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Synthesis and Characterization of Silver Nanoparticles from the Leaf Stalk Extract of *Moringa oleifera*

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Abstract

The exploitation of plant extract as a reducing agent for synthetic silver nanoparticles has become the main focus of researchers. This study aimed to synthesize silver nanoparticles (AgNPs) from *Moringa oleifera* leaf stalk extract via bio-reduction. The AgNPs were characterized through ultraviolet-visible absorption spectroscopy (UV-Vis), Fourier transforms infrared spectroscopy (FTIR), and X-ray diffraction (XRD). The research results indicated that an increase in the initial $AgNO_3$ concentration and incubation time had affected the absorption pattern of the surface plasmon resonance (SPR) trend of AgNPs. The results of the functional group analysis revealed the formation of the ketone group (1741 cm^{-1}), which was derived from hydroxyl groups (3415 cm^{-1}). The crystal structure of AgNPs was characterized using XRD. The estimation of the crystal measurement based on the Debye-Scherer equation was 15.59 nm, which was estimated as having a cube crystal form.

Keywords: Silver nanoparticle; Moringa oleifera; leaf stalk; surface plasmon resonance; green synthesis

INTRODUCTION

Green synthesis of nanoparticles, as known as biosynthesis, is a synthesis method with biological systems such as yeast, fungi, bacteria, and plant. The metal nanoparticles like silver, platinum, and gold nanoparticle were synthesized by biosynthesis technique. The synthesis of nanoparticles from plant extract has been revolutionized. The advantage of using biosynthesis is because of a large stock of plants, widely distributed, safe to use, minimizes waste, and is environment friendly (Roy et al, 2013). Indonesia is a country with high biological resources. As one of the largest tropical forests in the world, Indonesia has many types of flora and fauna. About 25.000–30.000 species of flowers and fruits grow in the natural forest of Indonesia, with 142 genus endemics (Maturahmah & Prafiadi, 2021).

One type of potential plant available in Indonesia's forest is Moringa. Moringa can grow well in tropical or subtropical areas, either in the lowlands or 1000 meters above sea level (Gopalakrishnan et al, 2016). Almost every part of the Moringa plant can be used for human life and has medicinal properties. (Hamzah & Yusuf, 2019). Besides being consumed, Moringa leaves are also used as medicine and water purification agent (Aminah et al, 2015). Moringa leaves contain vitamins, flavonoids and phenolic acids (Matinise et al, 2017). In addition, Moringa stems also contain ascorbic acid, saponins, polyphenols, alkaloids, tannins, steroids, flavonoids, niacin, retinol, quercetin, kaempferol, myricetin, ellagic acid, gallic acid, chlorogenic acid, and steroid acids. Natural compounds in Moringa have the potential as metal-reducing agents in the synthesis of nanoparticles (Gan & Li, 2012).

The synthesis of nanoparticles using Moringa plants has been widely reported. Moringa leaves are not only consumed but also used to synthesize ZnO nanoparticles (Matinise et al, 2017). Silver nanoparticles can also be synthesized through the bio-reduction method using Moringa leaf extract (Prasad & Elumalai, 2011). The antibacterial activity was shown by Fe nanoparticles synthesized from Moringa leaves (Katata-Seru et al, 2018). Moringa's flower has also been used in the synthesis of nanoparticles and is used for catalysts (Anand et al, 2016). However, only a few reports use Moringa leaf stalk in the synthesis of silver nanoparticles (Matinise et al, 2017). Therefore, in the present study, we discuss in detail the preparation and characterization of silver nanoparticle from the leaf stalk of *Moringa oleifera*.

Synthesis of silver nanoparticles with Moringa stalk extract is an environment-friendly method based on green chemistry principles. Synthesis of silver nanoparticles was carried out by reducing $AgNO_3$ compounds to silver nanoparticles using Moringa leaf stalk extract. The selection of Moringa leaf stalk is based on the few kinds of literature had been reported on the preparation of silver nanoparticles (Matinise et al, 2017). Therefore, it is necessary to research the synthesis of silver nanoparticles using Moringa leaf stalk extract, followed by the characterization of silver nanoparticles. The UV-Vis spectroscopy, FTIR, and XRD/XRF data have been used to verify the existence of silver nanoparticles.

METHODOLOGY

Materials and Instrumentals

Moringa leaf stalk was collected from local areas, Baubau (Indonesia), and the $AgNO_3$ was purchased from Sigma-Aldrich, Germany. A local pharmacy store supplied distillate water. All the chemicals were used without further purification. The instruments used include a UV-Vis spectrophotometer (UV-2600), an FTIR spectrophotometer (IRPrestige-21), and X-ray diffraction (Shimadzu 7000, Japan).

