

Plant-extract-mediated biosynthesis of silver nanoparticles using *Eleutherine americana* bulb extract and its characterization

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Manuscript received: 20 November 2021. Revision accepted: 30 November 2021.

Abstract. Manullang JR, Nugroho RA, Rohmah M, Rudianto, Qorysuchi A. 2021. Plant-extract-mediated biosynthesis of silver nanoparticles using *Eleutherine americana* bulb extract and its characterization. *Nusantara Bioscience* 13: 247-254. The plant-based biosynthesis of nanoparticles has gained increasing momentum due to being lower in cost and eco-friendly. This study aimed to biosynthesize nanoparticles from the ethanolic bulb extract of *Eleutherine americana* (Aubl.) Merr. Ex K.Heyne (Ea-AgNPs), then characterize Ea-AgNPs and determine their phytochemical content and antioxidant capacity. The Ea-AgNPs were synthesized using ethanolic extract of *E. americana* bulb along with various concentrations of AgNO₃ (0.5-4 mM). The Ea-AgNPs were then characterized using UV-VIS spectroscopic, Scanning Electron Microscopy/ Energy Dispersive X-ray (SEM/EDX), Transmission Electron Microscopy (TEM), X-ray Powder Diffractometry (XRD), and Fourier-Transform Infrared Spectroscopy (FTIR) techniques. The results indicated that *E. americana* could be used to reduce AgNO₃ to synthesize Ea-AgNPs, indicated by color change, and had optimum UV/VIS spectra at 400 nm. The FTIR analysis found that Ea-AgNPs showed peaks at 2919, 2850, 1586, and 1031 cm⁻¹, containing several important bio compounds. Additionally, the XRD results found an amorphous Ea-AgNP peak with maximum intensity and proportion of silver occurring at 24 Theta. The particle size distribution curve of Ea-AgNPs showed a size of 10⁵ nm. Furthermore, SEM/EDX analysis revealed an optical absorption characteristic peak at 3 keV. The EDX examination revealed three signals: a strong signal from the C atom (70.99 %), an O atom (28.95 %), and an Ag atom (0.06 %). The TEM imaging also showed the characteristics of Ea-AgNPs. Some phytochemicals such as flavonoids, tannins, alkaloids, and saponins were found in Ea-AgNPs, with IC₅₀ values of 45.30 ppm.

Keywords: Antioxidant properties, characterization, *Eleutherine americana*, nanoparticle

INTRODUCTION

Medicinal plant extracts were used in a bottom-up single-pot synthesis strategy using a wet chemistry method to conduct the green synthesis of silver nanoparticles. This method is environmentally benign, and low-nanotechnology has arisen from many disciplines of science and engineering, where novel concepts for modifying molecules and single atoms have been developed (Usman et al. 2020; Gottardo et al. 2021; Zhang et al. 2021). The application of nanomaterials is particularly popular within environmental and medical research in the fast-developing field of nanotechnology. Silver nanoparticles are distinctive among metallic nanoparticles (Crisan et al. 2021; Kaabipour and Hemmati 2021; Restrepo and Villa 2021); they are the most widely investigated by researchers worldwide due to their versatile applications, simplicity of synthesis, adaptability, shape, and large surface area.

Nanoparticles are also broadly applied in biomedicine, agriculture, and poultry feed fields. In biomedicine, nanoparticle plant-based extract demonstrated significant antibacterial activity against various pathogenic

microorganisms and cytotoxic action in the A549 and HepG2 cell lines (Acay 2021). Meanwhile, several studies have investigated the effects of nanoparticles on seed germination, growth stimulation, and metabolic rate changes (Goswami and Mathur 2019). However, nanoparticles also have negative consequences, such as suppressing plant development, inhibiting chlorophyll synthesis, and lowering photosynthetic efficiency (Goswami and Mathur 2019). In poultry, because of their huge surface area to volume ratio and rapid absorption in the body, nanoparticles may be introduced to animal feed, providing an ideal platform for incorporating a diverse range of substances such as vaccinations and vitamin supplements. Nanoparticles can also facilitate the direct delivery of chemicals to specific organs or systems while avoiding the rapid degradation caused by antibiotics, resulting in various health advantages (Singh and Lillard 2009).

Nanoparticles are often made using various chemical and physical processes that are costly and potentially harmful to the environment (Guo, 2012; Uzair et al. 2020). As a result, green synthesis approaches are gradually being linked with current scientific and industrial advancements

in the global endeavor to decrease hazardous waste production. This study costs and produces stable, well-dispersed silver nanoparticles with little aggregation and good size control (Irvani and Varma 2020; Velsankar et al. 2020).

