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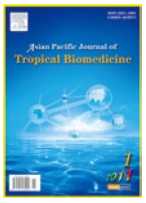

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Evaluation of the antimicrobial efficacy of phytogenic silver nanoparticles

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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

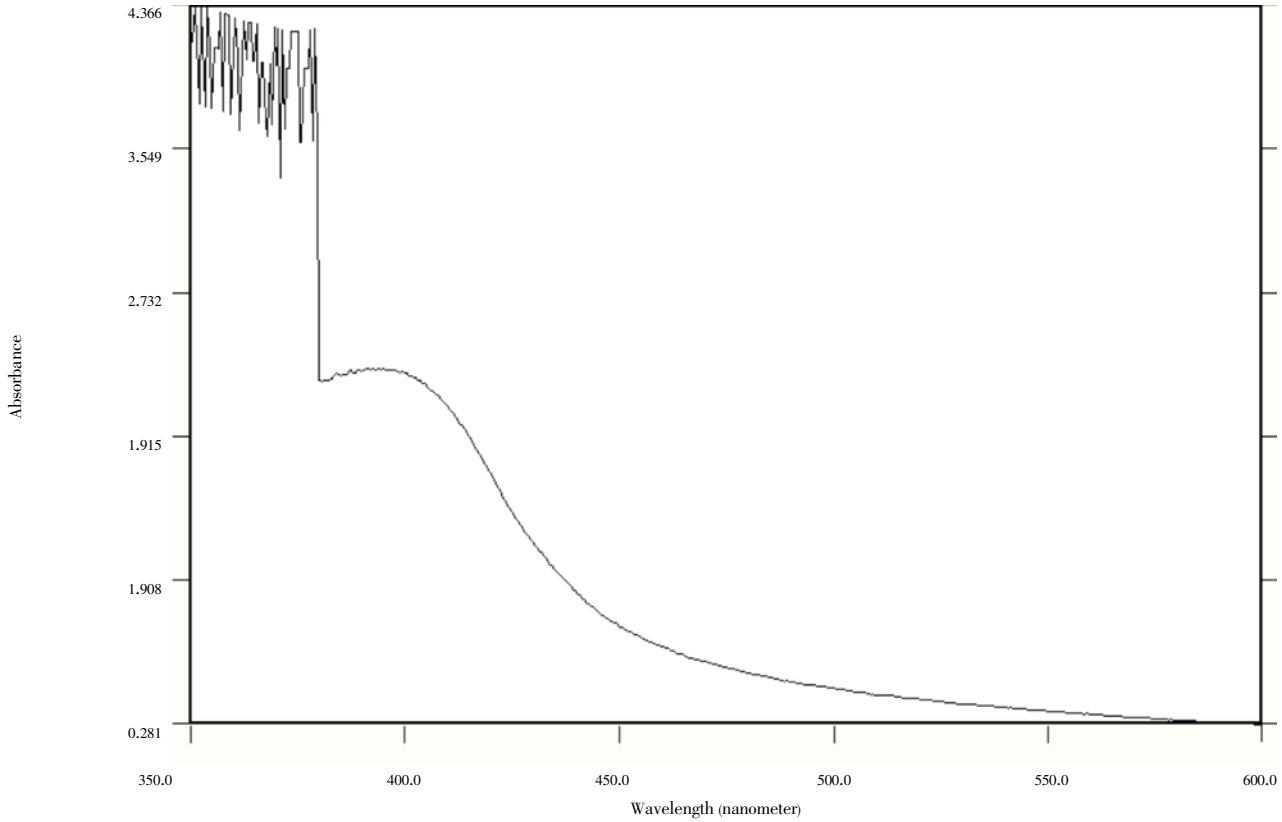

Figure 1. UV-Vis absorption spectra of silver nanoparticles (410 nm) synthesized from *P. guajava*.

The silver nanoparticles synthesized with the help of *P. guajava* extract were scanned using TEM (HITACHI, H-7500) from which we can conclude that the average mean size of silver nanoparticles was 59 nm and it seems to be spherical in morphology as shown in Figure 2.

3.3. Antibacterial studies

Silver nanoparticles were studied for antimicrobial activity against pathogenic microorganisms (clinical isolate) by using standard zone of inhibition microbiology assay, with a well size of 5 mm diameter and 30 μL of samples. Chloromphenical/ketoconazole of 10 mg/mL concentration was used as a control antimicrobial agent. The silver nanoparticles synthesized showed inhibition zone against

