Asian Journal of Pharmaceutical and Clinical Research

Vol 11, Issue 1, 2018

Online - 2455-3891
Print - 0974-2441

Research Article

PSIDIUM GUAJAVA: A NOVEL PLANT IN THE SYNTHESIS OF SILVER NANOPARTICLES FOR BIOMEDICAL APPLICATIONS

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Received: 14 August 2017, Revised and Accepted: 27 October 2017

ABSTRACT

Objective: Synthesis of silver nanoparticles (AgNPs) using a simple, cost-effective and environmentally friendly green route approach and to study the antibacterial activity of AgNPs against human pathogens.

Methods: Green route approach is used to synthesize AgNPs using *Psidium guajava* leaf extract. Fourier transform infrared (FTIR) was used to identify the presence of the functional group. X-ray diffraction (XRD) was used to analyze the structure of prepared AgNPs. Energy dispersive X-ray was used to the characteristic to the composition of the prepared nanoparticles. Size and morphology of the prepared AgNPs were investigated using field emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM) analysis. Antibacterials efficiency of prepared AgNPs was tested against *Escherichia coli* and *Staphylococcus aureus* by well diffusion methods.

Results: FTIR study shows the presence of different functional groups present in the leaves mediated AgNPs. The XRD studies yield diffraction peaks corresponding to face-centered cubic structure of Ag crystals. Spherical shaped AgNPs with a particle size of about ~55 nm were evidenced using FESEM and TEM analysis. Energy dispersive spectrum of the synthesized AgNPs confirms the presence of silver in the prepared nanoparticles. From UV-VIS analysis it is shown that the absorption band was red-shifted from 430 nm to 456 nm. The prepared AgNPs shows good antibacterial activity against *E. coli* and *S. aureus*.

Conclusions: *P. guajava* leaf extract is a potential reducing agent to synthesize AgNPs. The green synthesis approach provides cost-effective and eco-friendly nanoparticles, which could be used in biomedical applications.

Keywords: *Psidium guajava*, Silver nanoparticles, Fourier transform infrared, Transmission electron microscopy, Antibacterial activity.

 DOI: http://dx.doi.org/10.22159/ajpcr.2018.v11i1.21999

INTRODUCTION

Nanomaterials research has received the considerable attention of researchers worldwide due to its unique properties [1,2]. Nanomaterials are expected to have a wide range of applications in various fields such as electronics, optical communications, and biological system [3,4]. These applications are based on factors such as their physical property, huge surface area, and small size which offer the possibility for manipulation and room for accommodating multiple functionalities. The very promising and rapidly growing field of nanotechnology is in medicine and sustainable energy resources [5]. Silver nanoparticles (AgNPs) are widely being used because it is more effective against bacteria's. In the synthesis of AgNPs, the processing condition need to be controlled in such a manner that the resulting nanoparticles have an identical shape, identical size, and individually dispersed with no agglomeration [6,7]. AgNPs are mostly preferred for biomedical applications because; Ag^+ ions are highly compatible for the human body. Many methods have been reported for the synthesis of AgNPs [8-11], but only a few methods have produced a size controlled and monodispersed AgNPs. These monodispersed AgNPs have a great effect on their physical and chemical properties. All the methods have concentrated on preparing particles of very small size and definite shape which focuses for different applications [12-14].

The size of the metallic collides varies significantly with the type of reducing agent. To optimize AgNPs, the reducing agent was selected from plant extract by green process. Country like India has a rich source of medicinal plants and plant products. From centuries to till date the medicinal plants have been extensively used in Ayurveda. Among these medicinal plants, *Psidium guajava* (Guava) plant has showed high medicinal value. Studies have shown that AgNPs prepared from guava leaves were used in different application [15]. In most ethnopharmacological studies of *P. guajava* leaves showed that the leaf extract has antidiarrheal, antibacterial, anti-inflammatory, and anticancer activities [16-18]. *P. guajava* commonly called as guava plant has been studied for many years because the plant had interesting biological and pharmacological activities. The plant also has a potential antioxidant activity against free radical scavenging [19]. Hence, *P. guajava* leaf extract was used as a reducing agent in the preparation of AgNPs. Previous investigations on *P. guajava* plant extract have led to the isolation of different classes of bioactive constituents such as triterpenoids, flavonoids, tannins, and carotenoids [20-23]. These organic molecules are responsible for the reduction, stabilization and capping of the resulting nanoparticles [24].

