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One step green synthesis and anti-microbial and anti-biofilm properties of *Psidium guajava* L. leaf extract-mediated silver nanoparticles

Kuldeep Gupta, Shabiha N. Hazarika, Devabrata Saikia, Nima D. Namsa, Manabendra Mandal*

Department of Molecular Biology and Biotechnology, Tezpur University (A Central University), Napaam, Tezpur, Assam 784028, India

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ABSTRACT

This paper describes a single-step green synthesis of extracellular silver nanoparticles (AgNPs) through an in-situ bio-reduction of aqueous solution of silver nitrate using *Psidium guajava* L. UV–vis exhibits a characteristic surface plasmon resonance peak at around 487 nm. XRD analysis showed crystalline and face-centered cubic geometry of synthesized AgNPs. TEM results revealed stable and spherical particles with a mean diameter size of ~60 nm. Fourier transform infrared spectroscopy showed that the nanoparticles were capped with plant bioactive molecules. Phytosynthesized AgNPs showed broad-spectrum anti-microbial activity against Gram-positive, Gram-negative human pathogenic bacteria, and fungi. AgNPs showed prominent ability to inhibit the biofilms formed by *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* in a laboratory condition through crystal violet assay. The results suggest AgNPs as a promising candidate for the design of an effective antimicrobial and anti-biofilm agent in biomedical field.

1. Introduction

Silver nanoparticles (AgNPs) have become the focus of intensive research due to their widespread applications in areas such as catalysis, optics, antimicrobials, and biomaterial production. Recently nanoparticles have been successfully used for the delivery of therapeutic agents in chronic disease diagnostics, to reduce bacterial infections in skin and burn wounds, to prevent bacterial colonization on medical devices and in food and clothing industries as an antimicrobial agent [1–3]. A number of living organisms such as bacteria, fungi, actinomycetes and plants have been utilized for synthesis of nanoparticles with enhanced anti-microbial properties leading to more effective biomedical applications [4,5]. The recent increasing exploration of plants for the fabrication of nanoparticles has added advantages over chemical, physical, and microbial syntheses because there is no need of the elaborated process of culturing and maintaining the cells, hazardous chemicals, high energy requirements, and wasteful purifications [6,7]. The resistance to antimicrobial agents by pathogenic bacteria due to their ability to form biofilm that has emerged in recent years poses a major health problem, and there is a pressing need to develop silver-based nanoparticles with a broad-spectrum activity to lower microbial resistance to antibiotics [8].

The leaves and bark decoctions of *Psidium guajava* L. (Myrtacea) have traditionally been used to treat diarrhea, dysentery, vomiting, sore throats, skin problems, wounds, bacterial infections, etc. [9]. In this study, we developed an inexpensive and green method for synthesis of silver nanoparticles with *P. guajava* leaf broth and the as-prepared AgNPs showed broad-spectrum anti-microbial property against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Pseudomonas diminuta*, *Mycobacterium smegmatis*, *Fusarium oxysporum* and *Candida albicans*, and may have potential application in biomedical field.

2. Experimental sections

Ten milliliters leaf broth was added to 90 ml of 1×10^{-3} M aqueous $AgNO_3$ solution for the reduction of Ag^+ ions in a 250 ml conical flask and the formation was monitored periodically using UV–vis spectroscopy (Multiscan spectrophotometer, Thermo Scientific) [10]. The silver nanoparticles obtained from the solution were purified by repeated centrifugation at 12,000 rpm for 20 min

* Corresponding author. Tel.: +91 3712 267007/5419; fax: +91 3712 267005/267006.
E-mail address: mandal@tezu.ernet.in (M. Mandal).

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followed by dispersion of the pellet in deionized water to remove biomolecules such as proteins, free silver ions, and secondary metabolites [11]. The structure and composition of the freeze dried purified silver nanoparticles were characterized by EDX (energy-dispersive X-ray spectroscopy) equipped with a scanning electron microscope (SEM- JEOL JSM 6390LV, Japan), their crystallinity was characterized using an X-ray diffractometer (XRD Rigaku, Miniflex) employing CuKα radiation, and the possible participation of bio-active functional groups in capping and stabilization of bio-reduced Ag^{+} ions by the KBr pellet method by Fourier transform infrared (FT-IR, Perkin-Elmer, Spectrum 100). The size and morphology of silver nanoparticles were characterized using TEM (FEI Technai G2 F205-TWIN TEM). A 10 μl AgNP sample was placed on a carbon-coated copper grid and was allowed to dry at room temperature overnight. The sample-loaded copper grid was placed under an IR-lamp for 15 min to ensure complete dryness. The anti-microbial experiments using an agar-well diffusion method were carried out on Gram-negative, Gram-positive bacteria, and fungal strains, which were procured from the Institute of Microbial Technology (Chandigarh, India). Briefly, the microbial cultures were grown in an appropriate medium; 100 μl (10^5 CFU/ml) of the culture was spread uniformly and the wells were loaded with aqueous extract, AgNPs and standard antibiotics (Gentamicin and nystatin). The plates were incubated at 37 °C for 24 h and evaluated for antimicrobial activity by measuring the zone of inhibition (mm). The microbial biofilm inhibition assay was performed using a sterile 96-well polystyrene micro-titer plate as reported previously [12]. Overnight grown cultures were diluted 1:100 in a fresh medium. Different concentrations AgNPs were added to the 96-well micro-titer plate and incubated at 37 °C for 48 h. Thereafter the medium was removed and the wells were thoroughly washed with 1× PBS; 100 μL of 0.1% (w/v) crystal violet was added and incubated for 20 min. The crystal violet was removed and washed thoroughly with 1× PBS. For quantification of attached cells the crystal violet was solubilized in absolute ethanol and the absorbance was measured at 570 nm. Reduction of the biofilm was correlated with the cells grown in the absence of AgNPs in the medium as described previously.

