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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 (AgNO3) for the biosynthesis of AgNPs can be done by using a plant extract. This research was focused on the biosynthesize an eco-friendly, inexpensive, and simple method of AgNPs using aqueous bulb extract of Myrmecodia pendans. The formation of AgNPs can be proved by the changing of the color, 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 by using aqueous bulb extract of M. pendans which has a peak of absorption at the wavelength of 400 nm in a 1:8 ratio solution between AgNO3 and the 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 the XRD result. The FTIR analysis determined several functional groups of AgNPs.

INTRODUCTION

Nanoparticle fields are gaining in momentum, attracting researchers to explore their synthesize and characteristics. Nanoparticles can be synthesized by either physical or chemical methods. However, chemically nanoparticles synthesized cannot be used for medical purposes due to safety concerns regarding chemicals binding on its surface and by-product toxicity. Meanwhile, there are more disadvantages to synthesize nanoparticle by using a physical procedure that 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 the plant parts along with Silver (Ag+) is to produce nanoparticle (AgNPs) that may provide different advantages over conventional. The part of plants is abundant and the most preferred raw materials by

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scientists 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 the Hydnophytinae (Rubiaceae) family, consisting of five genera. According to Sudiono et al [16], *Hypnophytum formicarum, Myrmecodia pendans,* and *Myrmecodia tuberosa* which have an association with ants and have medical values.

Several studies investigating the biosynthesis of AgNPs have been also carried out by using the plant part 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 work is to determine the biosynthesized of AgNPs using the aqueous extract of *M. pendans* bulb and characterize the AgNPs.

METHOD

Plant Material and Nanoparticle Synthesis

The bulb of *M. pendans* was cleaned from extraneous matter by washing them 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, then heated at 70oC, and stirred 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 a 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 $AgNO_3$ addition was prepared as a control. The optical properties of *M. pendans* bulb crude extracts with $AgNO_3$ solution at different amount ratios were evaluated using Shimadzu UV-1800 Spectrophotometer. The wavelength range of the spectrophotometer was set from 300–700 nm. Either active acidity of the 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 SEM. Meanwhile, TEM analysis (model: JEM-1400) is using to observe the morphology of AgNPs at120V, 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 determine the 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. 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 the functional groups, molecular structure and, the chemical, including biomolecules structure that is responsible for reducing Ag+, Fourier transform infrared (FTIR) spectrometer was performed and based on an infrared absorption spectrum (Perklin Elmer Spectrum 100).

RESULT AND DISCUSSION

Nanoparticle Biosynthesizes

The current results indicated that the mixture of *M. pendans* bulb crude extracts (**FIGURE 1A**) and 0.5 M $AgNO_3$ solution at a specific amount ratio (1:8) resulting in the formation of the pink color 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.

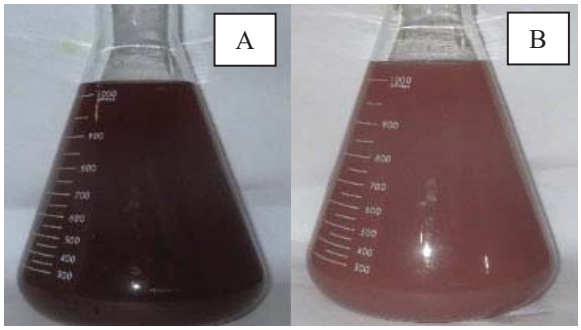

FIGURE 1. Colour change of silver nitrate ($AgNO_3$) to silver nanoparticles (AgNPs) by the addition of aqueous bulb extract of *Myrmecodia pendans*. (A). Aqueous bulb extract of *Myrmecodia pendans*; (B). AgNPs formation.

UV VIS-Spectroscopy Analysis

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

Furthermore, the biosynthesis of AgNPs that used several ratios of M. pendans bulb aqueous extracts like 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 was reduced and AgNPs were synthesized. Also, it was found that the pH of all AgNPs solution was not decreased significantly after repeating the pH measurements for several days later and 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].

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 by size between 20.70 nm and 37.88 nm. The current finding of SEM images shows that most of the AgNPs were spherical in shape. The average particle size that was found is around 26 nm. It was determined by using a software namely ImageJ and as a result, the particle size was similar to XRD analysis. Meanwhile, TEM imaging analysis of the AgNPs shows 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. The 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.

