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Research Article

Green synthesis of silver nanoparticles using *Barringtonia acutangula* (L.) Gaertn leaf extract as reducing agent and their antibacterial and antioxidant activity

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Abstract

The green synthesis of nanoparticles has been proposed as a cost effective and environmentally benevolent alternative to chemical and physical methods. In this work, a synthesis of silver nanoparticles (AgNPs) has been established using leaf extract of *Barringtonia acutangula* (L.) Gaertn to reduce an aqueous $AgNO_3$ solution. The obtained samples were characterized by various techniques including ultraviolet visible spectroscopy (UV-Vis), Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM) and laser particle size analyzer (LPSA). UV-vis spectra showed maximum absorption peak at 416 nm, which represents the characteristic surface plasmon resonance of the nanosilver. The structure of the particles was spherical and ellipsoidal as observed in TEM. FTIR analysis was carried out to probe the possible functional groups involved in the synthesis of AgNPs. The mean particle size calculated using LPSA was 60 nm. In addition, the synthesized AgNPs were tested for their antibacterial activity against two human pathogens including *Escherchia coli* and *Staphylococcus aureus*. The obtained AgNPs showed higher inhibitory activity on both bacterial species than the plant extract and the bare $AgNO_3$ solution. Moreover, the extract and the synthesized AgNPs were evaluated for the antiradical scavenging activity by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay.

Keywords: green synthesis, silver nanoparticles, *Barringtonia acutangula* (L.) Gaertn, antibacterial activity, antioxidant activity

Introduction

Nanotechnology has been applied to diverse fields with nanoparticles finding their uses in several applications from medicine and agriculture to energy and electronics (Prow et al., 2011). Silver nanoparticles (AgNPs) have attracted great interest among scientists because of their unique properties and their potential in biological applications. Green synthesis of nanomaterials is considered as a clean, nontoxic and environmentally-friendly method compared to other physical and chemical methods (Kumar et al., 2013). There has been a great deal of research focusing on the use of plant-based materials as reducing agents. Anti-microbial AgNPs have been successfully synthesized using plant based materials as reducing agents

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(Fatimah, 2016). Compounds in plants that act as reducing agents in the synthesis are generally flavonoids and polyphenols (Fatimah, 2016).

To date, there has been no report on the synthesis of silver nanoparticles by using the aqueous leaf extract of *B. acutangula* (L.) Gaertn, which is one of the important medicinal plants (Thamizh Selvam et al., 2016). An aqueous extract of *B. acutangula* has been investigated for its antioxidant and antibacterials activities (Kathirvel et al., 2012). Therefore, the present study aimed to characterize the silver nanoparticles synthesized using *B. acutangula* leaf extract as a reducing agent and study the optimization of parameters including temperature, pH and time. Moreover, the antioxidant and antibacterial activities of the synthesized silver nanoparticles were investigated.

Materials and methods

The leaves of *B. acutangula* (L.) Gaertn. were collected from Ban Pa-yang Tambon Tha-Ngio, Amphoe Mueang, Nakhon Si Thammarat Province, Thailand. All analytical reagents used in the study were of analytical grade and were purchased from Merck. Nutrient agar for bacterial culture and Mueller–Hinton broth and agar for antimicrobial activity were purchased from Hi-Media, Mumbai, India.

Preparation of leaf extract

10 grams of *B. acutangula* (L.) Gaertn leaves was weighed, cut into fine pieces, crushed with 100 mL of distilled water for 1 h at 60 °C, and filtered through Whatman No.1 filter paper. The filtrate aqueous extract was used as a reducing agent.

Phytochemical Analysis of leaf extract

Qualitative phytochemical tests for the identification of anthraquinones, flavonoids, steroids, terpenoids, saponins, alkaloids and tannins were carried out for the leaf extract by the method described in many studies.

Optimization of AgNPs synthesis and characterization of AgNPs

The aqueous solution of 1 mM silver nitrate ($AgNO_3$) was prepared in a flask. 2 mL of the leaf extract was mixed with 18 mL of the $AgNO_3$ solution under magnetic stirring. Silver nanoparticle formation was visually observed by the gradual change in color of the mixture, which was incubated under different pH, time and temperature. To achieve the maximum product yield, the optimization of these factors was analyzed by UV-visible absorption.