Many researchers have suggested that the reduction of metal nanoparticles was mainly due to plant phytochemical molecules and which is responsible for the size and shape of nanoparticles [24,25]. *P. guajava* leaf extract showed many phytochemical molecules and its hold high amount of novel caryophyllene based terpenoid (Guajadial-1) compound and its response for the various biomedical applications [21]. In this present work, an attempt has been made to prepare AgNPs using *P. guajava* leaf extract and study the antibacterial effect of the prepared nanoparticles against human pathogens.

METHODS

Chemicals and reagents

Silver nitrate (99% purity) was purchased from HiMedia, India Pvt. Ltd and the chemical used was of analytical grade purity. *P. guajava* leaves used in this experiment were collected from the local residence around Coimbatore, Tamil Nadu, India.

Preparation of leaf extract

Fresh leaves of *P. guajava* were collected and cut into small pieces. These leaves were washed thoroughly for several times with double distilled water to remove dust and other particles attached to the surface of leaves. *P. guajava* leaf extract was prepared by weighing 20 g of freshly cut leaves with 200 ml of double distilled water boiled at 60°C in Erlenmeyer flask for 20 min. The *P. guajava* leaf extract was then filtered through Whatman No.1 filter paper [26]. The filtered was used for the synthesis of nanoparticles in room temperature.

Green synthesis of AgNPs

AgNPs were synthesized by mixing 100 ml of 1×10^{-3} M, aqueous silver nitrate with 5 ml of *P. guajava* leaf extract and are stirred vigorously for 20 min in a magnetic stirrer at room temperature. It was observed that color of the reaction mixture changed from light green to reddish brown that indicating the formation of AgNPs [27].

Characterizations of AgNPs

The Fourier transform infrared (FTIR) spectra for *P. guajava* leaf extract, and AgNPs were obtained in range 4000–500/cm with IR-Affinity-1, Shimadzu, and FTIR Spectrometer. X-ray diffraction (XRD) (Shimadzu Lab, XRD 6000, Japan) was analyzed for the range of 10°–80° by powder XRD using CuKα radiation (1.5406Å). Full width half maximum (FWHM) (β) values and diffraction angles (θ) are used to calculate the crystallite size and peak boarding of prepared AgNPs. The surface morphology of the *P. guajava* leaf extract mediated AgNPs was carried out using JEOL-JSM-6490LA field emission scanning electron microscopy (FESEM) (JEOL, Tokyo, Japan) operated at 20 kV with a counting rate of 2838 cps. The composition of the prepared AgNPs was studied by energy dispersive X-ray (EDAX) spectroscopy (JEOL, JSM 6390, Japan). The atomic and weight percentage of the existing elements were calculated using EDAX spectrum. The optical properties of prepared AgNPs were characterized by UV-Visible Spectroscopy (CYBERLAB, UV-100) double beam spectrophotometer.

The antibacterial activity

The antibacterial activity of prepared AgNPs was investigated by a standard zone of inhibition microbiology assay against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) human pathogens. The clinically isolated Gram-negative bacteria *E. coli* and Gram-positive *S. aureus* were obtained from the clinical testing lab, Coimbatore, Tamil Nadu, India. Muller Hinton Agar plate was used to swab pathogenic bacteria culture (*S. aureus* and *E. coli*) from fresh culture (1.5×10^8 CFU/mL) by sterile cotton scrub. The surface of the medium was allowed to dry for about 3 min. The wells (6 mm) were punched over the agar plates using sterile gel puncher. Different concentrations (10, 25, and 50 μl) of prepared AgNPs were loaded into wells on test plates. The plates were incubated for 24 h at 37°C, and the zone of inhibition was measured in mille meter. The antibacterial efficiency of prepared AgNPs was compared with standard drug ofloxacin disc (5 μg/disc).