Methods

Preparation of Moringa Leaf Stalk Extract

Fresh and healthy leaf stalk was collected and rinsed in the tap water, followed by distillate water to remove all the dust and unwanted particles, cut into small pieces, and dried at room temperature. A 10 g of these finely Moringa leaf stalks transferred into a 500 mL beaker containing 100 mL distillate water and boiled for 20 min. The extracts were then filtered through Whatman filter paper under a vacuum condition. The filtered samples were then refrigerated at 4 ^{o}C for further experiment. The sterile condition should be maintained for effectiveness and accuracy in the result.

Synthesis of Silver Nanoparticles (AgNP)

Moringa leaf stalk extract (0.1 g/mL) with a volume of 1 mL was added to 90 mL of $AgNO_3$ solution of 1, 3, and 5 mM respectively. The mixture is then heated at 60-80 ^{o}C under stirring for 20 min. The color changes and UV-Vis spectrum measurements are carried out at certain intervals to monitor AgNP growth. The AgNP colloid was centrifuged at 10.000 rpm for 30 min to separate the nanomaterial from the colloid.

Analysis of Silver Nanoparticles

UV-Vis measurements were carried out to monitor the growth of silver nanoparticles (AgNP). UV-Vis analysis of AgNP colloids was performed using Shimadzu UV-2600 UV-Vis spectrophotometer for 1-5 days. AgNP colloids are characterized by measuring the maximum λ at 300-600 nm. UV-Vis measurements were carried out on AgNP synthesis with variations in concentrations of $AgNO_3$ of 1, 3, and 5 mM.

Identification of Silver Nanoparticle by FTIR

FTIR analysis determines the functional groups that reduce Ag^+ to silver nanoparticles (Ag^0). Moringa leaf stalk extract solution before and after synthesis of AgNP was centrifuged at 10.000 rpm for 30 min. The suspension is dried at 60-80 ^{o}C. Dry biomass was analyzed using the Shimadzu IRPrestige-21 FTIR spectrophotometer.

X-ray Diffraction and XRF Analysis

X-ray diffraction (XRD) and XRF analysis were carried out on AgNP solids. The AgNP solids were taken and characterized by XRD to confirm the crystal structure of AgNP. The crystal size of nanoparticles was confirmed using the *Scherrer* equation. XRD pattern analysis was obtained using a MiniFlex2 X-ray diffractometer, with Cu/30kv/15mA and radiation kα (wavelength λ=0.1542 nm). The scan distance is from 5000-7000 deg, with a 2000 deg/min speed.

RESULT AND DISCUSSION

The formation of silver nanoparticles

The formation of silver nanoparticles (AgNP) begins with a mixture of Moringa leaf stalk extract with silver nitrate solution of 1, 3, and 5 mM. The color change of the mixture from clear to brownish-yellow in the solution indicates the presence of nanoparticles (Figure 1a). Analysis of UV-Vis spectra also confirmed the formation of silver nanoparticles where the absorption band observed at a wavelength 425 until 448 nm was caused by AgNP surface plasmon resonance (SPR). SPR is a collection of oscillations in the conduction of electrons on the material's surface.

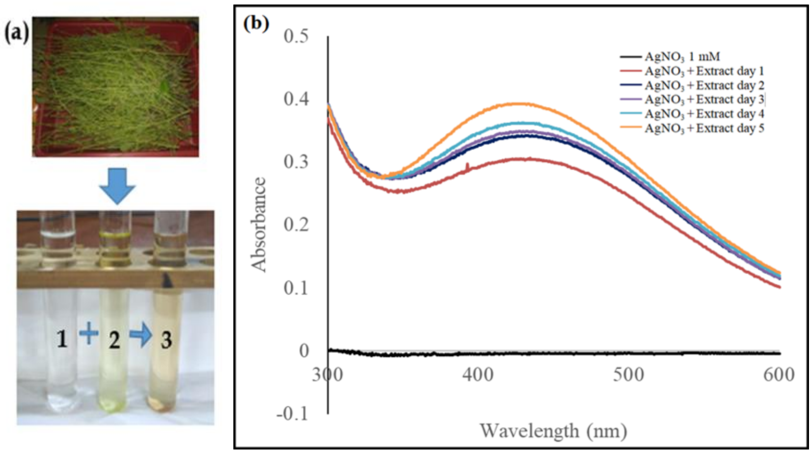

Figure 1. Biosynthesis of silver nanoparticles (a): *Moringa oleifera* leaf stalk waste, aqueous silver nitrat (1), aqueous leaf stalk extract (2), silver nanoparticles (AgNPs) (3); (b): UV-Vis absorbance spectra peak of AgNP at different incubation time.