In the present study, an extract of *Eleutherine americana* (Aubl.) Merr. Ex K. Heyne was employed as a reducing and stabilizing agent. *Eleutherine americana* (Dayak onion) is traditionally used as an ethnomedicine (Kamarudin et al. 2021); the bulb of this species is an essential traditional medicine used by Dayak tribes to treat heart illness, as an anti-inflammatory, to improve breast-milk production, diabetes, breast cancer, stroke, hypertension, sexual problems, and as a bacterial infection therapy (Ifesan et al. 2009; Song et al. 2009; Saragih et al. 2014; Nuryanto and Paramita 2018). The bulbs contain naphthalene, naphthoquinone, and anthraquinone (Mahabusarakam et al. 2010; Insanu et al. 2014) and phenolic compounds, which have powerful antioxidant effects and may be involved in the treatment of some diseases (Kuntorini et al. 2016). Furthermore, an oligosaccharide extract from *E. americana* has been utilized as a prebiotic to promote the development of gut flora (Phoem et al. 2019).

Plant extract as a reducing and stabilizing agent in the green production of silver nanoparticles is a new approach of interest to numerous scientists. Fatimah and Afrid (2019) worked on the biosynthesis of AgNPs using red spinach (*Amaranthus tricolor*) leaf extract and investigated its antibacterial activity, while Kedi et al. (2018) synthesized AgNPs from *Selaginella myosurus*. Another study performed by Jalilian et al. (2020) focused on the green synthesis of AgNPs using *Allium ampeloprasum* aqueous extract and investigated their characterization, antioxidant activity, and antibacterial and cytotoxicity effects. Finally, Vijayakumar et al. (2019) used garlic clove extract to produce AgNPs, and further assessed their antibacterial, antibiofilm, anthelmintic, anti-inflammatory, anticancer, and ecotoxicity characteristics. Other research on the biosynthesis of AgNPs using various plant extracts are as follows: *Oscillatoria* sp. extracts (Adebayo-Tayo et al. 2019), cauliflower extract (Oda et al. 2019), and the bulb of *Myrmecodia* sp. (Nugroho et al. 2021).

Despite the previous research in this field, the experimental biosynthesis and characterization of silver nanoparticles mediated using the *E. americana* bulb as a reducing and stabilizing agent are not fully understood. Thus, the present work aimed to biosynthesize silver nanoparticles from *E. americana* bulb-mediated extract (Ea-AgNPs), characterize the resulting Ea-AgNPs, evaluate their phytochemical content, and determine the DPPH radical scavenging assay for the Ea-AgNPs.

MATERIALS AND METHODS

Chemicals and extract preparation

All chemicals, such as AgNO₃, were obtained from Sigma Aldrich, MO, USA. The *E. americana* bulbs were

purchased from a local herbal market in Samarinda, East Kalimantan, Indonesia. The identity of the *E. americana* was confirmed by a taxonomist working at Universitas Mulawarman. Next, to obtain bulb extract of *E. americana*, the bulb was cleaned using distilled water, thinly chopped into small pieces, and soaked using ethanol (10 g per 100 mL). After 48 hours, the mixture was filtered and vacuum dried using a rotary evaporator. Finally, a dark brownish color *E. americana* bulb ethanolic extract was obtained and stored at 4°C until further use.

Ea-AgNPs synthesis

Furthermore, to synthesize the Ea-AgNPs, 10 mL of AgNO₃ solution at various concentrations (0.5, 1, 2, 3, and 4 mM) was prepared and added to 1 mL of *E. americana* bulb ethanolic extract. The synthesis was performed for 24 h at room temperature in a dark bottle to minimize the photo-activation of AgNO₃. The color shift of the solution from colorless to brown confirmed the reduction of Ag to Ag⁰; UV-Visible spectroscopy was also used to validate its formation.

Ea-AgNPs characterization

The Ea-AgNPs, which were obtained from maximum absorbance (1 mL extract of *E. americana* and 10 mL of AgNO₃ 4mM) freeze-dried and characterized using UV-Visible Spectroscopy (UV-VIS; Spectrophotometer SP-UN52N, Beijing China), Scanning Electron Microscopy (SEM; JEOL, Tokyo, Japan), Transmission Electron Microscopy (TEM; JEM 2100F JEOL, Tokyo, Japan), Fourier-Transform Infrared Spectroscopy (FTIR; 1000 FT-IR spectrometer, Perkin Elmer), and X-ray Fluorescence (XRF; EDAX Bruker, Bavaria, Germany).

Phytochemicals analysis of Ea-AgNPs

The presence of phenols, saponins, triterpenes, flavonoids, alkaloids, and steroids in the Ea-AgNPs was determined using phytochemical screening. The screening process was carried out according to the technique described previously by Dada et al. (2018) and Senguttuvan et al. (2014).

