

Biosynthesis of Silver Nanoparticles from Aqueous Extract of *Myrmecodia pendans* Bulb

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Abstract. Nanoparticles, especially the silver nanoparticles (AgNPs), have been widely used in different biomedical applications and nanotechnology fields. Bioreduction of silver nitrate (AgNO_3) for the biosynthesis of AgNPs respectively can be done by using a plant extract. Present research was focused on the biosynthesis of AgNPs using an eco-friendly, inexpensive, and simple method of AgNPs using aqueous bulb extract of *Myrmecodia pendans*. The formation of AgNPs was evidenced by color change, UV-VIS Spectroscopy, Scanning and transmission electron microscope (SEM and TEM), X-ray spectroscopy diffraction (XRD), and Fourier-transform infrared spectroscopy (FTIR). Current results confirmed that AgNPs could be synthesized using aqueous bulb extract *M. pendans* which has a peak absorption at the wavelength of 400 nm in 1:8 ratio solution between AgNO_3 and extract. The acidity of resulted AgNPs was ranged from 6.3 to 4.5 and stable for several days. Based on SEM and TEM image, the average particle size ranged from 20.70 nm to 37.88 nm and in line with XRD result. The FTIR analysis determined several functional groups of AgNPs.

INTRODUCTION

Nanoparticle fields are gaining in momentum, attracting researchers to explore their synthesis and characteristics. Nanoparticles can be synthesized by either physical or chemical methods. However, chemically synthesized nanoparticles cannot be used in medical purpose due to safety concern regarding chemicals binding on its surface and by-product toxicity. Meanwhile, there are more disadvantages to synthesize nanoparticle by using a physical procedure which is expensive and needs high energy and space [1-3].

Synthesizing nanoparticle using biological procedure has more advantages such as affordable, cost-effective, and free of hazardous components on their surface which is safe to be used for a medical purpose [4, 5]. For this biosynthesis, the use of part of the plant along with Silver (Ag^+) to produce nanoparticle (AgNPs) may give distinct advantages over conventional. The part of the plants are abundance and most preferred raw materials by scientist for preparing nanoparticles [6-11]. One of the plants that potential to be used as a capping and bioreduction agent for synthesizing AgNPs is *Myrmecodia pendans*.

The *Myrmecodia pendans* or known as ant nest plant is an epiphytic plant which has potent antioxidant properties [12-15]. This ant nest plant belongs to Hydnophytinae (Rubiceae) family, consisting of five genera. According to Sudiono et al [16], *Hypnophytum formicarum*, *Myrmecodia pendans* and *Myrmecodia tuberosa* which has an association with ants and has medicinal values.

Several studies investigating the biosynthesis of AgNPs have been also carried out by using part of the plant such as *Garcinia imberti* [17], *Tinospora cordifolia* [18], *Calliandra haematocephala* [19], *Psidium guajava* [20], and *Couroupita guianensis* [21]. In addition, Kumar, Mondal and Sakthivel [22] mentioned that the characteristics of resulted-AgNPs that synthesized using plant extract can be determined by using several tools, such as UV-Vis spectrophotometer, Scanning electron microscope (SEM), Transmission electron microscope (TEM), Xray Diffraction (XRD), and Fourier Transmission Infrared Spectroscopy (FTIR).

However, limited information is found in preparing AgNPs using a bulb of ant nest plant (*Myrmecodia pendans*). Thus, the aim of this present work was to determine the biosynthesis of AgNPs using the aqueous extract of *M. pendans* bulb and characterize the AgNPs.

RESEARCH METHOD

Plant material and nanoparticle synthesis

The bulb of *M. pendans* was cleaned from extraneous matter by washing using deionized water and dried in an oven at 50°C for 12 h. The bulb of *M. pendans* powder was obtained using a mill. The powder was extracted with

aquades, heated at 70°C, and filtered for 2 h. The extract was then filtrated and evaporated using a rotary evaporator and stored at 4°C until it was used as a crude extract. The synthesis of silver nanoparticles (AgNPs) was performed by mixing specific amount ratio (1:1; 1:2; 1:4 and 1:8) between 0.5 M AgNO₃ solution and *M. pendans* bulb crude extracts. This solution was shaken and placed in the incubator at 60°C for 24 h. The biosynthesis of AgNPs can be recognized with color changing after the addition of plant extract to the aqueous AgNO₃ solution [23-25].