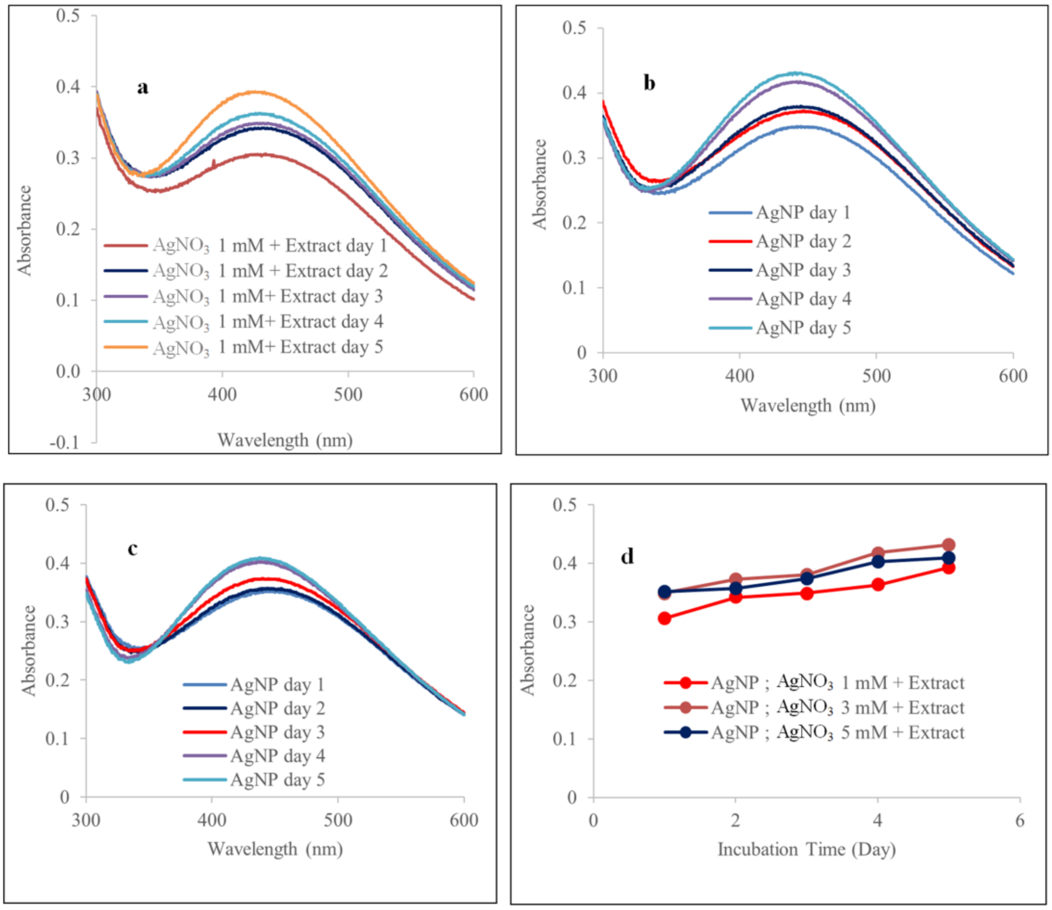

Figure 2. UV-Vis spectra of AgNP during incubation time (a) $AgNO_3$ 1 mM + *M. oleifera* leaf stalk extract, (b) $AgNO_3$ 3 mM + *M. oleifera* leaf stalk extract, (c) $AgNO_3$ 5 mM + *M. oleifera* leaf stalk extract; (d) the change of SPR intensity during reaction time

Electromagnetic fields generated by electron waves cause the excitation of a collection of electrons in silver nanoparticles. This phenomenon is called localized surface plasmon resonance (Willets et al, 2007). The changes in the pattern of SPR intensities during the reaction time are shown in Figure 2. The typical absorption peaks in the wavelength 425-448 nm region are suitable for the SPR of AgNP character, indicating the growth of AgNP (Rusnaenah et al, 2017; Jagtap & Bapat, 2013; Irwan et al, 2020).