Antioxidant activities

The antioxidant activity of Ea-AgNPs was determined using the DPPH radical scavenging test. The Ea-AgNPs' capacity to scavenge free radicals has previously been evaluated using the stable radical DPPH approach (Lateef et al. 2015). One milliliter of Ea-AgNPs in methanol at different concentrations (0.08, 0.1, 0.12, 0.14, and 0.16 mg/mL) was added to four milliliters of a 0.1 mmol L⁻¹ methanolic solution of DPPH. A blank was also prepared by diluting 1 mL methanol in 4 mL DPPH. The samples were incubated for 30 minutes in the dark at room temperature. The absorbance (517 nm) was measured against the prepared blank. The percent inhibition of free radicals by DPPH was estimated using the following formula:

$$\text{Inhibition \%} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100$$

Where A_{control} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound.

RESULTS AND DISCUSSION

Biosynthesis of Ea-AgNPs

The addition of *E. americana* bulb extract to the aqueous AgNO_3 solution resulted in a change in the color of the solution from dark yellowish to reddish-brown (Figure 1) during the reaction, owing to the excitation of surface plasmon vibrations in the silver nanoparticles (Ider et al. 2017). Furthermore, silver nanoparticles were produced at various AgNO_3 concentrations with ethanolic extract of *E. americana* bulb, as evidenced by the plasmon resonance

band found at 410 nm within the UV spectra (corresponding to Ea-AgNPs); AgNO_3 had an absorbance peak at 300 nm, and the ethanolic extract of *E. americana* bulb had a peak at between 500-600 nm. These findings are comparable with Pandian et al. (2015), stating that Ea-AgNPs shows a characteristic peak absorbance wavelength between 400-500 nm (Figure 2). Previous research has also noted that silver nanoparticles absorb light between 400 and 500 nm due to their surface plasmon resonance (Prathna et al. 2011). Therefore, the optimum peak of 410 nm for Ea-AgNPs derived from 4 mM AgNO_3 added with *E. americana* bulb extract indicates the biogenesis of Ag nanoparticles (Figure 3). The current work also shows that Ag nanoparticles are extremely stable in solution, even six days after their synthesis, which significantly confirms the suitability of *E. americana* ethanolic bulb extract for the production of AgNPs.

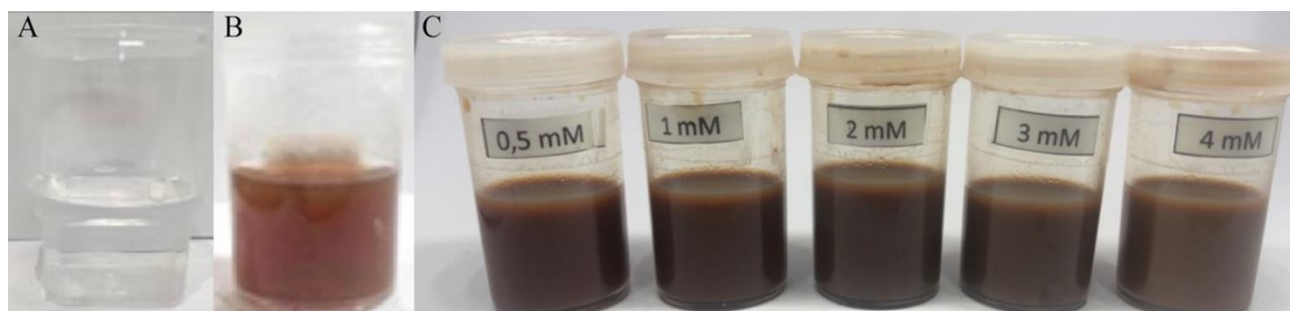


Figure 1. A. A solution of AgNO_3 , B. Dark yellowish ethanolic extract of *Eleutherine americana* bulb, C. Reddish-brown solution of Ea-AgNPs with different mixture concentrations of AgNO_3 (0.5-4 mM)

