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Evaluation of the antimicrobial efficacy of phytogenic silver nanoparticles

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ABSTRACT

Objective: To evaluate the antimicrobial activity of silver nanoparticles synthesized from *Psidium guajava* (*P. guajava*) against human pathogens. **Methods:** Ultraviolet-visible (UV-Vis) spectrophotometry and transmission electron microscopy (TEM) were performed to confirm the formation and stability of silver nanparticles. Antimicrobial activities of the synthesized Ag nanoparticles were determined using the agar well diffusion assay method. **Results:** UV-Vis spectrum of the aqueous medium containing silver nanoparticles showed absorption peak at around 410 nm. TEM showed the formation of silver nanoparticles with an average size of 59 nm. The formed silver nanoparticles showed good antimicrobial activity against *Escherichia coli*, *Bacillus cereus* and *Candida tropicalis*. **Conclusions:** *P. guajava* demonstrated strong potential for synthesis of silver nanoparticles by rapid reduction of silver ions (Ag^+ to Ag^0). Biological methods are a good competent for the chemical procedures, which are environment friendly and convenient.

1. Introduction

Nanotechnology has attracted a great interest in recent years due to its expected impact on many areas such as energy, medicine, and electronics. The development of new materials with nanometer size including nanoparticles, nanotubes, nanowires, *etc.*, is the major activity. Among all, nanoparticles with the unique properties in chemistry, optics, electronics, and magnetics have led to an increasing interest in their synthesis. Nanoparticles have been synthesized by sol-process, micelle, chemical precipitation, hydrothermal method, pyrolysis, chemical vapour deposition, bio-based protocols, *etc*[1]. Among the above, bio-based protocols are currently under exploitation. Recently, several authors have accomplished the biosynthesis of metal nanoparticles using biomass obtained from unicellular organisms like bacteria[2] and fungi[3], as well as extracts of plants, *e.g. Euphorbia hirta*[4], *Catharanthus roseus*[5], *Shorea tumbuggaia*[6], *Diopyros kaki*[7]. The rate of synthesis of nanoparticles by plant extracts is comparable to those of chemical methods and faster than green synthesis.

Psidium guajava (*P. guajava*) L, belonging to the Myrtaceae family, has been reported to have anti-diarrheal[8], anticancer[9], anti-inflammatory, analgesic[10], antiulcer[11], and antibacterial[12] activities. In this study we explored for the potential of the *P. guajava* to enlarge the scope of non-toxic biological systems for the biosynthesis of metallic nanomaterials.

2. Materials and methods

2.1. Materials

All chemicals used in this experiment were of highest purity and obtained from Sigma (Bangalore, India) and Merck (Mumbai, India). *P. guajava* leaves were collected from Regional Agricultural Research Station, Tirupathi, Andhra Pradesh, India.

2.2. Plant material and synthesis of silver nanoparticles

Plant leaf extract was prepared by mixing 10 g of dried powder with 100 mL deionized water in 500 mL of

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Erlenmeyer flask and boiled for 10 min. For the reduction of Ag^+ ions, 10 mL of leaf extract was mixed with 90 mL of 1 mM aqueous of $AgNO_3$ and then heated at 80 ℃ for 15 min. A change from brown to reddish color was observed.

2.3. Ultraviolet-visible (UV-Vis) spectra analysis

The reduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 h after diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer UV-2450 (Shimadzu).

2.4. Transmission electron microscopy (TEM)

TEM (HITACHI, H-7500) is a microscopy technique whereby a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through. An image is formed from the interaction of the electrons transmitted through the specimen. The image is magnified and focused onto an imaging device.

2.4. Antimicrobial activity study

Antimicrobial activities of the synthesized Ag nanoparticles were determined, using the agar well diffusion assay method[13]. Approximately 20 mL of molten and cooled media (NA/SDA) was poured in sterilized Petri dishes. The plates were left overnight at room temperature to check for any contamination to appear. The test organisms were grown in selected broth for 24 h. A 100 mL broth culture of each test organism (1×10^5 CFU/mL) was used to prepare lawns. Agar wells of 5 mm diameter were prepared with the help of a sterilized stainless steel cork borer. Two wells were prepared in the agar plates. The wells were labeled as A and B. 'A' well was loaded with 30 μL of Ag nanoparticles suspended hydrosol and 'B' well was loaded with 30 μL of positive control drugs (chloromphenical/ketoconazole). The plates containing the test organism and Ag nanoparticles were incubated at 37 ℃. The plates were examined for evidence of zones of inhibition, which appear as a clear area around the wells. The diameter of such zones of inhibition was measured using a meter ruler and the mean value for each organism was recorded and expressed in millimeter.