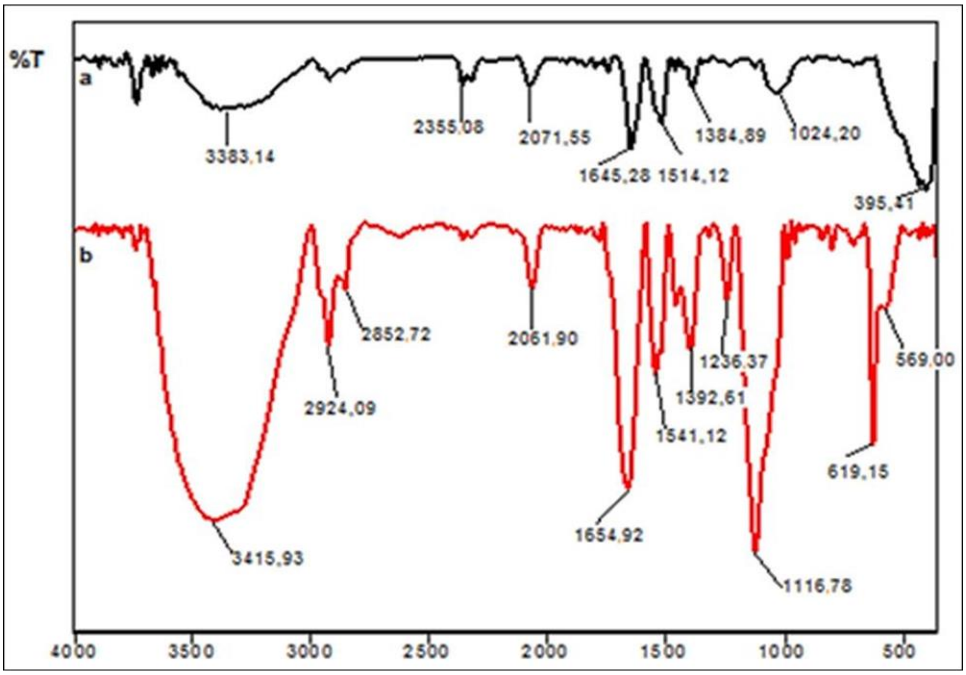

Figure 3. The infrared spectrum (a) silver nanoparticles, (b) Moringa leaf stalk extract

The absorption peak of AgNP SPR was at wavelengths of 434, 444, and 448 nm at incubation time for one day for each concentration of $AgNO_3$ 1, 3, and 5 mM with the concentration of Moringa leaf stalk extract of 0.1 g/mL. The difference in frequency and width of the SPR absorption band depends on the shape and size of the metal particles and the dielectric constant, and the medium around the metal particles (Wiley et al, 2006). The increase in SPR intensity during incubation time can be related to the increase in the number of particles in the reaction medium (Umadevi et al, 2013). Figure 2d shows the relatively higher absorption intensity from days 1 until 5 as an indication of the increasing number of particles in the incubation medium.

The stability of nanoparticles can also be seen qualitatively using the SPR absorption band (Huang et al., 2011). The stability of AgNP growth can be determined based on the SPR pattern over a certain time. The increasing SPR pattern from day 1 to day 5 indicates the stability of the nanoparticles during the incubation time. The increased SPR peak during incubation time also indicates the density of nanoparticles in the colloidal system (Ahmad et al, 2013).

Analysis of Fourier Transform Infrared Spectroscopic result

Based on the SPR data, colloid nanoparticles indicate to be stable during the incubation time. This shows that AgNP is stabilized by biomolecules contained in fresh extracts of Moringa leaf stalks. To find out the potential functional groups in AgNP formation, Fourier Transport Infrared (FTIR) spectroscopy is used. FTIR spectrum measurements were carried out to determine the possibility of functional groups involved in manufacturing nano-Ag. Figures 3 represent the spectrum of each IR absorption of silver nanoparticles (a) and Moringa leaf stalk extract (b).

Figure 3(b) is the IR spectrum of plant extracts. The width absorption peak at wave number 3415 cm^{-1} is indicated by the existence of a stretching -OH vibration, the absorption peak at 2924 and 2852 cm^{-1} is caused by the aliphatic -C-H group. The presence of a methylene group ($-CH_2$) and a methyl group ($-CH_3$) occurred at 1541 cm^{-1} and 1392 cm^{-1}, respectively. The absorption band at 1116 cm^{-1} and 619 cm^{-1} is caused by stretching vibration of =C-H aromatic. The presence of an aromatic C=C group is amplified by a sharp absorption band that appears at a wave number of 1654 cm^{-1}.

The absorption region at 1024-1741 cm^{-1} is caused by stretching vibrations of C=O, C=N, NH, and C=C aromatic. The absorption band is indicated by the content of flavonoids and phenol acids, which are considered to be the molecules responsible for AgNP synthesis (Matinise et al, 2017). The absorption band at 1778 cm^{-1} indicates the C=O ester group supported by C-O ester vibrations at 1236 cm^{-1} (Zakir et al, 2014).

