Original Article

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Heca Journal of Applied Sciences

Vol. 1, No. 2, 2023

Characterizing the Size Distribution of Silver Nanoparticles Biofabricated Using *Calotropis gigantea* from Geothermal Zone

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Article History

Received 30 May 2023
Revised 5 July 2023
Accepted 18 July 2023
Available Online 24 July 2023

Keywords:
Green synthesis
Metal nanoparticle
Nanoparticle size
Zeta potential analysis

Abstract

This research aims to synthesize silver nanoparticles (AgNPs) using an aqueous leaf extract of *Calotropis gigantea* obtained from the geothermal manifestation Ie Seu-Um, Aceh Besar, Aceh Province, Indonesia. The *C. gigantea* leaf extract was mixed with $AgNO_3$ solutions at concentrations of 2, 5, and 9 mM, respectively. The mixture was stirred at 80 rpm by a magnetic stirrer for 48 hours in the dark. The change in solution color indicated the reduction of Ag^+ to Ag^0. The resulting AgNPs synthesized using *C. gigantea* leaf extract (AgNPs-LCg) exhibited cloudy grey, reddish dark brown, and light brown colors when synthesized with $AgNO_3$ concentrations of 2, 5, and 9 mM, respectively. The particle sizes of AgNPs-LCg had maximum frequencies at 246.98 nm (synthesized using $AgNO_3$ 2 mM), 93.02 nm (synthesized using $AgNO_3$ 5 mM), and 171.25 nm (synthesized using AgNO3 9 mM). The zeta potential values of AgNPs-LCg using 2, 5, and 9 mM AgNO3 were -41.9, -40.1, and -31.4 mV, respectively. Based on the solution color, nanoparticle size, and stability value of AgNPs, it can be concluded that the use of $AgNO_3$ at 5 mM is optimal for the green synthesis process of AgNPs-LCg.

1. Introduction

AgNPs are a popular nanotechnology application [1–3]. AgNPs have developed as the most preferred option among other types of metal nanoparticles (such as Pd, Au, Cu, and Au) because of their ability to inhibit the growth of microorganisms [2, 4]. In addition to antibacterial properties, the principal applications of silver nanoparticles in the medical field include diagnostic and therapeutic applications [5].Various methods for producing metal nanoparticles include chemical, physical, and green synthesis. However, green synthesis is the most widely used than other methods since it is thought to be inexpensive, ecologically friendly, and low hazardous to eukaryotic cells [1, 2].

The green synthesis of metal nanoparticles is performed by reacting $AgNO_3$ with plant, bacterial, or fungal extracts over time and under specific conditions [1]. *Zingiber officinale*, *Nigella sativa*, *Plumbago auriculata, Hypericum perforatum, Amorphophallus paeoniifolius,* and *Tagetes erecta* were a few plants that have reportedly been used as AgNP bioreductors [6–10]. The leaf and flower *C. gigantea* extract have been utilized as a bioreductant for AgNPs [11–15].

Calotropis gigantea is a sap plant with thick leaves, white or purple flowers, and round fruit capsules [16]. This plant can survive both dry conditions and high concentrations of salt. The plant can rapidly spread its seeds through the air, causing a weed [17]. Several chemicals, including alkaloids, steroids, saponins, phenolics, and tannins, have been identified in leaf extracts of *C. gigantea* from the geothermal manifestation Ie Seu-Um. Alkaloids, terpenoids, flavonoids, tannins, phenolics, and saponins are present in its flower extract [15]. *C. gigantea* is a well-known Asclepiadaceae plant that has been used in traditional medicine for a long time [18]. Three distinct Indian tribes traditionally have used *C. gigantea* flowers to prevent seizures. Toxicology studies have shown that some parts of the plant (latex and fruit) contain toxins [19]. However, in-silico studies show that the leaves have anti-SARS-CoV-2 properties, suggesting that they could be developed as a drug against COVID-19 [20]. According to another study, the inside of the fruit of the *C. gigantea* plant contained fine fibers that have the potential to be used as a raw material for textile fibers due to their floatation (buoyancy) properties and oil spill absorbent [21].