The water-suspended nanoparticles were evaporated under vacuum. After the drying of the silver nanoparticles, their structure and composition were studied by SEM (Scanning electron microscopy), EDX (Energy-dispersive X-ray spectroscopy), TEM (Transmission electron microscope), LPSA (Laser particle size analysis) and FTIR (Fourier transform infrared spectroscopy).

Antioxidant activity on 2,2-diphenyl-1-picryl hydrazyl radical (DPPH)

The antioxidant activity of the leaf extract and the synthesized AgNPs were studied. Briefly, 2 mL of 2×10^{-4} mM of DPPH in 95% ethanol was added to 1 mL of the samples having different concentrations. The samples were kept at room temperature in the dark and after 5 min the absorbance was measured at 518 nm against a blank of 95% ethanol. Ascorbic acid was used as a standard compound.

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Antibacterial study

AgNPs biosynthesized from *B. acutangula* (L.) Gaertn leaf extract and the aqueous leaf extract were tested for their antibacterial activity against *E. coli* and *S. aureus* by using the Agar-well diffusion method. The bacteria culture was spread evenly on the nutrient agar plate using sterile cotton swab. Wells were prepared on agar plates. To these wells nanoparticles solution (30 mg/L) and standard antibiotic disc (chloramphenicol, 30 μg) were added. After incubation at 37 °C for 24 h, the diameter of inhibition zones around AgNPs were measured and compared with that around the commercial standard antibiotic chloramphenicol and aqueous leaf extract.

Results and discussion

AgNPs characterization

Photochemical analysis of the aqueous extract of *B. acutangula* (L.) Gaertn leaves showed the presence of different types of compounds, the main ones being flavonoids, steroids, terpenoids, saponins, alkaloids and tannins, which act as reducing agents for the reduction reaction of AgNPs. The reduction of silver ions into AgNPs by the plant extract was demonstrated by the visual color change of the solution from yellow to deep brown due to excitation of surface plasmon vibrations in AgNPs (Abdel-aziz et al., 2014). The surface plasmon resonance of AgNPs showed a peak centered near 416 nm at UV–vis spectra, which corresponds to the absorbance of AgNPs (Caroling et al., 2013) (Figure 1). This suggested that the active compounds in the leaf extract might reduce silver ions (Ag^+) to AgNPs.

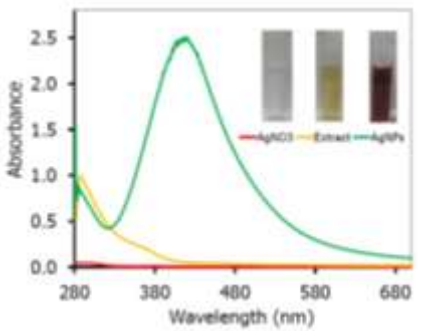

Figure 1. UV–vis spectra of *B. acutangula* (L.) Gaertn leaf extract, $AgNO_3$ and the AgNPs.

The FTIR spectroscopy of the leaf extract (Figure 2(a)) showed prominent peaks at 1042, 1350, 15617 and 3406 cm^{-1} attributed to C–N stretching (aliphatic amines), C-H group (aromatic) (Das et al., 2013), C=O stretching (Dinesh et al., 2012) and O–H stretching (Suman et al., 2013), respectively. The majority of the IR bands are characteristic of phenolic compounds present in the leaf extract. The FTIR spectroscopy of AgNPs shows a broad peak that decreases in intensity at around 3211 cm^{-1} corresponding to the OH stretching vibrations of phenolic compounds. The shift from 3406 to 3211 cm^{-1} may indicate the involvement of OH functional group in the reduction of Ag^+ ions (Vivek et al., 2012). The TEM micrograph of AgNPs is shown in Figure 2(b). It was clearly demonstrated that the shape of AgNPs was spherical and the LPAS analysis showed their average size was 60±0.026 nm. Furthermore, the SEM image showed uniformly distributed silver nanoparticles on the surfaces of the cells (Figure 2(c)). The silver nanoparticles were almost spherical in shape with smooth morphology.