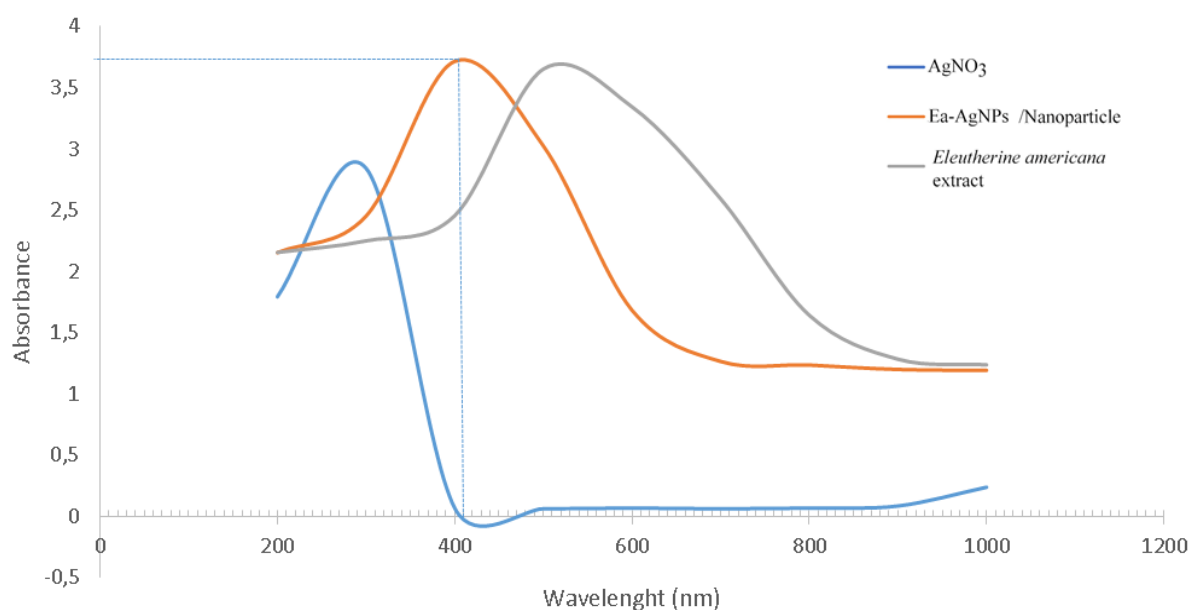


Figure 2. UV-VIS spectra of green synthesis of silver nanoparticles (Ea-AgNPs) using ethanolic extract of *Eleutherine americana* bulb and 4mM of AgNO_3

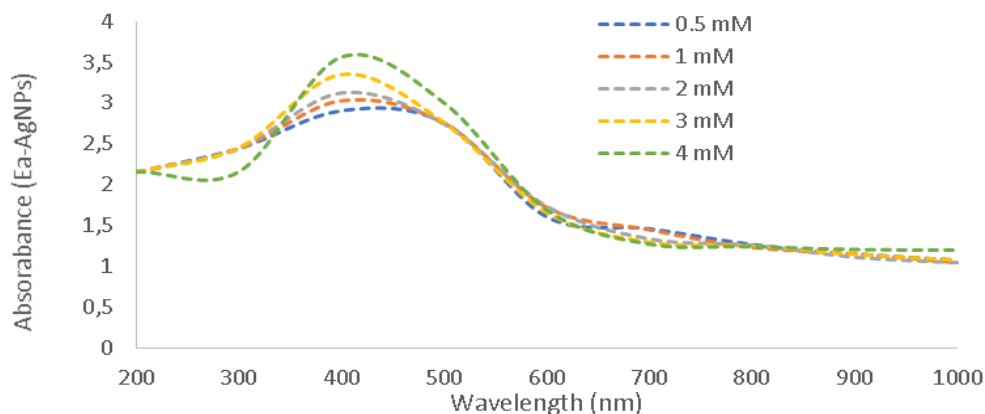


Figure 3. UV/VIS spectra characterization, showing the production of Ea-AgNPs at various concentrations of AgNO_3

Ea-AgNPs characterization

The FTIR analysis was used to determine the various functions of reducing metals to metal nanoparticles. Figure 4 shows the infrared spectrum of biogenic Ea-AgNPs, with four significant bands detected at wavenumbers 2919, 2850, 1586, and 1031 cm^{-1} . At 2919 and 2850 cm^{-1} , a wide peak corresponds to the asymmetric and symmetric stretching $\nu(\text{C-H})$ vibrations of the methylene group of aliphatic compounds, respectively. The absorption peak at 1586 cm^{-1} likely corresponds to the carboxyl group's $\nu(\text{-COO})$ stretching frequency. In contrast, the band at 1031 cm^{-1} is attributed to the C-O stretching vibration of the carbohydrate residues.

In addition, the X-ray Powder Diffractometry (XRD) pattern indicated that the Ea-AgNPs were naturally amorphous (Figure 5), which is consistent with previously published results (Umadevi et al. 2012). Based on the particle size analysis (Figure 6), the resulting Ea-AgNPs had a size of more than 1 μm , which might be caused by agglomeration, and the distribution of particles size of Ea-AgNPs was found to be 24.59 % of 6667.10 nm and 75.41 % of 7532.65 nm.

SEM and EDS Analysis of Ea-AgNPs

The presence of Ea-AgNPs was examined using Energy-Dispersive Spectroscopy (EDS) and SEM (Figure 7). The elemental composition of the Ea-AgNPs' nanocomposite film was determined using Energy Dispersive X-ray analysis (EDX), with the EDX spectrum of the Ea-AgNPs nanocomposite shown in Figure 8. The SEM image of the Ea-AgNPs (Figure 7) demonstrates that almost all nanoparticles are widely dispersed throughout the plant extract.