The benefits of *C. gigantea* in the textile and medicinal industries described above depended significantly on its secondary metabolites. The diversity and quantities of secondary metabolites depend on where the plant grows and how it grows, such as the temperature, the soil quality, the intensity of sunlight, and the volume of water available [22–24]. The ability of plants to survive and adapt in severe environments, such as geothermal zones, will improve the uniqueness of the secondary metabolites produced [23]. Several studies have also been reported evaluating the benefits of *C. gigantea* from the geothermal manifestation area of Mount Seulawah Agam. The ethanol extract of *C. gigantea* (from the geothermal manifestation Ie Jue) has highly antibacterial activity against Gram-negative and Gram-positive bacteria [25]. Another study found that the leaves of *C. gigantea* (from the geothermal manifestation Ie Brouk) have antiviral influenza and anti-SARS-CoV-2 potential based on in silico evaluation [26].

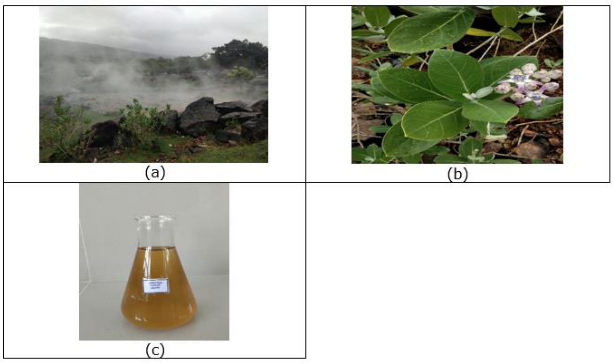


Figure 1. (a) The geothermal manifestation Ie Seu-Um; (b) Plants of *C. gigantea* growing in a geothermal environment; (c) The aquoeus leaf extract of *C. gigantea.*

In this study, silver nanoparticles (AgNPs) will be synthesized using *C. gigantea* growing in the geothermal manifestation Ie Seu-Um. It is known that the geothermal manifestation Ie Seu-um has a water temperature of 84.20 °C with a pH of 6.61 and even a significant concentration of chloride ions [16, 27] and the hot springs contain arsenic [28].

No specific investigations on the size distribution and stability of AgNP-leaves *C. gigantea* (AgNPs-LCg) from geothermal manifestation zones have been conducted. This study will investigate the connection between the concentration of $AgNO_3$ used and the size distribution of AgNPs-LCg.

2. Materials and Methods

2.1. Materials

Samples were collected in the close surroundings of the Ie Seu-Um hot spring (Figure 1a). The plant used was the leaves of *C. gigantea* (Figure 1b), growing in the geothermal manifestation Ie Seu-um. The metal precursor was $AgNO_3$ purchased at Sigma Aldrich (St. Louis, MO, USA). Distilled water was the solvent used to extract and produce the $AgNO_3$ solution.

2.2. Preparation of C. gigantea leaf extract

The preparation technique was carried out following [11, 29] in which *C. gigantea* leaves were taken and then washed to remove impurities. The leaf samples were cut into pieces and dried for several days at room temperature. The dried leaves were extracted by boiling them in 100 mL of distilled water for 20 minutes and filtering them through filter paper. The filtrate was kept at ±4 °C.

2.3. Biosynthesis of AgNPs-LCg and solution color observation

The biosynthesis of AgNPs-LCg using an aqueous extract of *C. gigantea* leaves was performed according to a previously established method [11, 14] with some modifications. 10 mL of *C. gigantea* leaf extract was mixed with 90 mL of varying concentrations of $AgNO_3$ solution (2, 5, and 9 mM). The reaction was performed in the dark condition and stirred at 80 rpm with a magnetic stirrer for 48 hours. Incubation was performed in a dark place to avoid photo activation reaction of silver nitrate. The AgNPs-LCg reaction solution was centrifuged (Nuve, model NF800R, Ankara, Turkey) at 13,000 × g for 10 min.

2.4. Particle Size and Zeta Potential Analysis

The characterization of the zeta potential value (mV) and nanoparticle size (nm) of the sample were analyzed using Zetasizer Nano (Horiba SZ-100, Horiba Mfg. Co., Ltd., Kyoto, Japan).

3. Results and Discussion

3.1. Plant extracted

The leaves of *C. gigantea* were extracted using a water-based solvent. Water was the most common solvent in plant extraction processes for AgNPs biosynthesis [13, 29–33]. Tannins and flavonoids were secondary metabolites reported as reducing agents of Ag [8, 34]. Secondary metabolites of plant in reducing Ag^+ to Ag^0 were compounds containing O-H and N-H [29, 35]. The two compounds had the characteristics of polar compounds that can be dissolved in water. The sample was boiled for 20 minutes, and then it was filtered to get a greenish-yellow *C. gigantea* leaf extract (Figure 1c).

