Green synthesis of silver nanoparticles using *Calotropis gigantea* and its characterization using UV-Vis Spectroscopy

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**Abstract.** The use of *Calotropis gigantea* from Ie Seu-Um, Aceh Besar geothermal area for silver nanoparticles (AgNPs) synthesized has been reported. The process of synthesis AgNPs can be carried out with chemical methods, physical methods, and green synthesis methods, but in this study, the process is focused on the green synthesis method using *C. gigantea* flowers and leaves extract from Ie Seu-Um geothermal area, Aceh Besar. Phytochemical analysis showed that *C. gigantea* leaves contain alkaloids, steroids, tannins, phenolics, and saponins, while the flowers of *C. gigantea* contain alkaloids, terpenoids, steroids, flavonoids, tannins, phenolics, and saponins. The reaction of the AgNPs formation was observed by colour change formed. AgNPs-*C. gigantea* flower extract dan AgNPs-*C. gigantea* leaves extract showed the reddish-brown and brownish-yellow solution respectively after 48 h incubation in dark condition at room temperature. The result of the reaction characterized using UV-Vis Spectrophotometry showed that the phenomenon of *Surface plasmon resonance* (SPR) occurs in the mixture of nanoparticles formed. The concentration of AgNO₃ as a reagent affected the SPR phenomenon. The result showed that particles formed are the same size and shape.

1. **Introduction**

Nanotechnology is an important field of modern research today. In the nanoparticle size range 1-100 nm, all physical properties and changes in atoms or molecules can be utilized more effectively and efficiently [1]. Nanotechnology is referred to metal nanoparticles. The use of nanoparticles increases because of their unique properties and better physical and chemical characteristics than ordinary sizes. Several studies have reported that nanoparticles through cell membranes and interact with cell biological systems with high oxidative activity. However, the widespread use of nanoparticles has risks to human health and the environment [2]. The application of metal nanoparticles as nanotechnology in biomedical therapy has attracted the attention of many researchers. Some of them are the use of silver nanoparticles synthesized using plants as antibacterial [3–6], antifungal [7,8], anticancer [9], antioxidant [6], and antiviral [10,11].
Silver nanoparticles (AgNPs) are the main focus of metal nanoparticles because AgNPs have the highest thermal and electrical conductivity and also have low boiling and melting points. AgNPs are known to be the most reactive metal among other metals and are used as agents of antimicrobial [12]. Metal nanoparticles can be synthesized in several ways, including using physical methods, chemical methods, and green synthesis methods. The green synthesis method is a popular method because it is environmentally friendly and relatively inexpensive compared to other methods [7]. This method is performed by using protein or secondary metabolite as an important agent in metal reduction reaction [Ahmed, 2016].

In Indonesia, the secondary metabolite is highly abundant in its biodiversity [13,14]. It is easily found in the plant, produced as a metabolite by itself, and stored in its anatomies like a leaf, root, bark [15–17], stem, flower, and fruit [18–20]. Other sources are endophytes, bacteria [21], or fungus [22]. The use of secondary metabolites in nanoparticle preparation has several advantages, including producing metal nanoparticles with lower toxicity for eukaryotic cells than others, because the secondary metabolites have functioned as a capping agent to neutralize metals (Ag²⁺→Ag³⁺) [12].

Some of the plants that have been reported in the AgNPs green synthesis process are Lampranthus coccineus and Malephora lutea [10], Andrographys paniculata, Phyllanthus niruri and Tinospora cordifolia [11], Calotropis gigantea [4,7], Platycodon grandiflorum [5], Memecylon umbellatum Burm [6], and Malva parviflora [8]. This study explores the use of plants that live in a geothermal area. Some research showed that the secondary metabolite content of plants taken from geothermal areas is higher in species diversity and yields than plants from ordinary areas [23–26]. In Aceh Province, there are several areas that have geothermal potential due to volcanic activity [27–29], one of which is Seulawah Agam Mountain in Aceh Besar District [30–32]. This mountain has several manifestations in several separate locations, namely the manifestation of Ie Brouk [33,34], Ie Seu-Um [35,36], and Ie Jue [37,38]. Lots of mineral-rich water in each of these manifestations spread to the surrounding environment so that it affects the mineral composition of the soil to the biosynthesis of plants that grow in that area [33].

