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Research Article

Cottonseed Oilcake Extract Mediated Green Synthesis of Silver Nanoparticles and Its Antibacterial and Cytotoxic Activity

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Agroindustrial byproduct mediated green synthesis of silver nanoparticles was carried out using cottonseed oilcake (CSOC) extract. The aqueous silver nitrate formed stable silver nanoparticles with CSOC extract as a reducing agent for Ag^{+} to Ag^{0}. The synthesized nanoparticles were characterized using energy dispersive X-ray spectroscopy (EDS), transmission electron microscopy (TEM), Fourier transform infrared (FTIR) spectroscopy, and X-ray diffraction (XRD) techniques. The synthesized silver nanoparticles (AgNPs) (4 mM) significantly inhibited the growth of phytopathogens, *Pseudomonas syringae* pv. *actinidiae* and *Ralstonia solanacearum*. Further, cytotoxicity of AgNPs was evaluated using rat splenocyte cells. The splenocyte viability was decreased according to the increasing concentration of AgNPs and 90% of cell death was observed at 100 μg/mL.

1. Introduction

Over the last few decades, nanoparticles have been widely applied in the fields of science and technology and medicine. In particular, silver nanoparticles (AgNPs) found applications in biology, medicine, and materials engineering due to their broad spectrum of physicochemical and optical properties. There are several physicochemical methods which have been espoused for the synthesis of silver and other metal nanoparticles [1, 2]. Particularly, the chemical synthesis of AgNPs cannot avoid the use of toxic chemicals [3]. Therefore, the biosynthesis of AgNPs gained considerable attention in the past decade owing to their perceived technological importance and environmental benefits [4]. Several natural resources like plant extracts [5], microorganisms [6], milk [7], oilcake [8], and panchakavyam [9] have been explored for the biological synthesis of AgNPs. Among the biological routes, plants based AgNPs synthesis gained more attention due to their rapid formation, easy handling, and large-scale production.

Cotton plant (*Gossypium hirsutum*) belongs to family *Malvaceae*, a perennial shrub cultivated as annuals. It is an economic plant, mainly grown for cotton lint, which is used in textile and clothing [10]. Apart from the fibers, cottonseeds also have an economical value. Cottonseed oil is a cooking oil extracted from the seeds of cotton plant. Cottonseed oilcake (CSOC) was generated as a byproduct of cottonseed oil manufacturing industry. Due to its rich protein content, it is used as animal feed. Recent studies reported synthesis of AgNPs using cotton plant leaves [10]. However, there is no report for the synthesis of AgNPs using CSOC.

Pseudomonas syringae pv. *actinidiae* and *Ralstonia solanacearum* are the pathovars responsible for bacterial blight in several grain crops worldwide. The disease is responsible for large production losses in Australia, Europe, America, and some Asian countries [11, 12]. Control measures for these pathogens remain a major challenge for farmers. With developments in science and technology, researchers focused on the application of nanomaterials for controlling plant diseases. AgNPs are reported to have both antibacterial and antifungal activity [7, 13]. Thus, the objectives of the study were to biosynthesize AgNPs using CSOC and to evaluate the antibacterial and cytotoxic activities of the synthesized AgNPs.

2. Materials and Methods

2.1. Materials. CSOC was procured from a local market in Mallasamudram, Tamil Nadu, India. Silver nitrate ($AgNO_3$) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade.

2.2. Oilcake Extraction and Biosynthesis of AgNPs. Two grams of CSOC was suspended in 100 mL of sterile ultrapure water (conductivity = 18 $\mu\Omega$/m, TOC < 3 ppb) (Barnstead, Waltham, MA, USA), and the flask was shaken at constant speed of 180 rpm for 6 h. Later, the mixture was filtered through Whatman No. 1 filter paper followed by 0.2 μm membrane filter. The filtrate was used for the synthesis of AgNPs. Synthesis of AgNPs was carried out according to Lee et al. [7]. Briefly, 40 mL of CSOC extract was mixed with 960 mL of 1 mM $AgNO_3$ solution and the resulting white colored mixture was incubated for 6–8 h in a rotary shaker (180 rpm) at 26°C. Reduction of Ag^+ ions to Ag nanocrystals was monitored by change in color of the reaction mixture from white to dark brown.