UV-VIS Spectroscopy analysis

The *M. pendans* bulb crude extract without AgNO₃ addition was prepared as a control. The optical properties of *M. pendans* bulb crude extracts with AgNO₃ solution at different amount ratio were evaluated using Shimadzu UV-1800 Spectrophotometer. The wavelength range of the spectrophotometer was set from 300–700 nm. Either active acidity of control solution (Primary extracts) or extracts with synthesized AgNPs was determined for seven days using Lutron pH 201 Electrode PE-03 pH meter (Lutron electronic enterprise co., ltd, Taiwan), with a measurement error of ±0.1.

SEM and TEM

The size and morphology of resulting AgNPs were determined using Evo MA 10 Carl Zeiss scanning electron microscope (SEM). Meanwhile, transmission electron microscope (TEM) analysis (model: JEM-1400) was used to observe the morphology of AgNPs at 120V, 20000x magnification.

XRD Analysis

XRD analysis of AgNPs was performed using a Bruker AXS D8 Advance diffractometer (X-rays of wavelength (λ)=1.54056 Å, 40 kV and 35 mA). The XRD patterns obtained were evaluated to find peak intensity, position and width. Based on the full width at half-maximum (FWHM) data, the mean particle size of AgNPs was characterized along with the Scherrer's formula. Respectively, the Scherrer's formula is $D = 0.9\lambda / \beta \cos\theta$, where: D = the mean of AgNPs diameter, λ = wavelength (XRD radiation source), β = the value of angular FWHM of the XRD peak, and θ = the diffraction angle.

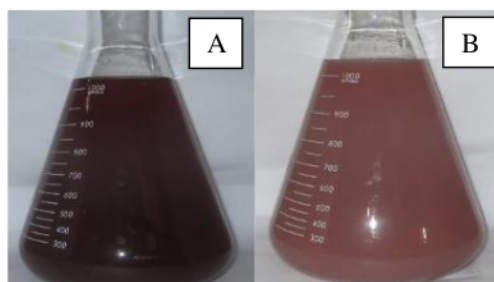
FTIR Observation

To analyze functional groups, molecular structure and chemical, including biomolecules structure that responsible for reducing Ag⁺, Fourier transform infrared (FTIR) spectrometer was performed, based on an infrared absorption spectrum (Perkin Elmer Spectrum 100).

RESULTS AND DISCUSSION

Nanoparticle biosynthesizes

The current results indicated that the mixture of *M. pendans* bulb crude extracts (FIGURE 1A) and 0.5 M AgNO₃ solution specific amount ratio (1:8) resulted in the formation of the pink colour solution (FIGURE 1B) which indicated the biosynthesis of silver nanoparticles (AgNPs). Similar results were revealed by Alagesan and Venugopal [26] that the first indicator of nanoparticle synthesis was detected by a color change.



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Figure 1. Colour change of silver nitrate (AgNO₃) to silver nanoparticles (AgNPs) by the addition of aqueous bulb extract of *Myrmecodia pendans*. (A). Aqueous bulb extract of *Myrmecodia pendans*; (B). AgNPs formation.

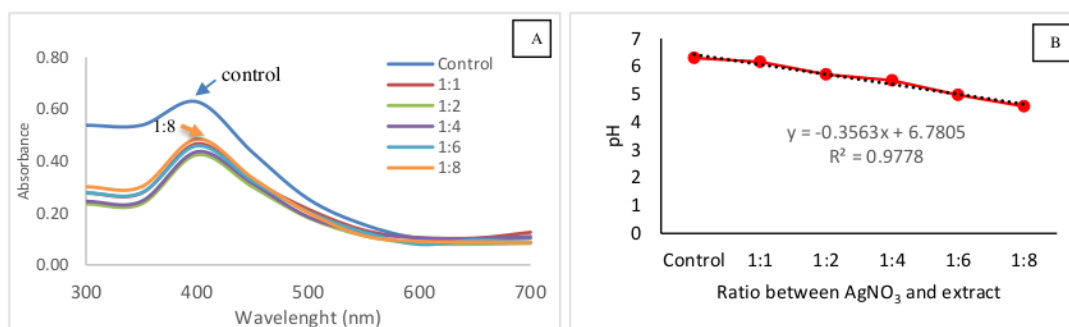
UV VIS-Spectroscopy analysis

Biosynthesis of AgNPs using different ratio of *M. pendans* bulb aqueous extract can be detected using UV-VIS spectral analysis, showing its formation and stability. Based on spectrum analysis, it is confirmed biosynthesis of AgNPs, and the peak absorption was obtained at wavelength of 400 nm, similar to control. It is also seen that the maximum absorption peaks of AgNPs was from samples with ratio 1:8 (FIGURE 2A). According to UV-VIS spectrum, it was revealed that peak maximum of the AgNPs was affected by the amount of *M. pendans* bulb aqueous extract. This finding is in line with past results stating that the peak maximum of AgNPs is around 400 nm and affected by the ratio of the plant extract [27, 28].