C. gigantea from Ie Seu-Um geothermal manifestation area, Aceh Besar, is the plant chosen in this study because the plant extract has high gram-positive and gram-negative antibacterial activity [35]. The plant was more fertile in the Ie Seu-Um geothermal manifestation area than other geothermal manifestation areas such as Ie Brok and Ie Jue. In addition, the use of C. gigantea flower extract has been used as a bioreductant of silver nanoparticles with antibacterial activity [4], and its leaf extract has been reported for the synthesis of silver nanoparticles with antifungal activity [7]. However, none of the research has been found on the synthesis of silver nanoparticles using C. gigantea plants which grow in geothermal manifestation areas.

2. Materials and methods

2.1. Collection and extraction of C. gigantea

Samples of C. gigantea flowers and leaves were taken from Ie Seu-Um, Aceh Besar geothermal manifestation area. The sampling locations at N05°32’50.97”, E095°32’55.10’ with an altitude of 97 m above sea level. The samples were separated between flowers and leaves, then washed with water flow and cut into small pieces to be dried in shade condition for several days. Sample extraction was carried out in different ways for flowers and leaves. The process of extracting C. gigantea leaves follows a reported procedure [7], whereas the dried leaf samples 10 g were taken to be boiled with 100 mL of distilled water for 20 minutes and filtered using filter paper. The dried C. gigantea flowers were put in 200 mL of distilled water while stirring at room temperature for 15 minutes and filtered [4]. Extract of flowers and leaves C. gigantea can only be stored at 4°C for a week.
2.2. Phytochemical Analysis
Phytochemical analysis was carried out to test the content of alkaloids, saponins, phenolics, tannins, flavonoids, steroids, and terpenoids in flowers and leaves of *C. gigantea*. The phytochemical screening followed standardized methods [39].

2.3. Green Synthesis AgNPs
The process of synthesizing silver nanoparticles followed the method reported by [40] in which 90 mL of AgNO₃ (2 mM and 9 mM respectively) was mixed with 10 mL of *C. gigantea* leaves and flowers extracted separately. The reaction was incubated at room temperature and kept under constant stirring using a magnetic stirrer 60 rpm for 48 hours in dark conditions. The colour formed of the reaction was observed. The reaction was centrifuged and characterized by UV-Vis spectrophotometry.

2.4. Characterization AgNPs Using UV-Vis Spectrophotometry
The formation of silver nanoparticles (AgNPs) is characterized by discolouration that occurs at the time of incubation. Each mixture of AgNPs reaction with leaves and flowers of *C. gigantea* was measured at a wavelength range of 200-600 nm.

3. Results and discussion

3.1. Plant Extraction
*C. gigantea* leaves and flowers extract from Ie Seu-Um, Aceh Besar geothermal area was washed first to remove impurities. These samples were cut into small pieces to expand the sample surfaces so the compounds of the samples could come out easily when water was added. The synthesis of AgNPs using plant extracts was reported to use water as solvent [4–7,10,11,41]. But the process of the leaves extraction was used at high temperature while the flowers extraction was not. The result of extraction obtained a pink solution of *C. gigantea* flower extract and a dark yellow solution of *C. gigantea* leaves extract.

3.2. Phytochemical Analysis
*C. gigantea* is a gummy plant that is found at Ie Seu-Um Outflow Geothermal Area, Aceh Besar [35], and almost all locations in Aceh. The plant is drought and salt tolerant. It can grow quickly as a weed because it can spread seeds by the wind [42]. The bioactive compounds in the genus Calotropis used in
medicine include cardenolides in leaves; flavonols in Arial; proteinase, triterpene ester and cysteine proteinase in the sap; triterpenoids in flowers; cardiac glycosides, terpenes, aromatic products (essential oils), cardenolides, sterols, and the non-protein amino acid at the root [43]. Analysis of phytochemicals on the leaves, stems, and flowers of *C. gigantea* from the normal environment showed that the plant contains saponins, tannins, alkaloids, glycosides, flavonoids, cyclicides, and steroids [44].

*C. gigantea* from Ie Seu-Um, Aceh Besar geothermal manifestation area was tested for phytochemicals to find out the secondary metabolites in the plant. There is a difference in the content of secondary metabolites between the flowers and the leaves of the plant. Table 1 shows a comparison of the phytochemical *C. gigantea* from Ie Seu-Um, Aceh Besar geothermal manifestation area.