3.2. Biosynthesis AgNPs-LCg

To produce AgNPs-LCg, $AgNO_3$ as a metal precursor was mixed with an aqueous extract of *C. gigantea* leaves [11, 15, 29]. The reduction from Ag^+ to Ag^0 was shown by the color solution change from colorless to yellow-brown [31, 32] or reddish brown [13, 30, 36]. The color of the solution formed before and after the reaction incubation process is shown in Figure 2. The mixture of $AgNO_3$ and *C. gigantea* extract at the beginning of the reaction was almost colorless. After incubation, the color of the solution changed depending on the concentration of $AgNO_3$ used. AgNPs-LCg synthesized using 2 mM $AgNO_3$ appeared cloudy grey. AgNPs-LCg solution synthesized using 5 mM $AgNO_3$ had a reddish dark brown color, whereas AgNPs-LCg synthesized with 9 mM $AgNO_3$ had a light brown solution. Based on the color observation of the solution, the concentration of $AgNO_3$ 5 mM was the best concentration to be used in the green synthesis AgNPs-LCg because the color indicates the success of the Ag^+ reduction reaction. The color change of the solution to brown during the green synthesis reaction of AgNPs was also reported using *Picea abies* L. [37], *Zingiber officinale* [10], *Nigella sativa* L. Seed [10], beech tree [38, 39], *Plumbago auriculata Lam* [7], and *Hypericum perforatum* [6].

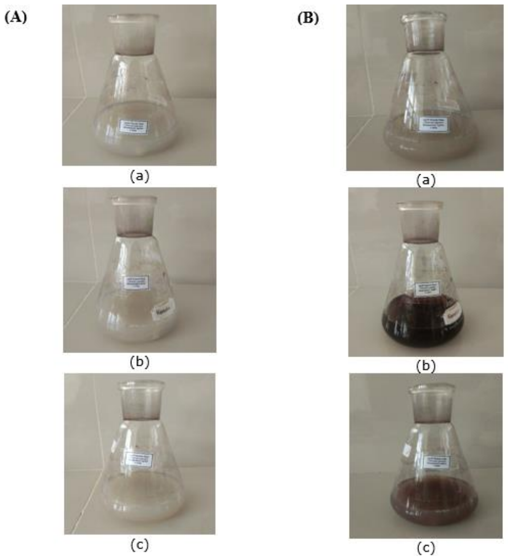
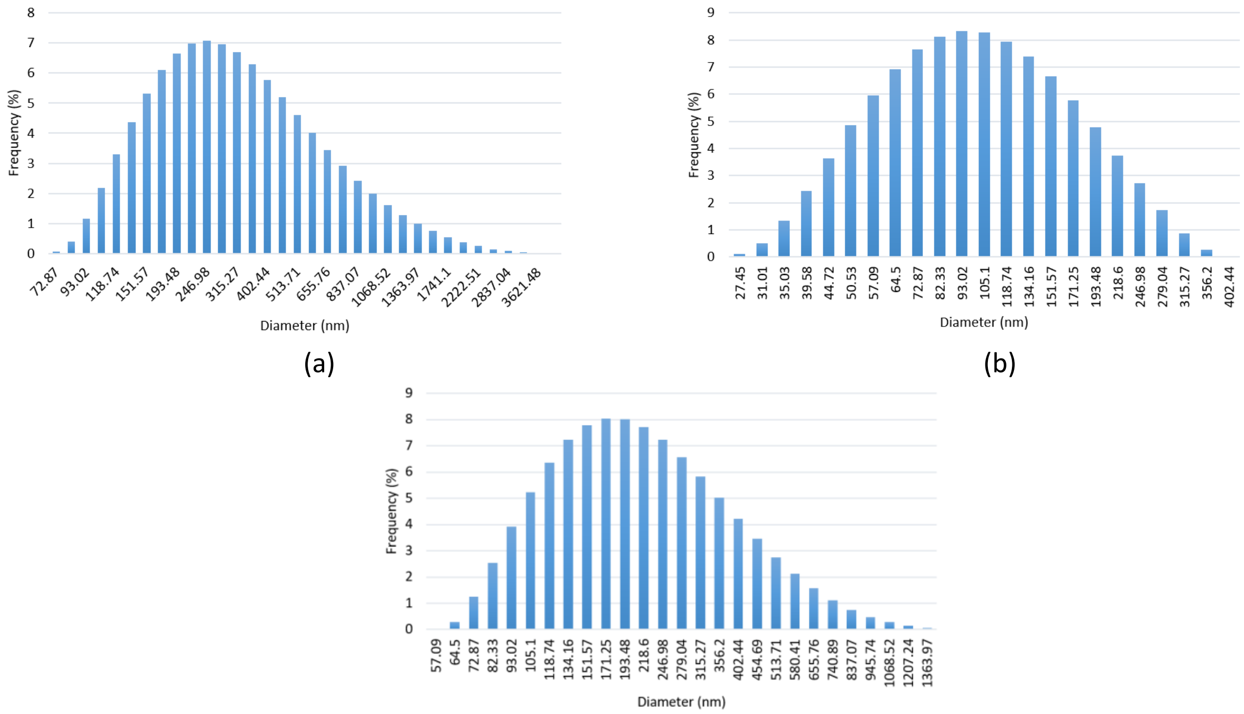