Further, the biosynthesis of AgNPs using several ratios of *M. pendans* bulb aqueous extracts, active acidity (pH) was evaluated for seven days. The pH of AgNPs solutions ranged from 6.3 to 4.5 (FIGURE 2B) and decrease with the increase of extract ratio. These results revealed that Ag⁺ in the *M. pendans* bulb aqueous extract were reduced and AgNPs were synthesized. In addition, it is also found that the pH of all AgNPs solution was not significantly decreased after repeated pH measurements for several days later, stating that the AgNPs solutions were stable. Previous results also revealed that the AgNPs remained stable between 410 nm to about 420 nm and in acidic and alkaline pH conditions [29].

12 SEM and TEM Imaging

The shape and size distribution of AgNPs can be characterized by using SEM (FIGURE 3A) and TEM (FIGURE 3B). The nanoparticles were characterized with a size between 20.70 nm and 37.88 nm. Current finding of SEM images showed that most of the AgNPs were spherical in shape. The average particle size was found around 26 nm, determined by using a software namely ImageJ and the resulted particle size was similar to XRD analysis. Meanwhile, TEM imaging analysis of the AgNPs indicated the spherical shape of nanoparticles. The nanoparticles were also surrounded by a thin layer, showing capping organic materials from the aqueous bulb extract of *M. pendans* which is useful to stabilize the nanoparticles. Present result is in agreement with previous studies by Moldovan, Sincari, Perde-Schrepler and David [30] revealing that the AgNPs were capped and stabilized by organic bioactive molecules derived from the *Ligustrum ovalifolium* fruits extract.



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Figure 2. (A) UV-VIS Spectra of silver nanoparticle synthesized using aqueous extract of ant nest plant (*Myrmecodia pendans*) bulb. Control = *Myrmecodia pendans* bulb crude extract without AgNO₃ addition. Ratio 1:8 = 0.5 M AgNO₃ and 10 % of *M.*

pendans bulb crude extracts. (B) Degree acidity of silver nanoparticles solution system synthesized using different ratio aqueous extract of ant nest plant (*Myrmecodia pendans*) bulb.

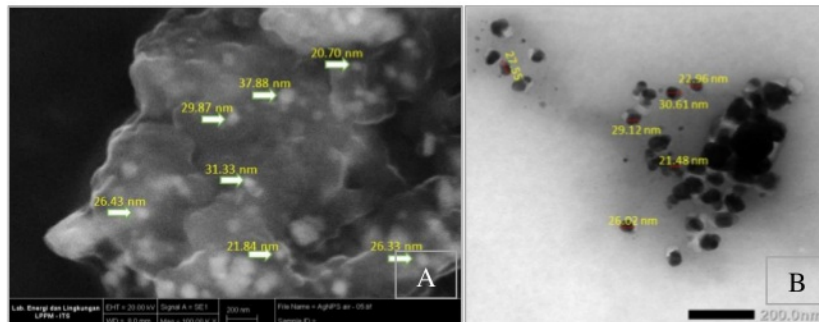


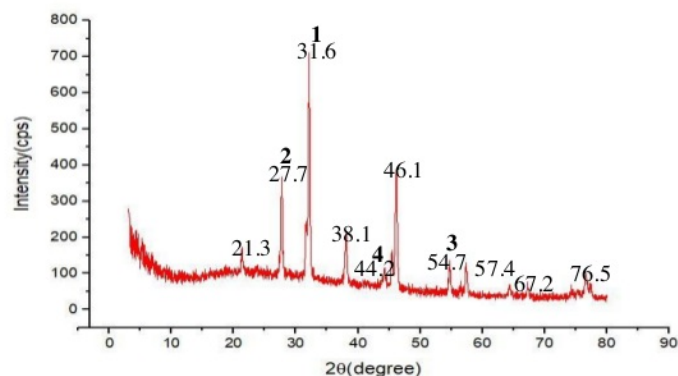
Figure 3. (A) SEM and (B) TEM analysis of AgNPs using green synthesis from aqueous extract of ant nest plant (*Myrmecodia pendans*) bulb.