The SEM/EDX was used to determine the nanoparticles' surface morphology and elemental composition (Anake et al. 2016). Figure 7 depicts the morphology of the Ea-AgNPs produced as amorphous aggregates. The EDX analysis was used to determine the elemental components and relative abundance of biosynthesized Ea-AgNPs, as shown in Figure 8. The purity and entire chemical composition of the Ea-AgNPs are shown by the EDX spectrum (Figure 8). The percentage content of Ag metal present in association with

other chemical elements was discovered to be significant. The EDX spectrum revealed an optical absorption characteristic peak corresponding to Ag at 3 keV. Overall, the EDX examination revealed three signals: a strong signal from C atoms (70.99 %), in addition to signals from O atoms (28.95 %) and Ag atoms (0.06 %). Other elements or impurities did not exhibit obvious peaks. For example, metal silver nanoparticles have a typical optical absorption peak at about 3.7 keV; however, the additional peaks for C and O indicate that the nanoparticles are mixed into precipitates in the plant extract.

TEM characterization

Following confirmation of the formation of Ea-AgNPs through color change observation and UV/VIS absorption spectra, the size, shape, and morphology of Ea-AgNPs were investigated using TEM examination. The TEM images show that the powder particles were amorphous in form and agglomerated (Figure 9); the Ea-AgNPs were successfully formed, with an average particle size of ~1 μm .

The phytochemical contents and DPPH assay

The phytochemical contents in the Ea-AgNPs are summarized in Table 1. The presence of alkaloids, flavonoids, phenolics, tannins, and coumarin was observed in the Ea-AgNPs.

The results indicate that the EA-AgNPs contain several important phytochemicals, including alkaloids, flavonoids, phenolics, tannins, and coumarin (Table 1). These results are similar to findings from previous studies, which revealed that silver nanoparticles from *Sargassum tenerrimum* contain several phytochemicals (Kumar et al. 2012). Furthermore, some phytochemicals, such as flavonoids, tannins, alkaloids, and saponins, which are found in Ea-AgNPs, may exhibit antibacterial properties (Rai et al. 2020; Nahar et al. 2021). Meanwhile, DPPH assays were used to assess the antioxidant activity of Ea-AgNPs; the results indicate that the Ea-AgNPs have a higher free radical inhibition percentage, with IC_{50} values of 45.30 ppm (Table 2).