3. Results and discussion

Fig. 1a shows the UV–vis absorption spectrum of AgNPs with a broad surface plasmon resonance (SPR) peak at 487 nm, an indication of silver nanoparticles formation and stabilization within 2 h of incubation at room temperature. In a previous study using carob leaf extract (*Ceratonia siliqua*), the formation of silver nanoparticles was obtained within 2 min of reaction time [13]. The rapid reduction of silver ions into silver nanoparticles by carob leaf extract could be ascribed to the variable presence of possible bioactive molecules involved in the process of reduction. XRD data indicated the crystalline nature and the estimated size of AgNPs ranged between 11 and15 nm (Fig. 1b, see the inset table). There is a good correlation between the observed experimental diffraction angle [2θ] and the standard diffraction angle [2θ]. The diffraction patterns of our sample can be indexed to face-centered-cubic silver (JCPDS file no. 04-0783), where the diffraction peaks at 2θ values of 38.70°, 44.84°, 64.89° and 77.79° can be attributed to the reflection of (111), (200), (220), and (311) planes of the face-centered cubic silver, respectively. As observed through TEM micrographs (Fig. 1c), the as-synthesized AgNPs were mostly spherical shaped and monodispersed, and the average mean particle size was ~60 nm (Fig. 1d). An EDX profile shows strong silver signal along with a weak oxygen, sulfur, and carbon peak, which may originate from the biomolecules that are bound to the surface of the silver nanoparticles (Fig. S1a). Analysis of the total phenolic content demonstrated the attachment of lower amounts (56 ± 2.3 mg GA/g) of polyphenolic compounds in AgNPs as

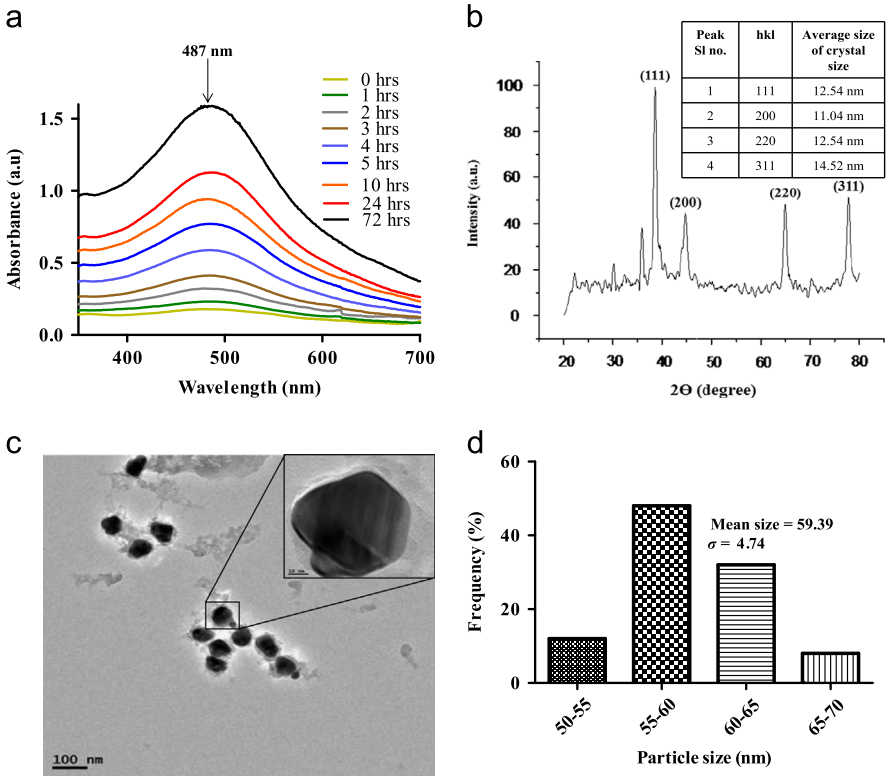

Fig. 1. (a) UV–vis spectra of silver nanoparticles using *P. guajava* plant extract as a function of time, indicated by an absorbance peak at 487 nm, (b) X-ray powder diffraction pattern of the green synthesized silver nanoparticles, (c) representative TEM micrographs of phytosynthesized silver nanoparticles, and (d) histogram frequency for the particle size distribution derived by counting over multiple images.