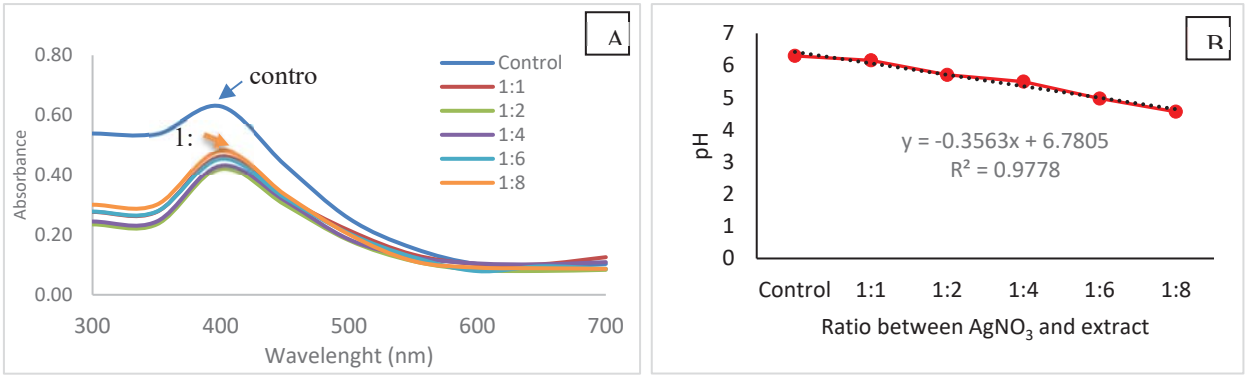

FIGURE 2. **(A)** UV-VIS spectra of silver nanoparticle synthesized using aqueous extract of ant nest plant (*Myrmecodia pendans*) bulb. Control = *Myrmecodia pendans* bulb crude extract without $AgNO_3$ addition. Ratio 1:8 = 0.5 M $AgNO_3$ and 10 % of *M. pendans* bulb crude extracts. **(B)** The degree acidity of the silver nanoparticles solution system synthesized using different ratio aqueous extract of ant nest plant (*Myrmecodia pendans*) bulb.

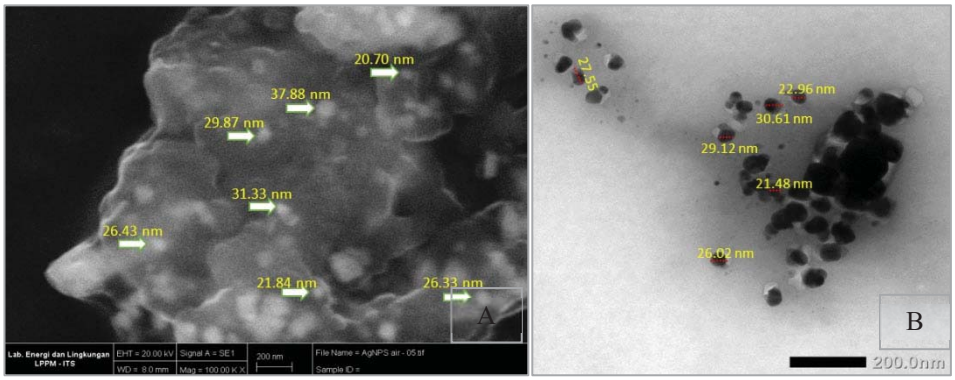

FIGURE 3. **(A)** SEM and **(B)** TEM analysis of AgNPs using green synthesize from aqueous extract of ant nest plant (*Myrmecodia pendans*) bulb.

XRD Analysis

The result of The XRD spectra reveals the crystalline nature of the AgNPs is synthesized by using the bulb of *M. pendans***.** The patterns are shown in **FIGURE 4**. Furthermore, the Bragg reflections 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 each. This is in line with the result of TEM analysis. The XRD spectra of AgNPs results also revealed the presence of C and O that derived from organic compounds of *M. pendans* aqueous bulb extract as the capping of AgNPs. There is no occurrence either on the N signal due to the 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** which shows the 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}, which indicates the occurrence of O-H stretching and H-bonded of alcohols and phenols. The peak at 3394 cm^{-1} represents the Hydroxy group, the H-bonded OH stretch. Absorption bands at 2924 and 2854 cm^{-1} were caused by the strain vibrations of the C−H group. The peaks observed at 2283 cm^{-1} correspond to the aliphatic cyanide/nitrile. The stretch of the aromatic ring of C=C-C occurred at 1527 cm^{-1} and 1612 cm^{-1}. While the peaks of the alkanes bend on at 1381 cm^{-1} C-H 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 the C-C vibrations tertiary alcohol, C-O stretch at 1111 cm^{-1}. The alcohol, OH out-of-plane bend occurred at the wavenumber 609 cm^{-1}.