3. Results

3.1. UV-Vis spectra analysis

The color change showed the presence of Ag nanoparticles in the *P. guajava* leaf extract and it was characterized by UV-Vis spectrophotometer and monitored by taking readings at regular time intervals in UV-Vis spectrophotometer UV-2450 (Shimadzu). The strong broad peak located at 410 nm was observed for Ag nanoparticles (Figure 1).

3.2. TEM analysis of silver nanoparticles

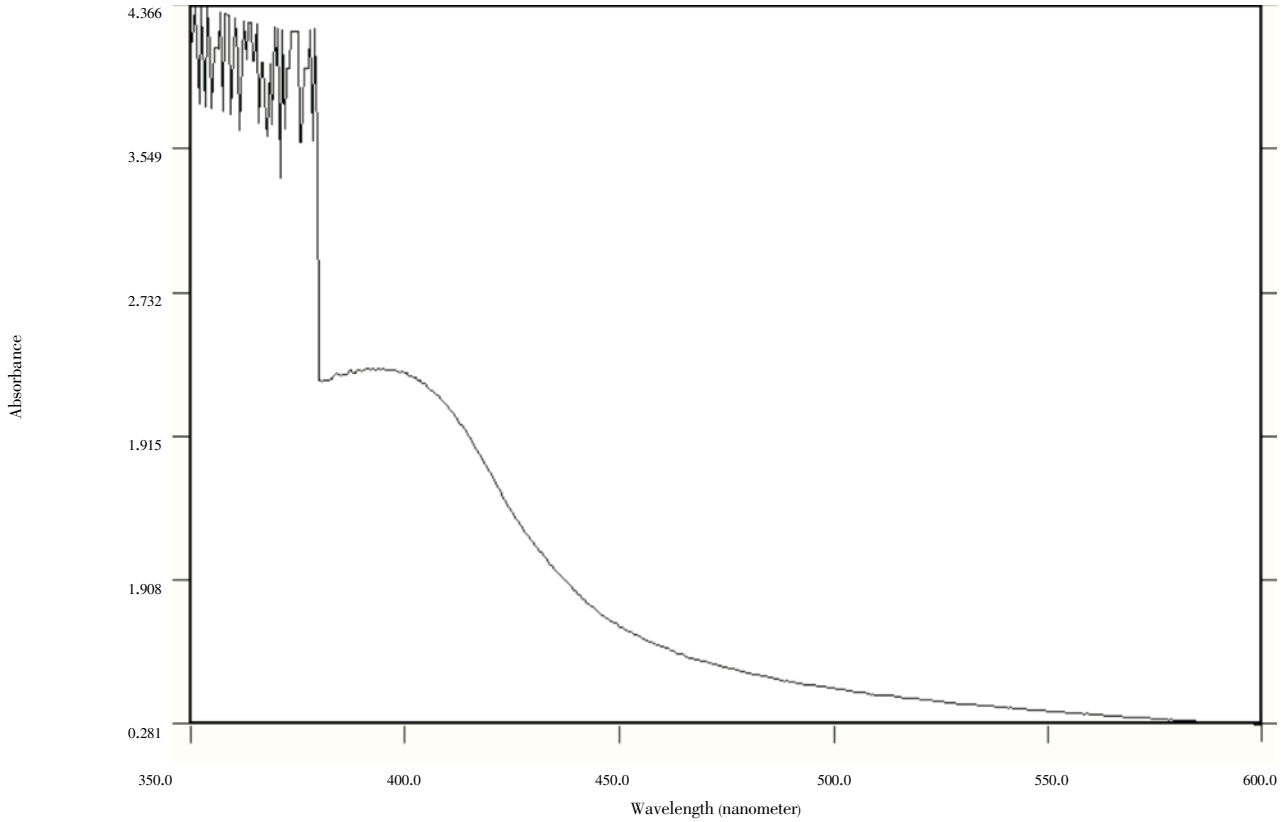

Figure 1. UV-Vis absorption spectra of silver nanoparticles (410 nm) synthesized from *P. guajava*.