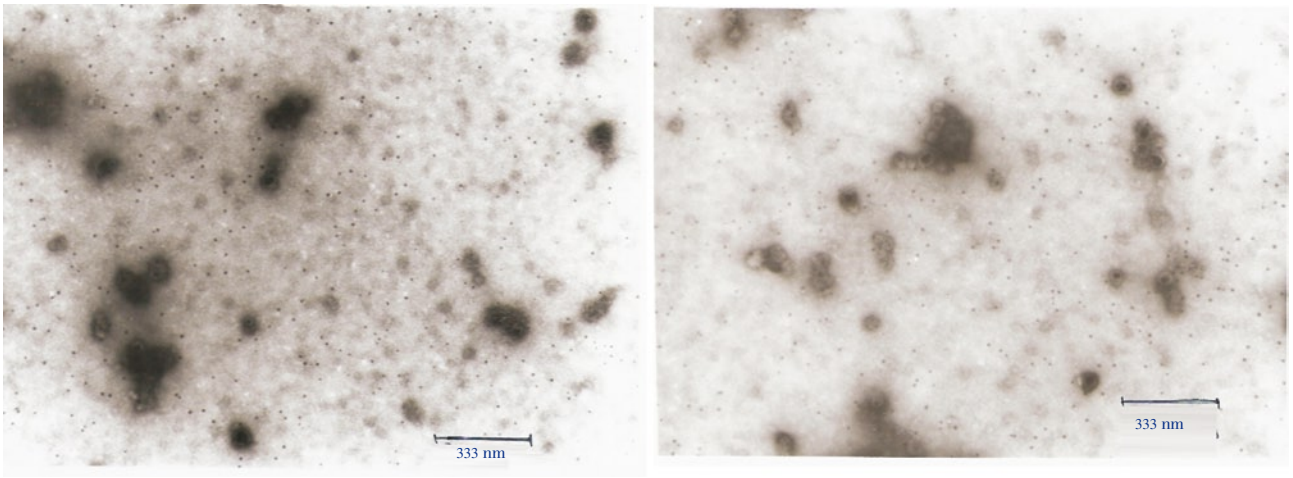

Figure 2. TEM image of the silver nanoparticles synthesized from *P. guajava* leaf.

all the test organisms. Maximum zone of inhibition was found to *Escherichia coli* (*E. coli*) (12 mm), *Bacillus cereus* (*B. cereus*) (14 mm), *Candida tropicalis* (*C. tropicalis*) (11 mm), *Candida albicans* (*C. albicans*) (10 mm), and minimum zone of inhibition was found to *Klebsiella pneumoniae* (*K. pneumoniae*) (6 mm) in all the test organisms (Table 1 and Figure 3).

Table 1
Antimicrobial activity of silver nanoparticle synthesized from *P. guajava*.

Name of the test organisms	Zone of inhibition (mm)	
	Ag nanoparticle	Reference drugs
S. aureus	–	19
E. coli	12	20
K. pneumoniae	6	21
B. cereus	14	18
C. albicans	10	23
C. tropicalis	11	21
C. krusei	–	19

Reference drugs: chloromphenical/ketoconazole.

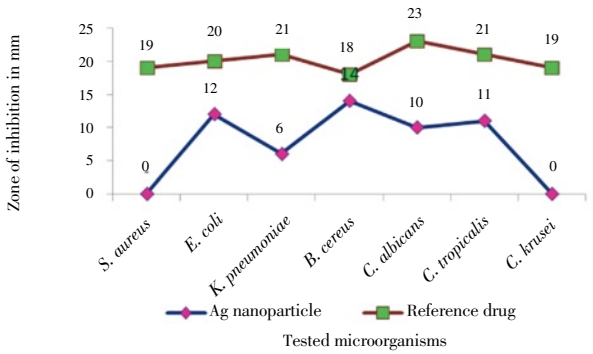

Figure 3. Antimicrobial activity of silver nanoparticle synthesized from *P. guajava*.

4. Discussion

4.1. UV-Vis spectra analysis

Formation and stability of silver nanoparticles in aqueous colloidal solution are confirmed using UV-Vis spectral analysis. It is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles[14,15]. As the *P. guajava* leaf extract was mixed with aqueous solution of the silver nitrate, it started to convert the color from watery to reddish brown due to reduction of silver ion, which indicated the formation of silver nanoparticles. It is generally recognized that UV-Vis spectroscopy could be used to examine size and shape-controlled nanoparticles in aqueous suspensions[16]. UV-Vis spectra was recorded from the reaction medium after heating the solution at 80 ℃ for 15 min. Absorption spectra of silver nanoparticles formed in the reaction media has absorbance peak at 410 nm and broadening of peak indicated that the particles are polydispersed.

4.2. Antimicrobial activity study

Silver nitrate which is readily soluble in water has been exploited as an antiseptic agent for many decades. Dilute solution of silver nitrate has been used since the 19th century to treat infections and burns. The exact mechanism of the antibacterial effect of silver ions is partially understood. Literature survey reveals that the positive charge on the Ag ion is crucial for its antimicrobial activity. The antibacterial

activity is probably derived, through the electrostatic attraction between negative charged cell membrane of microorganism and positive charged nanoparticles[17].

Shrivastava *et al*[18] studied antibacterial activity against *E. coli*, *S. aureus*, and *Salmonella typhi*. They reported that the effect was dose dependent and was more pronounced against gram-negative organisms than gram-positive ones. They found that the major mechanism through which silver nanoparticles manifest antibacterial properties was either by anchoring or penetrating the bacterial cell wall, and modulating cellular signaling by dephosphorylating putative key peptide substrates on tyrosine residues[18]. The antibacterial efficacy of the biogenic silver nanoparticles reported in the present study may be ascribed to the mechanism described above but it still remains to clarify the exact effect of the nanoparticles on important cellular metabolism like DNA, RNA and protein synthesis[19,20].

A critical need in the field of nanotechnology is the development of a reliable and eco-friendly process for synthesis of silver nanoparticles. We have demonstrated that use of a natural, low cost biological reducing agent *i.e. P. guajava* leave extracts (aqueous) can produce nanoparticles, through efficient green methodology, avoiding the presence of hazardous and toxic solvents. The biosynthesized silver nanoparticles using guava leaves extract proved excellent antimicrobial activity. The present study showed a simple, rapid and economical route to synthesize silver nanoparticles.

Conflict of interest statement

We declare that we have no conflict of interest.

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