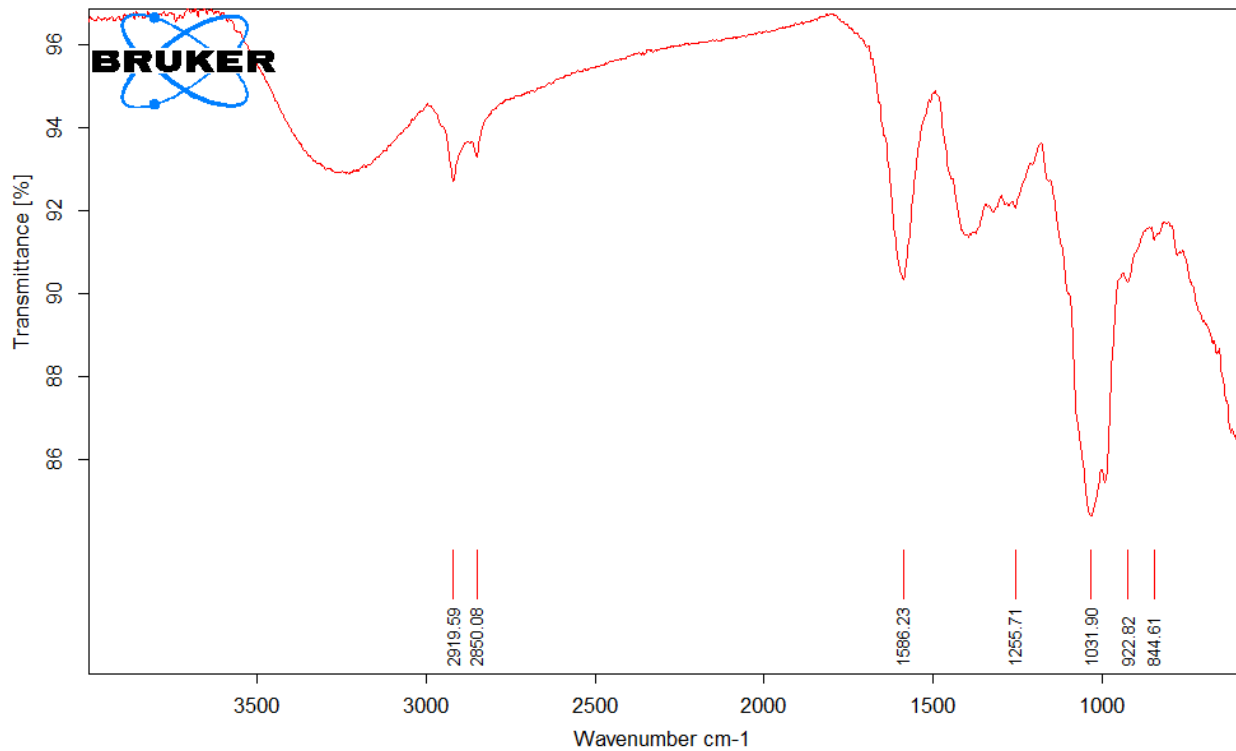


Figure 4. Fourier-transform infrared spectroscopy (FTIR) spectra of green synthesized silver nanoparticles (Ea-AgNPs) using ethanolic extract of *Eleutherine americana* and 4 mM AgNO_3 ; peaks at 2919, 2850, 1586, and 1031 cm^{-1} were due to C-H methylene group of aliphatic compounds, (-COO) stretching frequency of carboxyl group, and C-O stretching vibration of the carbohydrate residues, respectively

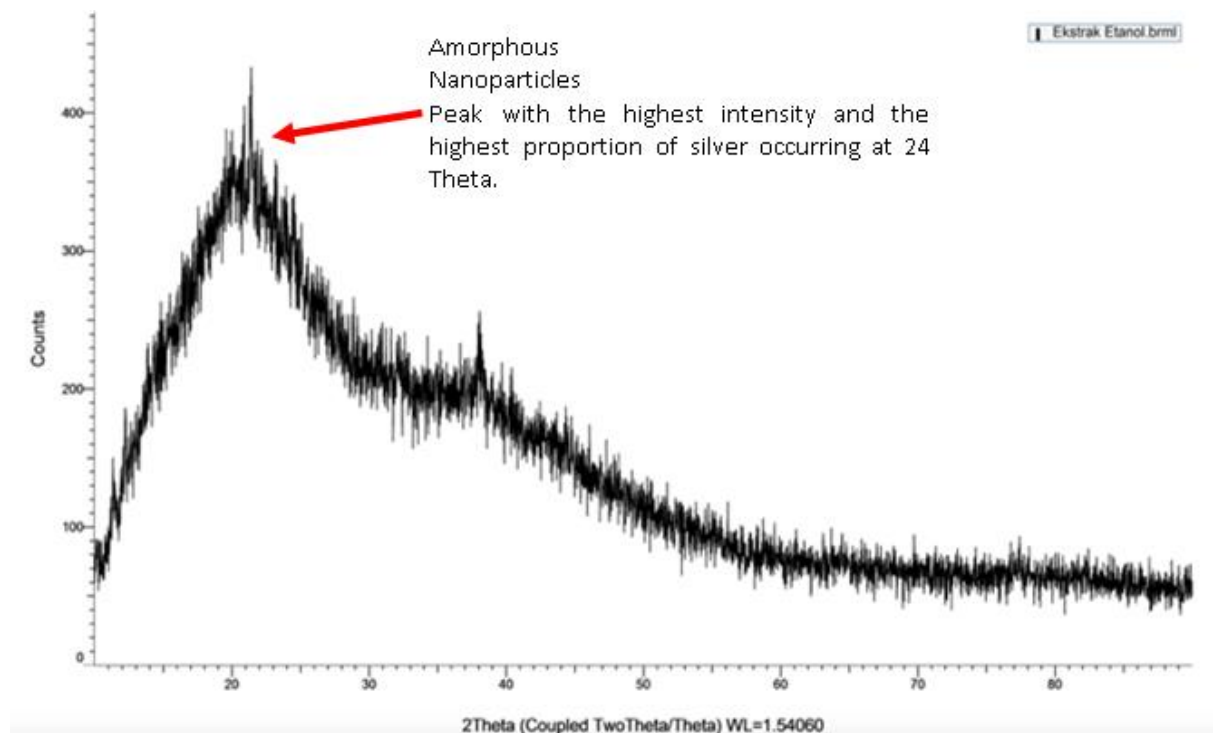


Figure 5. The X-ray Powder Diffractometry (XRD) pattern of the green synthesized silver nanoparticles mediated by *Eleutherine americana* (AgNPs); there was no characteristic peak which revealed the amorphous structure of nanoparticles