Figure 2. The color of the AgNP-LCg solution using (a) $AgNO_3$ 2 mM; (b) $AgNO_3$ 5 mM; and (c) $AgNO_3$ 9 mM; before (A) and after (B) 48-hour incubation.

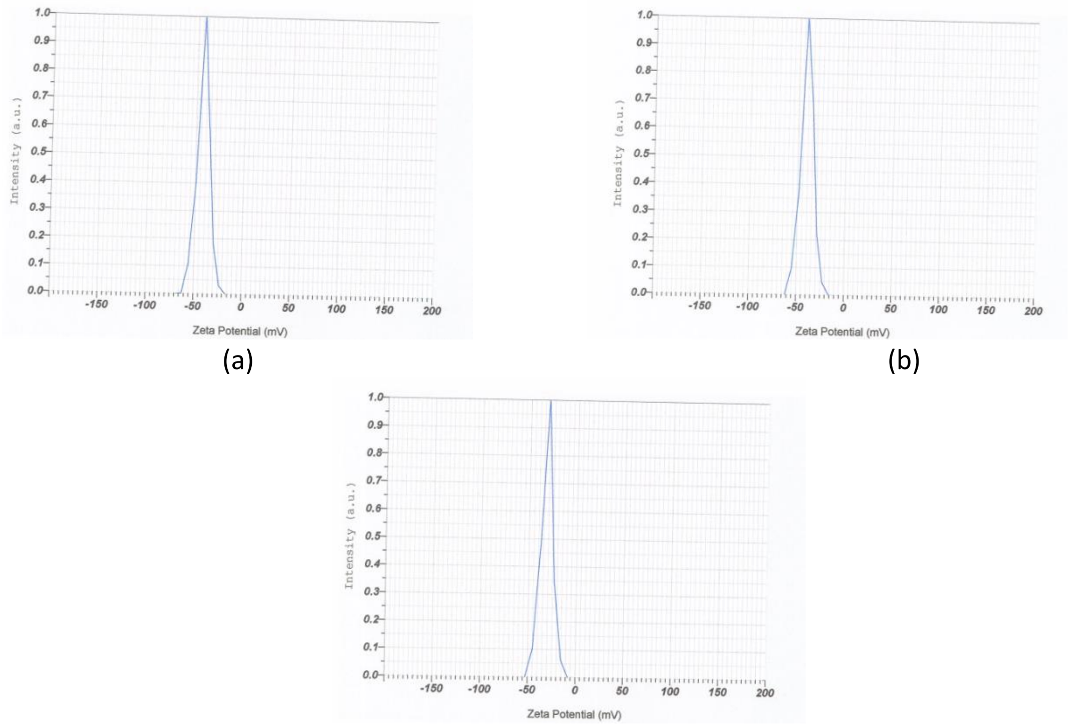
3.3. Nanoparticle Size and Zeta Potential Analysis