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The EDX profile displayed a strong peak at the energy of 3 keV for silver and also some of the weak peaks for C and O were observed which may have initiated from the biomolecules bound to the surface of the silver nanoparticles. The emission energy at 3 keV indicates the reduction of silver ions to the elemental silver (Ag^0).

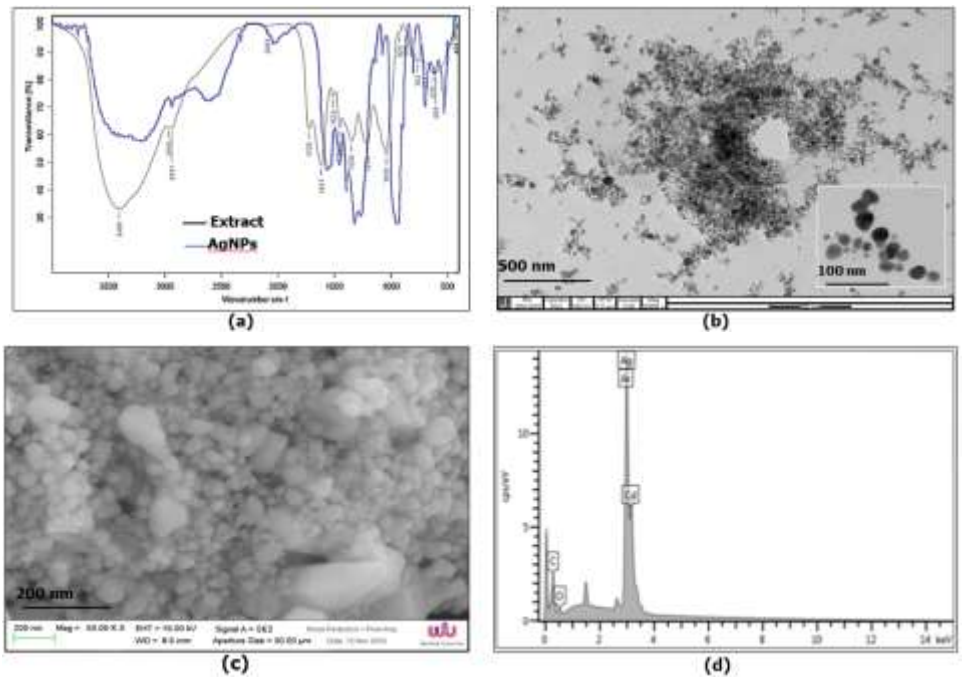

Figure 2. AgNPs Characterization (a) FTIR spectra of AgNPs vs leaf extract, (b) TEM micrograph, (C) SEM micrograph and (d) EDX spectrum.

Effect of pH

The effect of pH was studied at three different conditions; acidic, neutral and basic. The UV–vis spectra of the silver nanoparticles were measured at pH 3.0, 5.0, 7.0 and 9.0. The particle size of AgNPs was measured under the best condition. The result showed that the synthesis of the silver nanoparticles occurred in a neutral condition and yielded more products in a basic condition. Figure 3(a) shows the effect of the pH on the synthesis of AgNPs. At pH 3, the colloid solution was colorless and it changed to brown at pH 9. It was found that as the values of the pH increased, the color of the solution became more intense indicating that the formation of AgNPs was more effective at higher pH.

Effect of time

The time is one of the most important factors in the realization of nanoparticles during the green synthesis. Figure 3(b) showed an increase in the reaction time resulted in a gradual increase in the absorbance at 420 nm and it was found that the color intensity increased with the duration of the incubation with the maximum absorbance reached at 3 h of the reaction time. The incubation time at 5 h, the absorbance decreased and there was a redshift of

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the peak, a condition that is unstable for the formation of AgNPs, which may be due to increased particle aggregation and precipitation when the reduction time increased (Zhang et al., 2013).

Effect of temperature

Figure 3(c) shows the UV–vis spectra of the silver nanoparticles at three different temperatures; at room temperature, 40 °C and 60 °C, respectively. We found that the rate of silver nanoparticle formation increased with increasing temperature. The maximum synthesis of silver nanoparticles occurred at 60 °C. However, when the reaction was carried out at a temperature more than 60°C, the leaf extract will be degraded.