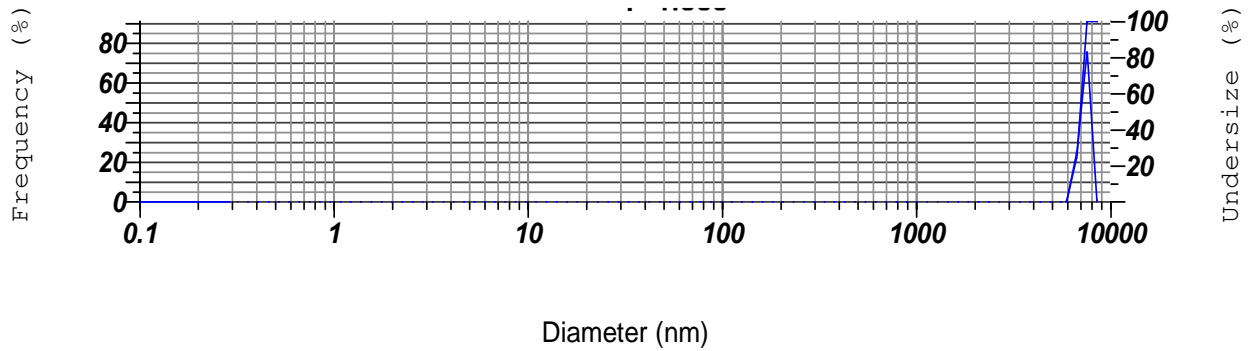


Figure 6. The particle size distribution curve of silver nanoparticles (Ea-AgNPs) complexes was obtained by particle size analyzer; the Ea-AgNPs were obtained from the combination of ethanolic extract of *Eleutherine americana* and 4 mM AgNO₃

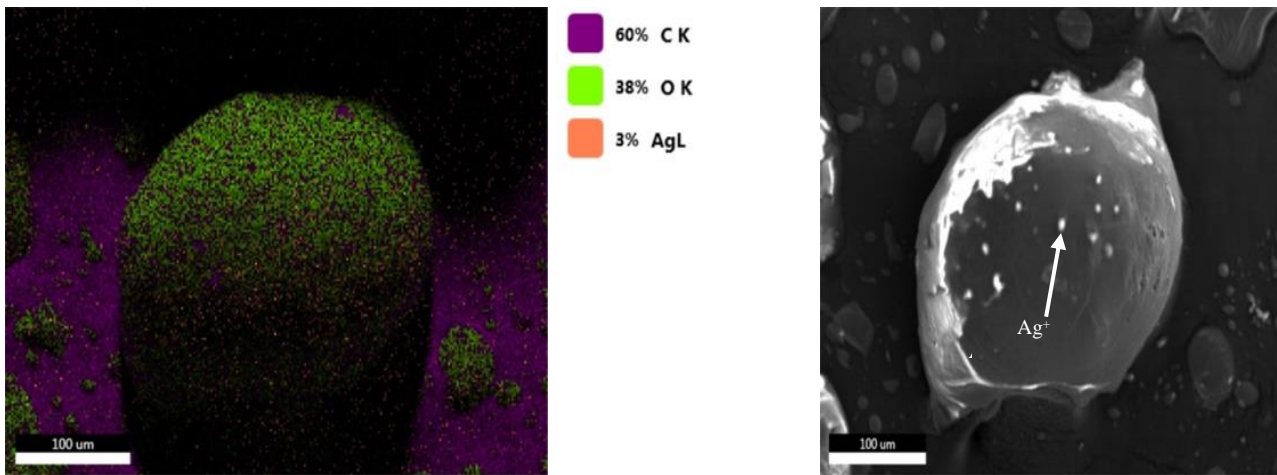


Figure 7. Scanning Electron Microscopy (SEM) images of the reduction of Ag⁺ to Silver nanoparticles