The synthesized AgNPs-LCg was continued with the characterization process. The purpose of characterization is to determine the physical and chemical properties of AgNPs-LCg, especially the nanoparticle size. A particle is counted in nano size at 1-100 nm [2, 35]. The size distribution of AgNPs-LCg with various concentrations of $AgNO_3$ used is presented in Figure 3. AgNPs-LCg synthesized using $AgNO_3$ 2 mM are not likely to form nanoparticles. The particles formed are mainly in size range at 193.48-402.44 nm. The sizes have been predicted because the color of the solution formed did not change significantly. The AgNPs-LCg that was synthesized using 5 mM $AgNO_3$ showed the nanoparticle size distribution becoming surprisingly good with a frequency maximum at 93.02 nm. $AgNO_3$ concentration of 5 mM is the recommended optimum concentration in this study. AgNPs-LCg synthesized using 9 mM $AgNO_3$ is known to have nano-sized particles with little frequency. The formation of AgNPs is a complex process. The size of AgNPs strongly depends on the concentration of Ag^0 in the solution, which directly correlates to the number of metal precursors available in the solution. However, the increased metal precursor concentration generally



(a) (b) (c)

Figure 3. Size distribution AgNPs-LCg synthesized using AgNO₃ (a) 2 mM; (b) 5 mM; and (c) 9 mM.



(a) (b) (c)

Figure 4. Zeta Potential of AgNPs-LCg synthesized using AgNO₃ (a) 2 mM; (b) 5 mM; and (c) 9 mM.

causes the bigger AgNPs size [2]. The concentration of $AgNO_3$ directly affects the formation process of AgNPs. A higher $AgNO_3$ concentration generally leads to more nucleation occurrences. The increase in growth causes more AgNPs to be produced and a faster rate of Ag^+ reduction reaction. However, the exact correlation between $AgNO_3$ concentration and AgNP formation can also be affected by the concentration of the extract. In this study, $AgNO_3$ with a concentration of 5 mM is the most appropriate condition to react with *C. gigantea* leaf extract with a ratio between reactants of 9:1.

The zeta potential test was carried out to evaluate the stability of AgNPs. Generally, the more negative the zeta potential value, the more stable the AgNPs are [31]. The zeta potential of silver nanoparticles ranged from very negative to highly positive, depending on the production technique and surface coating [40]. The zeta potential values of AgNPs-LCg obtained using 2, 5, and 9 mM $AgNO_3$ were -41.9; -40.1; and -31.4 mV, respectively. The results showed that the optimum particle stability value is obtained in the green synthesis reaction of AgNPs using 5 mM $AgNO_3$. This result indicates the effect of concentration on the stability of the synthesized AgNPs. The stability of AgNPs-LCg is presented in Figure 4. In general, the AgNPs formed are relatively stable compared to the AgNPs-*A. paniculata* (-21,4 mV), AgNPs-*P. niruri* (-20 mV), AgNPs-*T. cordifolia* (-17.0 mV), AgNPs-*M. parviflora* (0,412 mV), and AgNPs-*P. grandiflorum* [31, 32, 41].

4. Conclusions

This research focused on the size distribution and zeta potential analysis of AgNPS-LCg. AgNPs-LCg has been synthesized through the green synthesis reaction using *C. gigantea* leaf extract, which grows in the geothermal manifestation Ie Seu-Um, Mount Seulawah Agam, Aceh Province, Indonesia. The *C. gigantea* leaf extract can be used as a bioreductant of the Ag^+ to Ag^0 reaction, indicated by the color change in the solution after 48 hours of incubation. Based on the study, the concentration of $AgNO_3$ directly affects the formation process of AgNPs, especially nanoparticle size distribution and zeta potential value.

Author Contributions: Conceptualization, P.K., K.K., M.R. and R.I.; methodology, G.M.I, and P.K.; software, G.M.I.; validation, K.K., M.R., P.K., D.S.N and R.I.; formal analysis, P.K. and T.E.T.; investigation, P.K. and T.E.T.; resources, P.K.; data curation, P.K., D.S.N. and R.I.; writing—original draft preparation, P.K. and K.K.; writing—review and editing, P.K., M.R., T.E.T. and R.I.; visualization, P.K. and E.M.; supervision, K.K., M.R., and R.I.; project administration, R.I.; funding acquisition, R.I. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Universitas Syiah Kuala, Kementerian Pendidikan, Kebudayaan, Riset dan Teknologi Indonesia through “Program Riset Unggulan USK Percepatan Doktor” grant number 487/UN11/SPK/PNBP/2022.

Ethical Clearance: Not applicable

Informed Consent Statement: Not applicable.

Data Availability Statement: Data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgments: We thank Universitas Syiah Kuala and the LPPM at Universitas Syiah Kuala for their help and support.

Conflicts of Interest: The authors declare no conflict of interest.

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