XRD Analysis

Present result of The XRD spectra revealed the crystalline nature of the AgNPs synthesized by using the bulb of *M. pendans* and the patterns are shown in FIGURE 4. Further, the Bragg reflection of AgNPs were obtained at 2θ values of 31.62° , 27.71° , 54.73° , and 44.28° for *M. pendans* bulb. According to the Debye Scherrer equation, it is stated that the average sizes of the AgNPs biosynthesized by bulb extract of *M. pendans* was around 26.15 nm, respectively, which is in line with the result of TEM analysis. The XRD spectra resulted of AgNPs also revealed the presence of C and O that came from organic compounds of aqueous bulb extract of *M. pendans* as the capping AgNPs. There is no occurrence either N signal due to complete reduction of Ag^+ from $AgNO_3$ in the biosynthesis of AgNPs.

FTIR Spectral Analysis

FTIR spectral of *M. pendans* of AgNPs aqueous bulb extract was given in FIGURE 5, showing different functional groups. The spectral was set up in the wavenumber area between 300 and 4000 cm^{-1} . The highest FTIR spectrum peak at 3873 cm^{-1} , indicating the occurrence of O-H stretching and H-bonded of alcohols and phenols. The peak at 3394 cm^{-1} represented Hydroxy group, H-bonded OH stretch. Absorption bands at 2924 and 2854 cm^{-1} are due to stretching vibrations of C-H group. The peak observed at 2283 cm^{-1} correspond to aliphatic cyanide/nitrile. The C=C aromatic stretch occurred at 1527 cm^{-1} and 1612 cm^{-1} . Meanwhile, the peak at 1381 cm^{-1} C-H bend of alkanes and 1442 cm^{-1} represent methyl C-H asym./sym. bend, respectively. The aliphatic phosphates (P-O-C stretch) was indicated at peak 1288 cm^{-1} while C-C vibrations tertiary alcohol, C-O stretch at 1111 cm^{-1} . The alcohol, OH out-of-plane bend was occurred at wavenumber 609 cm^{-1} .



2

Figure 4. XRD pattern of AgNPs biosynthesized from aqueous extract of ant nest plant (*Myrmecodia pendans*) bulb. Ag peaks are marked 1-4 and 2θ values are given.

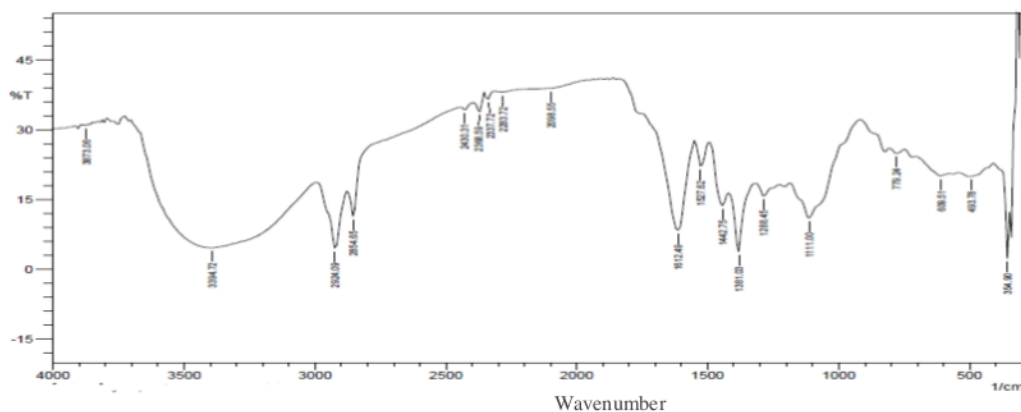


Figure 5. FTIR spectral of AgNPs synthesis by aqueous extract of *Myrmecodia pendans* bulb.

CONCLUSION

Present research confirmed that aqueous extract of *M. pendans* bulb has capability for the biosynthesis of AgNPs which reduction of Ag ion is occurred from AgNO_3 due to bulb extract. The SEM and TEM image analysis confirmed the particle shapes which is crystalline. The average crystalline size of AgNPs that has been characterized by XRD is estimated to be 26 nm, representing the AgNPs resulted from the biosynthesized. The resulted XRD data shows the sample as silver crystalline particles that has values corresponding to FCC silver. Different functional groups of resulting AgNPs is identified by The FTIR analysis. This study highlights promising finding for the biosynthesis of nanoparticle using plant extract which has stability and several functional groups.

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