RESULTS AND DISCUSSION

The AgNPs which are less likely to cause ecological effect have been identified as a potential replacement of synthetic chemical insecticides; hence, the need to use green synthesized AgNPs for the control of disease vectors. *P. guajava* leaf extract showed different classes of bioactive constituents, which is responsible for the biological applications. Previous researcher reported that the *P. guajava* leaf extract showed terpenoids compounds in high concentration (>50%) when compared with other plant phytochemicals such as flavonoids, tannins, etc. [15,20]. Guajadial (1) is a novel caryophyllene based meroterpenoid, was isolated from the leaves of *P. guajava* [21], which may be responsible for the reduction of AgNPs. The possible reduction of Ag from Guajadial (1) is shown in Fig. 1.

FTIR spectroscopy

To support the possible pathway of green synthesized AgNPs the FTIR analysis for the guava leaf extract and the synthesized AgNPs were carried out. Fig. 2 shows the FTIR spectrum of the *P. guajava* leaf extract and the synthesized AgNPs.

FTIR peaks at 3373 (-OH), 2788 (CH-O), 1645 (C=O), 1451 (C-C=C), 1363 (N-O), 1023 (C-O), 825 (alkenes), and 702 (aromatic rings), represents the different functional groups present in the *P. guajava* leaf extract. FTIR peaks at 1529 (C-C), 1341 (N-O), 962 (C-C), and 716 (R-CH) represents the different functional groups present in AgNPs after reduction. From the FTIR spectrum it is clearly seen that the AgNPs have been accommodated into the –OH stretching and have been reduced into O^- and Ag^+ ions. The intensity of all the other peaks in the synthesized nanoparticles has been reduced and is shifted toward the lower wavelength.

XRD analysis

The XRD peaks of the green route synthesized AgNPs are shown in Fig. 3. The XRD spectrum shows four main predominant Braggs diffraction peaks which are positioned at 2θ values of 38.0°, 44.1°, 64.4°, and 77.4° which corresponds to 111, 200, 220, and 311facets of the face-centered cubic structure of AgNPs. These diffraction peaks are consistent with the standard Ag crystals (JCPDS Card No.4-0783), indicating that the synthesized AgNPs are of crystalline in nature. These results are in good correlation with previously reported results of AgNPs [28]. The unassigned peaks (*) observed in the XRD spectrum indicates that the crystallization of plant phytochemicals phase presence in *P. guajava* leaf extract occurred on the surface of the AgNPs.

The crystallite size of the green synthesis AgNPs was calculated by the Debye-Scherrer equation, $D = K\lambda/\beta \cos \theta$. Where D = average crystallite size, K - shape factor (0.9), λ - wavelength of Cu Kα radiation (1.541 Å), β - FWHM of refection (in radians) located at 2θ and θ - angle of reflection (in degrees) was used to relate the crystallite size to the line broadening. The average crystallite size of prepared AgNPs was found to be about 25.8 nm.

FESEM and transmission electron microscopy (TEM) analysis

FESEM micrographs of AgNPs synthesized using *P. guajava* plant extract shows that the AgNPs are spherical in shape with average grain size

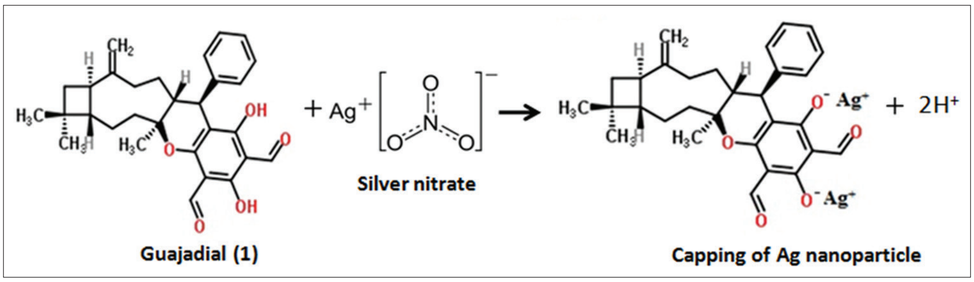

Fig. 1: Possible reduction of silver nanoparticles from Guajadial (1)

