High Antioxidant activity of silver nanoparticles using novel rout bio reducing from *Terminalia catappa* leaf extract

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Abstract

Green synthesis of nanoparticles from plant extracts is receiving enormous interest due to their biomedical applications and cost effective and eco-friendly alternative in the current research. In this study, the reducing potential of *Terminalia catappa* leaf extract was used as the novel reducing agents to synthesize silver nanoparticles (AgNPs). The phytochemical constituent of extract was investigated by standard method and it possesses anthraquinones, flavonoids, steroids, terpenoids, saponins, alkaloids and tannins. The synthesized silver nanoparticles were characterized by UV–vis, SEM-EDS, LPSA and FT-IR analysis. UV-vis spectra showed maximum absorption peak at 423 nm, which represents the characteristic surface plasmon resonance of the nanosilver. The structure of the particles was spherical as observed in SEM and 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 48±0.60 nm. The antioxidant properties of the obtained biomaterial were assessed in vitro, using the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical. AgNPs containing leaf extract showed a higher antioxidant activity compared to *Terminalia catappa* leaf extract. It could be concluded that *Terminalia catappa* leaf extract can be used effectively in the production of potential antioxidant AgNPs for commercial application.

Keywords: Green synthesis, Silver nanoparticles, *Terminalia catappa*, Antioxidant activity

Introduction

Silver nanoparticles (AgNPs) have a wide range of applications in various fields from electronics to biology, pharmaceuticals to medical diagnosis and therapeutics to biosensor development. Various physical and chemical methods were extensively used to synthesize monodispersed AgNPs. Eventually, it is necessary to minimize the time required to reduce Ag^+ ion using plant extract to compete with chemical approaches to fulfill its high demand in consumers industries. This green chemistry approach towards the synthesis of AgNPs has many advantages such as, cost effectiveness, ease of scale up, economic viability and most importantly eco-friendly nature.

The main objectives of the present study were to synthesis Ag NPs using aqueous extract of *Terminalia catappa* leaf extract and characterize Ag NPs using UV–vis spectroscopy, FTIR, SEM-EDS, LPSA and FT-IR analysis. More over to check its antioxidant activity by using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay

Materials and methods

The leafs of *Terminalia catappa*. were calm 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

Terminalia catappa leafs 10 g 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.

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 measured 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 by using DPPH assay. 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.

Results and discussion

AgNPs characterization

Photochemical analysis of the aqueous extract of *Terminalia catappa* leafs 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. The surface plasmon resonance of AgNPs showed a peak centered near 423 nm at UV–vis spectra, which corresponds to the absorbance of AgNPs (Fig. 1). This suggested that the active compounds in the leaf extract might reduce silver ions (Ag^+) to AgNPs.

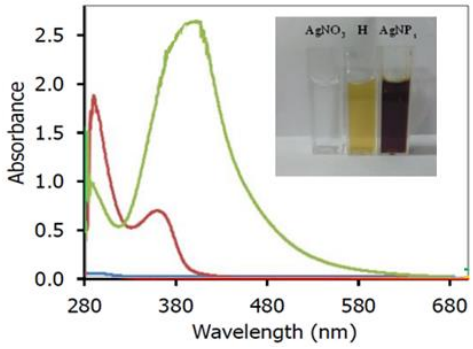

Fig. 1 UV–vis spectra of *Terminalia catappa* leaf extract, $AgNO_3$ and the AgNPs.

The majority of the FTIR 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 3346 cm^{-1} corresponding to the OH stretching vibrations of phenolic compounds. The shift from 3400

to 3346 cm^{-1} may indicate the involvement of OH functional group in the reduction of Ag^{+} ions. The TEM micrograph of AgNPs is shown in Fig. 2(b). It was clearly demonstrated that the shape of AgNPs was spherical and the LPAS analysis showed their average size was 48±0.60 nm. Furthermore, the SEM image showed uniformly distributed silver nanoparticles on the surfaces of the cells (Fig. 2(c)). The silver nanoparticles were almost spherical in shape with smooth morphology. The EDS 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}).

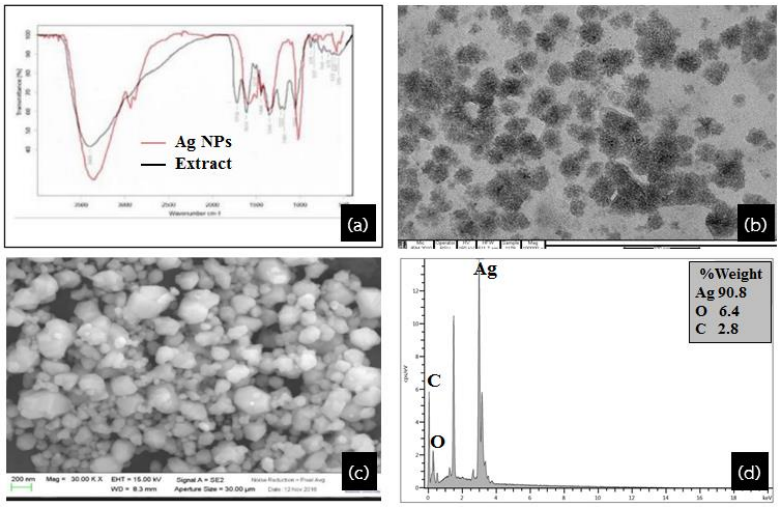

Fig. 2 AgNPs Characterization (a) FTIR spectra of AgNPs vs leaf extract (b) TEM micrograph (c) SEM micrograph (d) EDX spectrum.

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 22.54 μg/mL and 10.16 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.

Conclusion

We reported a simple and efficient green synthesis of AgNPs using the aqueous leaf extract of *Terminalia catappa*. 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 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 48±0.060 nm. The obtained AgNPs showed higher inhibitory activity on both bacterial species than the extract and the bare $AgNO_3$. Moreover, the AgNPs showed high 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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