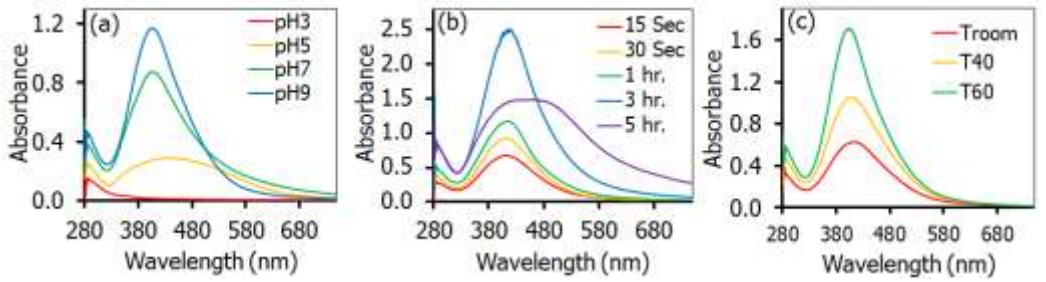

Figure 3. UV spectra showing (a) the effect of pH, (b) the effect of time and (c) the effect of temperature on the nanoparticles synthesis

Antioxidant activity

The antioxidant activity of the aqueous extract and the synthesized AgNPs was evaluated using DPPH scavenging assay. The IC_{50} value of the leaf extracts and the AgNPs was 89.12 μg/mL and 13.40 μg/mL, respectively. The result indicated that the AgNPs had a better antioxidant activity in comparison to the leaf extract. Moreover, the DPPH free radical scavenging assay of AgNPs when compared with that of the standard ascorbic acid showed a promising result. It was found that the bioconjugated AgNPs exhibited a comparable free radical scavenging activity to that by the drug ascorbic acid, which has an IC_{50} value of 10.41 μg/mL.

Antibacterial activity

The antibacterial activity of the AgNPs, $AgNO_3$, leaf extract and distilled water was studied by the well diffusion method according to the protocol as described in the previous section. The zones of inhibition are shown in Figure 4. 30 mg/L of the synthesized AgNPs displayed an efficient antibacterial activity against both *E. coli* and *S. aureus*. The AgNPs showed the largest zone of inhibition, at around 16 mm for *S. aureus* and 12 mm for *E. coli*. As expected, the negative control (distilled water) and the leaf extract did not exhibit any zone of inhibition. The positive control ($AgNO_3$) also had an antibacterial activity, with the zone of inhibition being 14±0.07 mm for *S. aureus* and 12±0.45 mm for *E. coli* (12 mm). We found that the zone of inhibition was larger in Gram-positive bacteria than in Gram-negative bacteria.

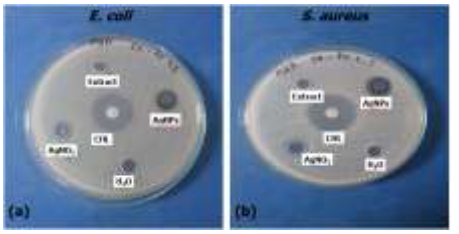

Figure 4. Zone of Inhibition Area (in mm) exhibited by the formed AgNPs against (a) *E. coli*. and (b) *S. aureus*.

Conclusion

We reported a simple and efficient green synthesis of AgNPs using the aqueous leaf extract of *B. acutangula* (L.) Gaertn. The synthesized AgNPs were characterized by UV–Vis spectroscopy, FTIR, LPSA, SEM, and TEM analyses. The UV-vis spectra showed a maximum absorption peak at 416 nm, which represents the characteristic surface plasmon resonance of nanosilver. Observed in TEM and SEM, the particles were spherical and ellipsoidal in shape. FTIR analysis was carried out to probe the possible functional groups involved in the synthesis of AgNPs. The mean particle size calculated using LPSA was 60±0.026 nm. In addition, the synthesized AgNPs were tested for *E. coli* and *S. aureus* inhibition. The obtained AgNPs showed higher inhibitory activity on both bacterial species than the extract and the bare $AgNO_3$. Moreover, the AgNPs showed significant antioxidant activity for DPPH radicals. We found the single-step green method to be effective and economical providing an alternative to the rapid production of the silver nanoparticles, which could be used in various fields of applications ranging from biomedicine to environment.

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