2.3. Characterization of AgNPs. The absorption spectra of the synthesized AgNPs were obtained using a Shimadzu UV-1800 UV-Vis spectrophotometer (300–800 nm). Biological transmission electron microscopy (Bio-TEM; H-7650, Japan, Hitachi) was used to observe the morphology of the synthesized AgNPs. The elemental composition of the synthesized AgNPs was confirmed by scanning electron microscopy-energy dispersive spectroscopy (SEM-EDS; JEOL-64000, Japan). The X-ray powder diffraction (XRD) analysis was carried out using Rigaku X-ray diffractometer (Rigaku, Japan). Scanning was performed in the region of 2θ = 30 to 80° at 0.041°/min with a time constant of 2 s. The Fourier transform infrared (FTIR) spectra of the AgNPs were measured using a Perkin-Elmer spectrum (USA) in the diffuse reflectance mode at a resolution of 4 cm^{-1} in KBr pellets.

2.4. Antibacterial Activity of AgNPs. *Pseudomonas syringae* pv. *actinidiae* and *Ralstonia solanacearum* were procured from the Korean Agriculture Culture Collection (KACC), South Korea, and the cultures were maintained on Luria-Bertani (LB) agar. The cultures were independently allowed to grow in 100 mL of LB broth supplemented with different concentrations (1–4 mM) of AgNPs. The optical density was measured every 4 h to determine the growth of the bacteria using the Shimadzu UV-1800 spectrophotometer. The culture without AgNPs was used as a control.

2.5. Splenocyte Cells. Adult 8–12-week-old male Sprague-Dawley rats were purchased from Koatech, South Korea. The animals were maintained in a specific pathogen-free facility. Fresh splenocytes of the rats were obtained by teasing the spleen under aseptic conditions according to Lu et al. [14]. Single cell suspensions were prepared according to Aravinthan et al. [15]. The splenocytes were cultured in Roswell Park Memorial Institute (RPMI) medium containing 10% fetal bovine serum and maintained at confluence of 80% and thus used for cytotoxicity assays.

2.6. MTT Assay. The cytotoxic effect of AgNPs was assessed by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole) cell viability assay. In brief, rat primary spleen cells grown in 96-well tissue culture plates were treated with different concentrations of AgNPs (10–100 μg/mL) for 24 h at 37°C. The stock concentration (5 mg/mL) of MTT was prepared and 20 μL of MTT was added to each AgNPs treated well and incubated for 4 h. Purple color formazone crystals were observed and these crystals were dissolved with 200 μL of dimethyl sulphoxide (DMSO) and read at 595 nm in a multiwell plate reader (Epoch microplate spectrophotometer, Biotek, USA).

3. Results and Discussions

3.1. Characterization of AgNPs. The present study focused on green and rapid AgNPs synthesis using CSOC extract as a bioreductant. The initial indication for AgNPs formation is color change in the reaction mixture. A clear dark brown color was formed within 4 h when CSOC extract was added to the 1 mM $AgNO_3$, which indicates the formation of AgNPs. This color change was attributed to excitation of surface Plasmon vibrations within the synthesized AgNPs [16]. The UV-visible absorbance of the reaction mixture was scanned between 300 and 800 nm (Figure 1). The characteristic surface plasmon resonance (SPR) band was observed at 450 nm. It is suggested that the sharp SPR peak for AgNPs appeared around 450 nm, which suggested the spherical shape of AgNPs [17]. TEM images of the biosynthesized AgNPs showed that the particles were spherical in shape with an average size of 10 to 90 nm (Figure 2(a)). The particle size distribution of the synthesized AgNPs is shown in Figure 2(b). Several studies have reported smaller size spherical AgNPs [8, 18].