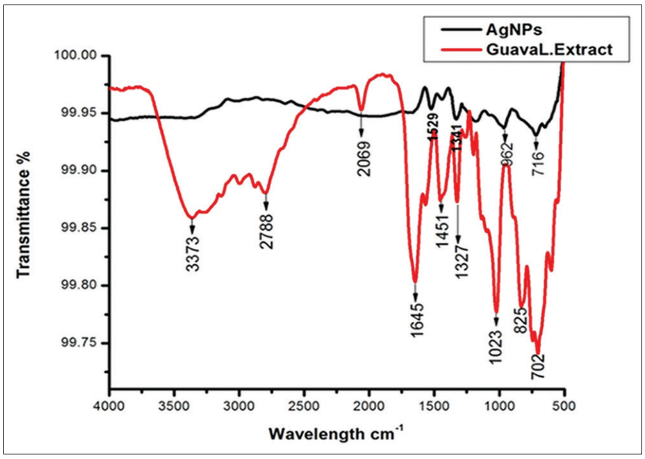

Fig. 2: Fourier transform infrared spectrum of silver nanoparticles and *Psidium guajava* leaf extract

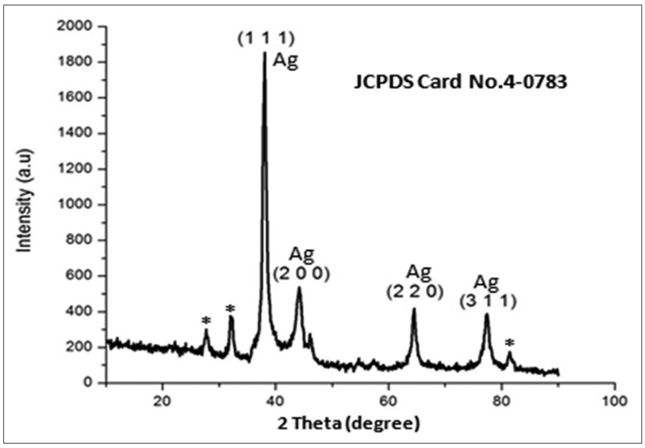

Fig. 3: X-ray diffraction spectrum silver nanoparticles

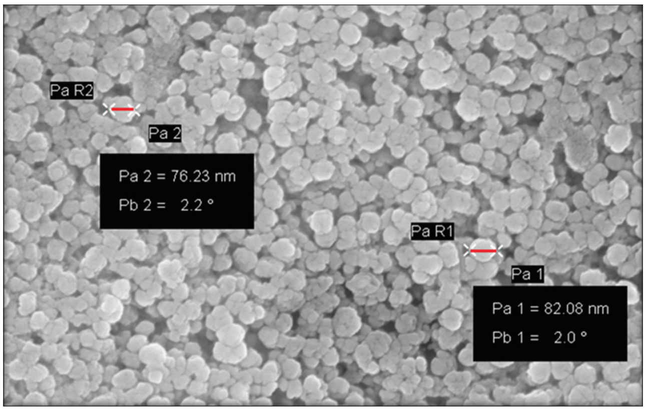

Fig. 4: Field emission scanning electron microscopy images of green synthesized silver nanoparticles

