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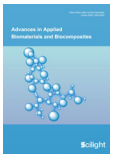

Supplementary Materials

Green Fabrication of Silver Nanoparticles via Lichen Secondary Metabolites: A Sustainable Bioreduction Approach

Vasim-Akram, Hajeebasha[1]., Sri Ashwathi Thangaraj[1]., Subhashini Selvakumar[1]., Swetha Pushpanathan[1,3], Keerthivasan Sivasubramaniyan-Murugesan[1], Rajaprabu Nagaraj[2,4], Velavan Viswakethu*[1,5]. Moorthy Maruthapandi*[6,7]

[1]*Department of Biotechnology, Bishop Heber College (Autonomous), Tiruchirappalli–620017, Tamil Nadu, India.*
[2]*Biomedical Research Laboratory, Department of Botany, Bharathiar University, Coimbatore-641046, Tamil Nadu, India.*
[3]*School of Biosciences and Technology, SRM Institute of Science & Technology (Deemed University), Tiruchirapalli-621105, Tamil Nadu, India*
[4]*Department of Botany, Bharti Vishwavidyalaya (Deemed University), Durg, 491001. Chhattisgarh, India*
[5]*Postharvest Science Department, Institute of Postharvest and Food Sciences (Volcani Institute-Agricultural Research Organization), Rishon LeZion 7505101, Israel*
[6]*Department of Chemistry, Bar-Ilan University, Ramat-Gan 52900, Israel*
[7]*Bar-Ilan Institute for Nanotechnology and Advanced Material, Bar-Ilan University, Ramat-Gan 52900, Israel*

Corresponding Authors *:velavan.sv@gmail.com, maruthapandimartin.m@gmail.com

Materials and Methods

Specimen (lichen) collection and identification

The lichen specimens were collected from the Western and Eastern Ghats in Tamil Nadu, India. Specimens were obtained from rock-inhabiting structural components [30]. For thalli attached to rocks, chisel and hammer were used to detach them from the substrate [45]. Terricolous lichens were extracted from soil and submitted to the Lichen Herbarium, Department of Botany (Biomedical Research Laboratory), Bharathiar University, Coimbatore, Tamil Nadu, India. GC–MS analysis of the lichens revealed the presence of various bioactive phytochemicals [45].

Biosynthesis of lichen-based silver nanoparticles (AgNPs)

Lichen species such as L. austroamericanum, P. melanothrix, and H. diademata (Figure 1) were used for nanoparticle synthesis. Air-dried samples (5–10 g) were mixed with 100 mL of 100% acetone and processed using a cold extraction method, followed by filtration through Whatman filter paper. The lichen samples were eluted with acetone and incubated on a rotary shaker at 150 rpm for 24 h at 27 °C. Following the method of [30], the extracts of *L. austroamericanum*, *P. melanothrix*, and H. diademata were used for AgNP biosynthesis using different ratios (1:9, 1:3, 1:2, 1:1), $AgNO_3$ concentrations (1 mM and 10 mM), and incubation periods (24 h, 48 h, and 72 h) at 25 °C and 40 °C. Silver nanoparticles were predominantly synthesized at 40 °C. Particle size analysis was performed using a Zetasizer.

Synthesis and characterization of silver nanoparticles (AgNPs)

AgNPs were synthesized by mixing 5 mL of lichen acetone extract (10 mg/mL) with 15 mL of silver nitrate solution (10 mM) in a flask. The mixture was gently shaken for 3.5 h under dark conditions and then incubated at 40 °C for 72 h to facilitate nanoparticle formation. The resulting biogenic AgNPs were characterized using various analytical techniques.

UV–Visible spectroscopy

UV–Visible spectroscopy was employed to analyze the optical properties of the synthesized nanoparticles in the wavelength range of 300–600 nm, using deionized water as a blank. This technique is particularly useful for characterizing plasmonic nanoparticles such as gold and silver, as surface plasmon resonance (SPR) peaks provide insights into particle size, shape, and aggregation. For green synthesis, 2 g of lichen was added to 30 mL of distilled water and heated at 80 °C for 1 h. The mixture was then filtered, and the filtrate was used as the lichen extract for nanoparticle biosynthesis.

Fourier transform infrared spectroscopy (FTIR)

FTIR analysis was used to identify functional groups in the lichen extract that may be involved in the reduction and stabilization of AgNPs. This technique measures the absorption of infrared radiation at different wavelengths, providing detailed information about surface chemistry. The spectra obtained helped identify chemical bonds and organic functional groups attached to the nanoparticle surface, which are important for understanding their interactions with the surrounding environment.

Particle size analysis (zeta potential analysis)

The particle size of the synthesized nanoparticles, ranging from sub-nanometers to several micrometers, was determined using dynamic light scattering (DLS). The size distribution and stability of nanoparticles were further evaluated through zeta potential measurements using a Zetasizer. Higher absolute zeta potential values indicate stronger electrostatic repulsion and better colloidal stability, which is essential for assessing nanoparticle dispersibility.

X-ray diffraction (XRD) analysis

The crystalline structure of AgNPs synthesized from acetone extracts of L. austroamericanum, P. melanothrix, and H. diademata were analyzed using X-ray diffraction (XRD). Measurements were performed over a 2θ range of 5°–80°. Key parameters such as peak position, d-spacing, full width at half maximum (FWHM), and relative intensity were recorded to confirm nanoparticle formation and crystallinity (Ibrahim, 2015).

Scanning electron microscopy (SEM)

The morphology and size distribution of AgNPs were examined using a scanning electron microscope (SEM; Hitachi S-4500). A thin layer of freshly prepared sample was deposited onto a silicon wafer and dried at 37 °C in a cooled incubator. For SEM analysis, thin films were prepared by placing a drop of the sample onto a carbon-coated copper grid. Excess solution was removed using blotting paper, and the film was exposed to a mercury lamp for 5 min. The samples were then analyzed at 20 kV to determine nanoparticle size and surface morphology.