The presence of silver atoms in nanoparticles was confirmed using SEM-EDS. The optical absorption peak was observed at 3 keV (Figure 3) which is typical for the absorption of silver nanocrystallites due to SPR [19]. The XRD profile of biosynthesized AgNPs is shown in Figure 4(a). The diffraction peaks (111), (200), (142), (220), and (311) correspond to the reflections of face centered cubic structure of synthesized AgNPs. The results are consistent with previous studies reporting similar diffraction peaks for AgNPs obtained by green synthesis [18, 20]. Some additional as yet assigned peaks are also observed in the XRD analysis due to the presence of bioorganic matters and capping agents for AgNPs synthesis [21].

FTIR spectroscopy is an important tool for the identification of functional groups and interactions between molecules. The FTIR spectrum of the biosynthesized AgNPs showed strong absorption peaks at 3299, 2918, 1653, and 1051 cm^{-1}, respectively. The peak at 3299 cm^{-1} corresponds to O–H stretching vibrations of alcohols and phenols. C–H bonds at 2918 cm^{-1} arise from metabolites present in the CSOC. The sharp absorption peak at 1653 corresponds to C=C stretching vibrations of the metabolites present in the CSOC extract [22]. The absorption peak at 1051 cm^{-1} could be of N–H stretching of primary and secondary amines

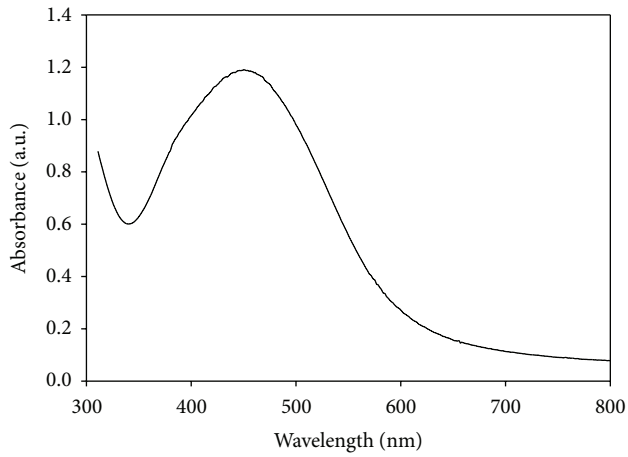

Figure 1: UV-Vis absorption spectrum of the AgNPs prepared from 1 mM $AgNO_3$ solution.

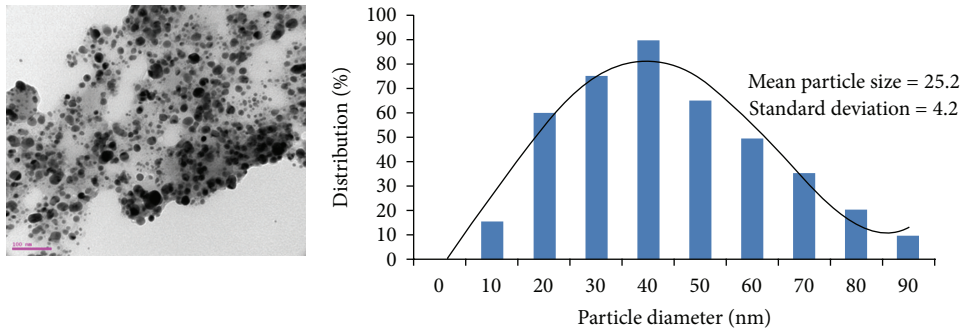

Figure 2: (a) Transmission electron microscopic images of AgNPs. (b) Particle size distribution of the synthesized AgNPs.

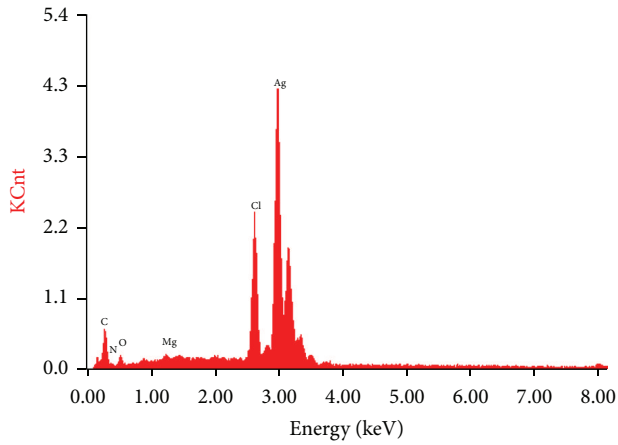

Figure 3: SEM-EDS spectrum of AgNPs. A strong peak at 3 keV confirming the presence of Ag.

and amides (Figure 4(b)), which mostly come from the sugars present in the CSOC extract. The FTIR spectra results indicated that the phytocompounds of the CSOC extract, such as phenols, alkaloids, sugars, and amino acids, might be participating in the process of AgNPs synthesis [23].

