cm\(^{-1}\) (aromatic C–H stretching), 590.92 cm\(^{-1}\) (alkyl halide C–Br stretching) and band at 2926.81 cm\(^{-1}\) suggests that the presence of alkanes group bound by C–H stretching of individual protein were measured respectively. Based on the TEM

Fig. 3. FTIR analysis of green synthesized AgNPs from B. cylindrica leaf extract.

Fig. 4. TEM image of biosynthesized AgNPs from B. cylindrica leaf extract.
Table 1
Percentage of Cells Viability on MCF-7 Cells was measured after treatment with biosynthesized AgNPs for 12, 24 and 36 h by MTT assay.

| Treatment                              | 12 h Dead (%) | 12 h Viable (%) | 24 h Dead (%) | 24 h Viable (%) | 36 h Dead (%) | 36 h Viable (%) |
|----------------------------------------|---------------|-----------------|---------------|-----------------|---------------|----------------|
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