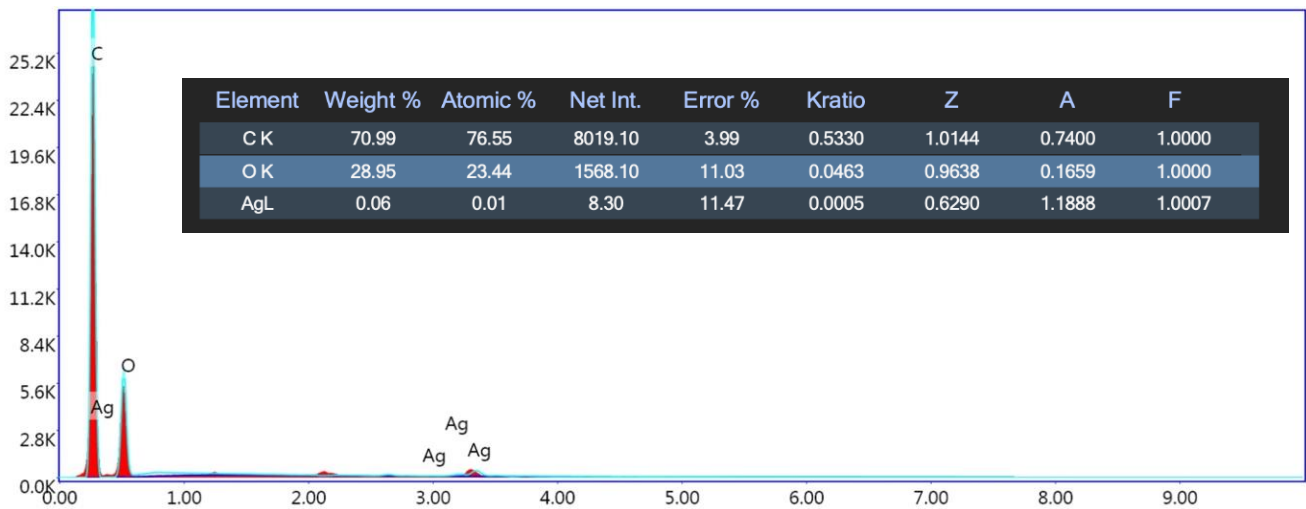


Figure 8. The Energy Dispersive X-ray (EDX) spectrum analysis of *Eleutherine americana* silver nanoparticle (Ea-AgNPs)

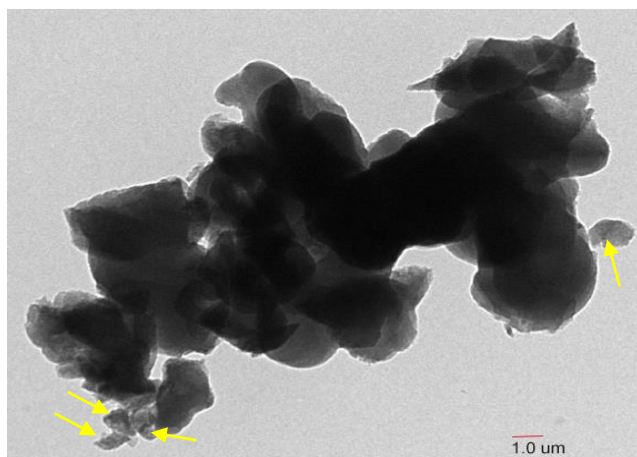


Figure 9. Transmission Electron Microscopy (TEM) micrographs of the green synthesized silver nanoparticles (AgNPs), mediated by *Eleutherine americana* bulb aqueous extract

Table 1. Ea-AgNPs phytochemicals content

Phytochemicals	Indicator
Alkaloid	+
Flavonoid	+
Phenolic	+
Saponin	-
Triterpenoid	-
Steroid	-
Tannin	+
Glycoside	-
Coumarin	+
Carotenoid	-

Note: (+) Present, (-) Absent

Table 2. Antioxidant activity

Antioxidant (%)	Sample concentration (ppm)
49.67685398	80
57.31063763	100
58.4287385	120
59.37676585	140
64.46053971	160
IC ₅₀	45.30 ppm

Plants are well-known to contain many phenolic and flavonoid compounds, which may have superior antioxidative properties and are regarded as potent free radical scavengers (Mustafa et al. 2010). The present IC₅₀ value showed that the Ea-AgNPs have significant antioxidant action. Large molecular weight values and the closeness of multiple aromatic rings and hydroxyl groups are crucial for bioactive compounds' free radical scavenging action (Hagerman et al. 1998). Further, Panneerselvam et al. (2011) demonstrated the in vitro antioxidant capacity of synthesized silver nanoparticles mediated by *Andrographis paniculata* (Acanthaceae) using a DPPH radical scavenging test, which corroborates the findings of our study.

In conclusion, this study demonstrates the successful synthesis of Ea-AgNPs using ethanolic extract of *E. americana* bulb. An optimum UV/VIS spectrum absorption peak at 400 nm was found, corresponding to the resulting Ea-AgNPs. The FTIR analysis revealed several important bio compounds, and XRD characterization found an amorphous Ea-AgNP peak at 24 Theta. Meanwhile, the particle size distribution curve of Ea-AgNPs showed a size of 10⁵ nm, with an optical absorption characteristic peak at 3 keV observed via SEM/EDX analysis. The EDX examination revealed three signals: a strong signal from C atoms (70.99 %) and signals from O atoms (28.95 %) and Ag atoms (0.06 %). The TEM imaging also showed the characteristics of Ea-AgNPs. Phytochemical screening tests identified the presence of some phytochemicals such as flavonoids, tannins, alkaloids, and saponins in the Ea-AgNPs and showed IC₅₀ values of 45.30 ppm.

ACKNOWLEDGEMENTS

The authors thank the Department of Veterinary and the Department of Biology, Universitas Mulawarman, Indonesia, for all their kind support. The authors are also grateful thankful the ministry of research and technology, research and innovation, Indonesia, for the financial funding (2021), through Hibah PTUPT contract number: (No. 297/E4.1/AK.04.PT/2021).

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