3.2. Antibacterial Activity of AgNPs. The bactericidal activity of the synthesized AgNPs was evaluated against phytopathogenic bacteria, namely, *Pseudomonas syringae* pv. *actinidiae* and *Ralstonia solanacearum*, at different concentrations (1–4 mM) and the results are shown in Figures 5(a) and 5(b). The results clearly indicated that 4 mM concentrations of AgNPs effectively inhibited the bacterial population in the medium. The potential reason for the bactericidal property of silver is that AgNPs may attach to the surface of the cell membrane, disturbing permeability and respiration functions of the cell, which leads to cell death [24]. The results are consistent with our previous study reporting the antibacterial activity of AgNPs against phytopathogens [8, 15].

3.3. Cytotoxicity of AgNPs. The cell viability test is one of the important methods for toxicology investigation which explain the cellular response to a toxic material and it can provide information on cell death, survival, and metabolic activities [25]. Thus, in vitro cytotoxicity of the silver nanoparticles was evaluated against rat splenocytes at different concentrations (10–100 μg/mL). Our results clearly demonstrated that there is a direct dose dependent relationship with the tested cells at higher concentrations. In contrast, the presence of 100 μg/mL of AgNPs significantly inhibits the cells growth

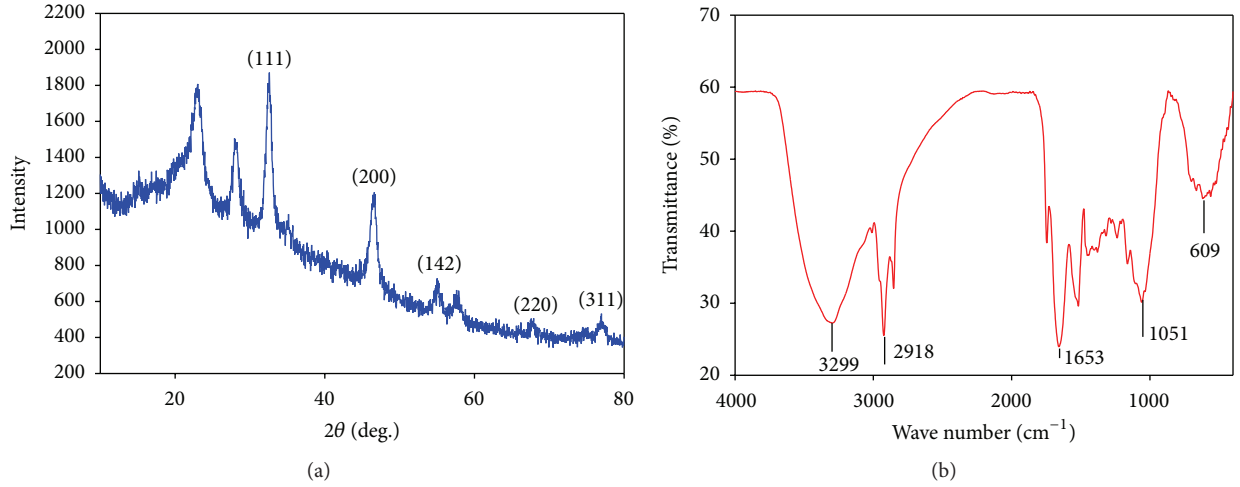

Figure 4: (a) XRD pattern and (b) FTIR spectra of AgNPs synthesized from CSOC extract.