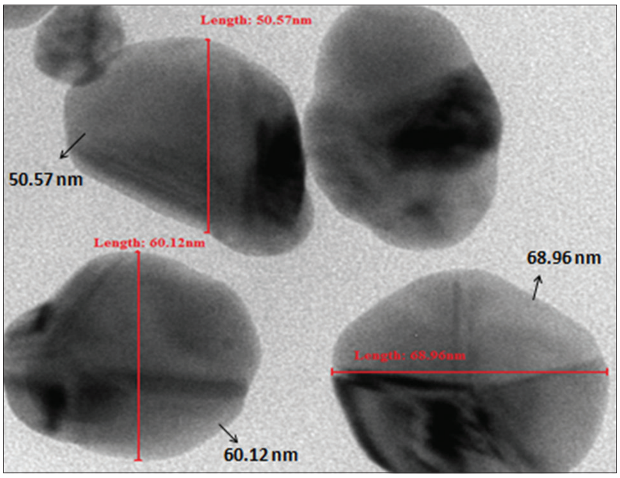

Fig. 5: Transmission electron microscopy image for the green synthesized silver nanoparticles

of ~80 nm. The FESEM micrographs of different magnifications are shown in Fig. 4.

TEM image of green synthesized AgNPs shown in Fig. 5. The micrograph technique shows that the resultant nanoparticles are very fine and well dispersed. The nanoparticles obtained are all spherical in shape with an average size of 55 nm. Constituents present in *P. guajava* leaves were capped in the AgNPs, and these constituents might have prevented the nanoparticles from agglomeration.

The energy dispersive spectrum (EDS) was done along with the FESEM analysis. The EDS spectra of the green route prepared AgNPs are shown in Fig. 6. The spectrum reveals the presence of silver at ~3 keV. In general, elemental silver gives a signal in this energy region [29,30]. The EDS composition analysis confirmed the presence of Ag (93% wt) in the prepared AgNPs.

UV-visible spectroscopy

Absorption spectral analysis is the most commonly used technique in the identification of nanoparticles formation. Reduction of silver ions into AgNPs using *P. guajava* leaf extract was evidenced visually by a change in color from light yellow to dark brown [31]. Color intensity increases with increase in incubation time. Initially, when adding 100 ml of 1×10^{-3} M aqueous silver nitrate with 5 ml of leaf extract, the color of the solution was found to be light yellow after 15 min the change of color from light yellow to reddish brown was evidenced, and after 1 hour the color changed from reddish brown to dark brown, this effect may be due to surface plasmon vibration [32,33]. The color of the colloidal solution of AgNPs depends on the size and shape of the nanoparticles. Usually, metal nanoparticles were analyzed in the range from 300 to 800 nm wavelength, and formation of AgNPs can be detected commonly from the absorption measurement in the wavelength ranges of 400–450 nm [34]. In this present study, the absorption band was observed from 430 to 456 nm for different incubation time (Fig. 7). The intensity of the absorption peak increases steadily as a function of time and was highest for 1 h. The UV-spectra recorded after 1 h does not show any increase in the intensity of absorption spectrum, from which it is confirmed that the reaction was completed within 1 h.

Antibacterial activity of AgNPs

The agar well diffusion method was employed for screening the antibacterial activity of AgNPs against Gram-positive and Gram-negative bacteria. The green route synthesized AgNPs showed antibacterial activity in all concentrations (10, 25, and 50 μL) against both test pathogens such as *S. aureus* and *E. coli* and also it indicated the dose depended on mechanisms. The antibacterial activity of AgNPs was increasing with the increasing the concentration of AgNPs against test pathogens. The prepared AgNPs showed a maximum zone of inhibition in all three concentration against *E. coli* compare to *S. aureus* (Fig. 8).

At the concentration of 50 μL, AgNPs solution shows a maximum zone of inhibition around 12 mm for both test pathogens. Whereas, 25 μL showed a zone of inhibition around 11 mm for *E. coli* and 10 mm for *S. aureus*. The lowest concentration of 10 μL AgNPs solution observed 9 mm for both tested human pathogens. The present study clearly indicates the antibacterial activity was dose-depended manner. The standard drug (ofloxacin) (5 μg/disc) observed 19 and 12 mm against