The intensity of the C=C aromatic group decreased and shifted from 1654 cm^{-1} to 1645 cm^{-1}. It is indicated the reduction of Ag^+ to Ag^0 is caused by Moringa leaf stalk extract. The IR absorption intensity decreases in the -OH group with decreased absorption intensity after synthesis, and the IR absorption band shifted from 3415 cm^{-1} before synthesis to 3383 cm^{-1} after AgNP synthesis (Figure 3 a). The shift of the C=O ester group also occurred to being C=O ketone at 1741 cm^{-1} in the AgNP spectrum, originally at the wave number 1778 cm^{-1} with a small absorption intensity. The reduction of absorption intensity of the C=C aromatic group and the appearance of absorption of Ag at wavenumbers of 420 cm^{-1} and Ag0 at 3700 cm^{-1} indicate Ag^+ has been reducing to be Ag^0. Estimates of the mechanism for reducing $AgNO_3$ are very suitable as proposed by Matinise et al (2017).

Analysis of XRD Pattern

To confirm the crystal phase of AgNP, X-Ray Diffraction (XRD) pattern analysis was performed. The peak characteristics observed in the XRD pattern of AgNP through reducing of $AgNO_3$ 1 mM by using Moringa leaf stalk extract at 0.1 g/ml at 60-80 ^oC confirmed the presence of silver nanoparticles (Figure 4).

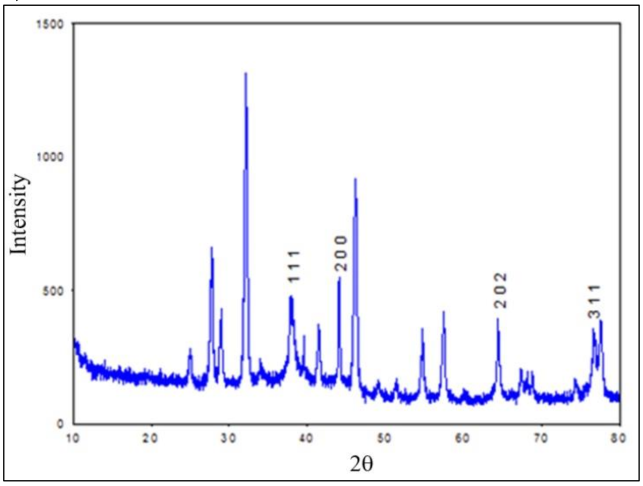

Figure 4. XRD pattern of silver nanoparticle (aqueous $AgNO_3$ 1 mM + *M. oleifera* leaf stalk aqueous extract)

Diffraction peaks at angles of 37.82, 44.04, 64.80 and 77.65^o correspond to the fields of (1 1 1), (2 0 0), (2 0 2), and (3 1 1) silver nanoparticles (Suh, Ohta, & Waseda, 1988). The diffraction pattern with the index miller of (1 1 1) corresponds to the AgNP face-center-cubic (FCC) crystal system (Zakir et al, 2014). The average crystallite size of AgNP was obtained using Debye-Scherrer's equation. The diameters of silver nanoparticles were estimated to be in the range of 15.59-49.03 nm. Several diffraction peaks also appear in XRD patterns of 32.11, 46.10 and 27.71^o, suggesting the presence of other compounds besides AgNP. This is confirmed by XRF data where the elements like chlorine, potassium, sulfur, and calcium are still contained in the AgNP with values of 45.63, 10.13, 9.44, and 0.60%, respectively (Table 1). The highest chlorine at the Moringa leaf stalk must be considered a reason for the reduced percentage of Ag. Therefore, we suggest a further experiment to separate the chlorine from Moringa before the silver nanoparticle synthesis from plant extract.

Table 1. XRF data of silver nanoparticles

Element	m/m%
Cl	45.63
Ag	33.56
K	10.13
Sx	9.44
Ca	0.60
Rh	0.155
Nb	0.133
Mo	0.109
Fe	0.083
Ru	0.0789
Zn	0.031
Br	0.0272
Te	0.0145

CONCLUSION

Silver nanoparticles were successfully fabricated using *Moringa oleifera* leaf stalk extract. The synthesized silver nanoparticle was estimated at 15.59 nm in size, characterized by XRD. The analysis of UV-Vis spectra showed the absorption in the region of 425-448 nm wavelength with a color change from clear to brownish-yellow. The hydroxyl groups are responsible for reducing metal to silver nanoparticles.

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