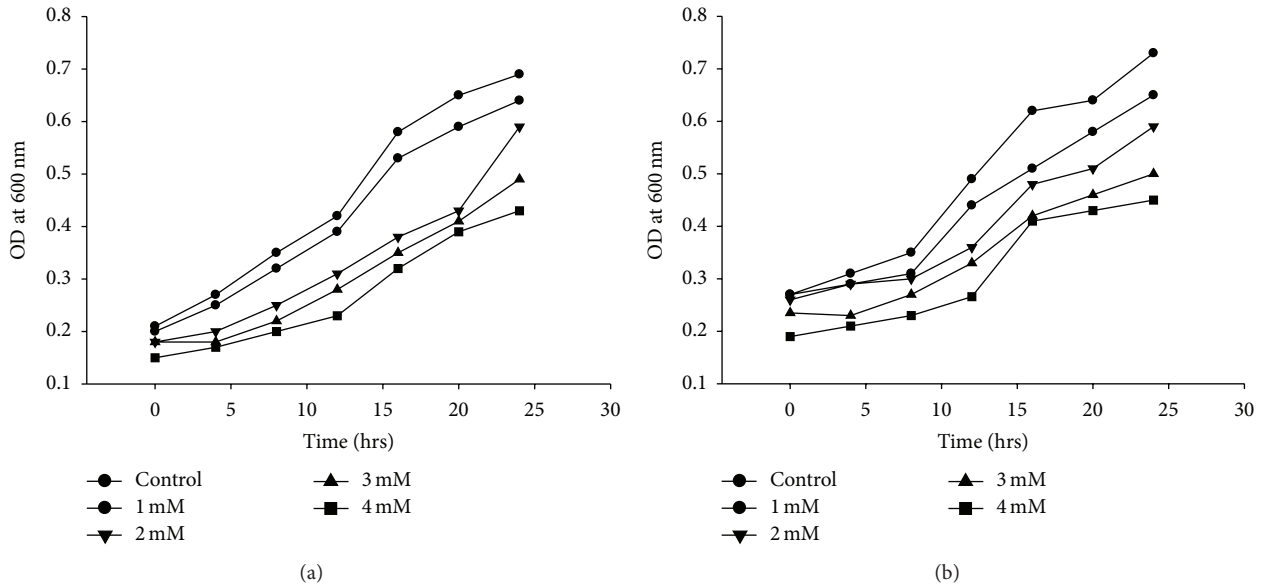

Figure 5: Effect of AgNPs on growth of phytopathogens. (a) *Pseudomonas syringae* pv. *actinidiae* and (b) *Ralstonia solanacearum*.

by more than 90% (Figure 6). Several studies have reported that AgNPs may induce reactive oxygen species and cause damage to cellular components leading to cell death [26–29]. Nevertheless, this is the first report on cytotoxicity effects of green synthesized AgNPs using CSOC extract against rat splenocytes.

4. Conclusion

This study reported simple, cost-effective, and ecofriendly agroindustrial waste mediated synthesis of AgNPs using CSOC extract. The green synthesized AgNPs were characterized by UV-Vis spectrometry, FTIR, XRD, and TEM. In addition, the prepared AgNPs had significant antibacterial activity against phytopathogenic bacterial strains. However, the results of the cytotoxicity study indicate that more studies are warranted before applying the AgNPs in agricultural and biomedical sciences.

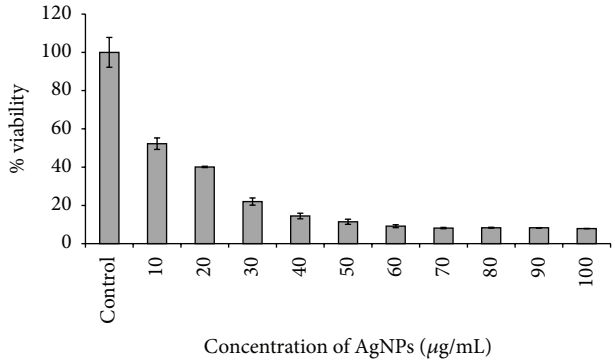

Figure 6: Cytotoxic effects of AgNPs on rat splenocytes.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

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