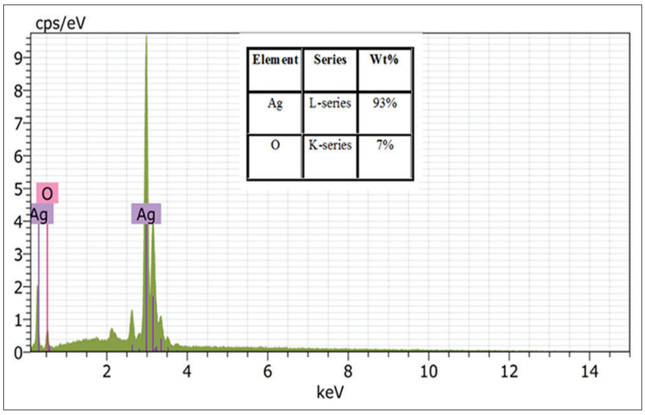

Fig. 6: Energy dispersive spectrum of silver nanoparticles

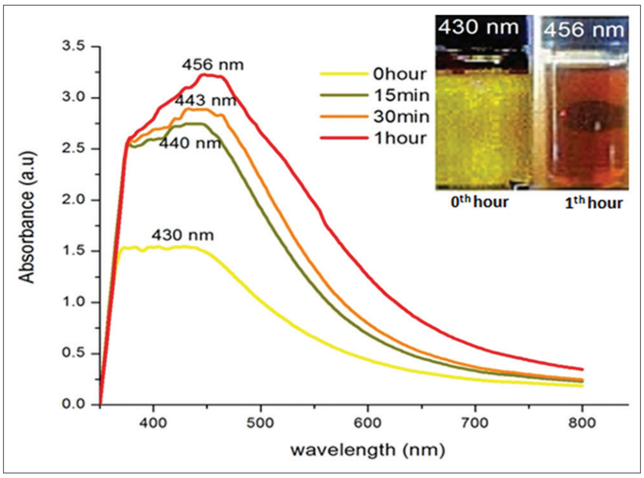

Fig. 7: UV-visible absorption spectra of silver nanoparticles for different time intervals

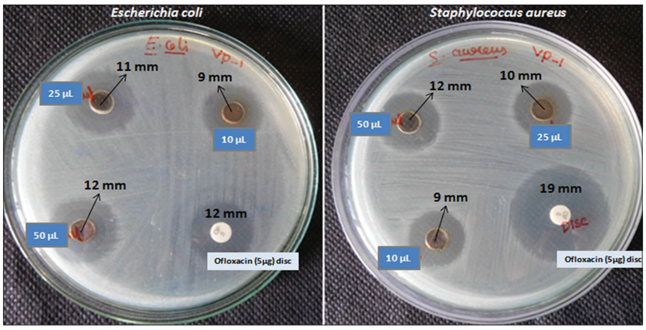

Fig. 8: Antibacterial activity of silver nanoparticles

S. aureus and *E. coli.* Nanoparticles may infiltrate the cells causing intracellular loss leading to cell death, and this inhibition depends on the concentration of the AgNPs [35,36]. The observed value of inhibition is in good agreement with recently reported values against *E. coli* and *S. aureus* using inorganic nanoparticles by our group [26,37]. In this present study reveals that the *P. guajava* leaf extract mediated green synthesized AgNPs are good antibacterial material against Gram-negative and Gram-negative pathogens.

CONCLUSION

The AgNPs were synthesized at room temperature using *P. guajava* leaf extract. The plant phytochemicals molecules are responsible for the reduction of AgNPs and enhanced antibacterial activity. FTIR analysis supports the presence of functional groups of biomolecules capped on the prepared AgNPs. These green routes synthesized AgNPs were preliminary identified by visual inspection where the change in color was evidenced, and then different characterization techniques were implemented to prove the presence of AgNPs. The absorption spectrum and XRD studies evidenced the presence of AgNPs. Electron microscope technique such as FESEM and TEM analysis confirmed the spherical shape and size of about ~55 nm of the un-agglomerated AgNPs. The elemental analysis also confirms the presence of Ag in the prepared sample. The antibacterial activity of AgNPs was carried out against *E. coli* and *S. aureus* and its zone of inhibition was dose-dependent manner. Moreover, the present study demonstrated fast, eco-friendly, cost-effective and simply route to synthesize AgNPs from *P. guajava* leaf extract for biomedical applications.

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