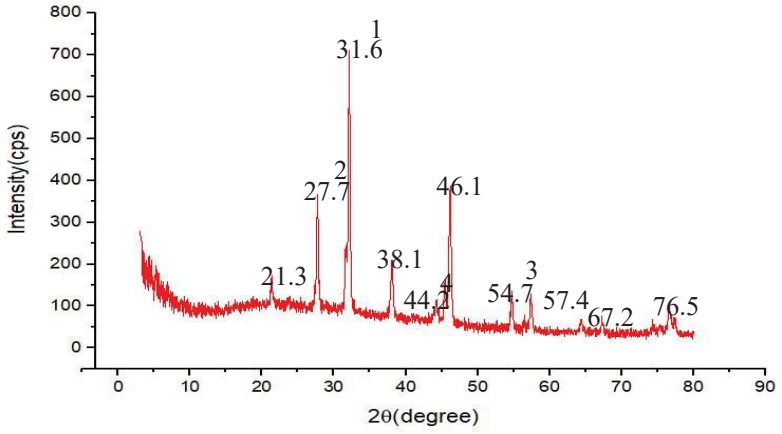

FIGURE 4. XRD pattern of AgNPs biosynthesized from aqueous extract of ant nest plant (*Myrmecodia pendans*) bulb. The Ag peaks are marked with 1-4 and 2θ values are given.

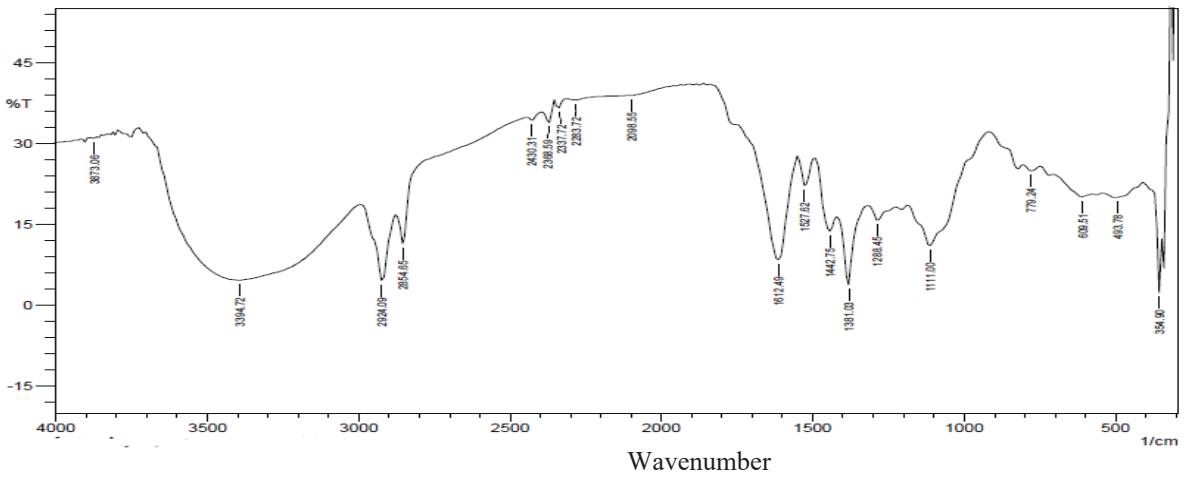

FIGURE 5. FTIR spectral of AgNPs synthesis by aqueous extract of *Myrmecodia pendans* bulb.

CONCLUSION

This research confirmed that aqueous extract of *M. pendans* bulb has the capability for the biosynthesis of AgNPs where there was a reduction of Ag ion from $AgNO_3$ due to bulb extract. The SEM and TEM image analysis confirmed that the particle shape is crystalline. The average crystal size of AgNPs that has been characterized by XRD wad estimated to be 26 nm, represent the AgNPs result from the biosynthesized. The result of XRD data shows that the sample of silver crystalline particles has values similar to FCC silver. Different functional groups from AgNPs results are identified by The FTIR analysis. This study highlights promising findings for the biosynthesis of nanoparticle using plant extracts that have stability and multiple functional groups.

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