**Table 1.** Phytochemical screening analysis of *C. gigantea* flowers and leaves extract from Ie Seu-Um, Aceh Besar geothermal area.

|                  | Flowers | Leaves |
|------------------|---------|--------|
| Alkaloids        | +       | +      |
| Terpenoids       | +       | -      |
| Steroids         | -       | +      |
| Flavonoids       | +       | -      |
| Tannins          | +       | +      |
| Phenolics        | +       | +      |
| Saponins         | +       | +      |

3.3. Green Synthesis AgNPs

The silver nanoparticle (AgNPs) synthesis reaction was carried out using two samples: flower extract and leaves extract of *C. gigantea*. The reaction is carried out in a dark condition to avoid the photoactivation reaction of silver nitrate [11]. An indication of AgNPs formation is the occurrence of discoloration in the solution. After some incubation time, it was reported that the AgNPs solution has discoloration to brownish-yellow [5,11] or reddish-brown [4,8,10]. The discoloration is an indication that Ag⁺ has been reduced to AgNPs in the solution [11].

The study showed a change in the colour of the solution after 48 h of incubation. A pink solution of *C. gigantea* flowers extract will form a reddish-brown solution after mixing AgNO₃. A dark yellow solution of *C. gigantea* leaves extract will form brownish yellow after reacting with AgNO₃. Based on the results, it can be concluded that silver nanoparticles synthesized using *C. gigantea* from Ie Seu-Um, Aceh Besar geothermal area have been formed.

![Figure 2](image_url)

**Figure 2.** (a) *C. gigantea* flowers extract; (b) AgNP- *C. gigantea* flowers extract; (c) *C. gigantea* leaves extract; (d) AgNP- *C. gigantea* leaves extract.
3.4. Characterization UV-Vis Spectrophotometry

UV-Vis characterization is one of the common characterization performed to test AgNPs formed. Previously, it has been discussed that all synthesis processes of AgNPs have discoloration to brownish yellow or reddish-brown. The presence of chromophores in the organic components affects the visible light while UV-Vis light absorption extinction of metal AgNPs is connected to Localized Surface Plasmon Resonance (LSPR) appearance. LSPR is a complex process and describes excitation and coherent electron oscillation under an electromagnetic field of incident light. The complexity of the LSPR phenomenon implies the influence volume, composition, structure, shape, and also an aggregation of AgNPs [12]. Alsalhi et al. (2019) explained when plant extract is mixed with silver nitrate solution, SPR dominates and changes the electron density. SPR spectrum is also known as making redshift because of increasing temperature. This is presumably because the heating during the reaction process will cause AgNPs to agglomerate and become larger particles [5].

![Figure 3](image1.png)

**Figure 3.** Characterization UV-Vis AgNPs using AgNO₃ 9 mM as a reagent (a) AgNP- *C. gigantea* leaves extract; (b) AgNP- *C. gigantea* flowers extract.

![Figure 4](image2.png)

**Figure 4.** Characterization UV-Vis AgNPs using AgNO₃ 2 mM as a reagent (a) AgNP- *C. gigantea* leaves extract; (b) AgNP- *C. gigantea* flowers extract.

Figures 3 and 4 showed that AgNO₃ concentration affected the SPR phenomenon. When using AgNO₃ 9 mM as a reagent in AgNPs synthesis reaction, AgNP- *C. gigantea* flowers extract with SPR peak at 282 nm, whereas AgNP- *C. gigantea* leaves extract with SPR peak at 264 nm. It is different from
using AgNO$_3$ 2 mM as a reagent, AgNP-C. gigantea leaves extract showed an SPR peak at 256 nm, while AgNP-C. gigantea flowers extract showed an SPR peak at 260 nm. The broadening peaks in the UV-Vis spectra indicate that silver nanoparticles formed are polydispersed in nature due to the slow reduction of silver ions. In addition, none of the AgNPs reactions showed a maximum absorption of SPR above 500, so that indicates that the particle formed have the same size and shape [11].

4. Conclusions
Silver nanoparticles (AgNPs) have been synthesized using C. gigantea flowers and leaves extracts from Ie Seu-Um manifestation geothermal area, Aceh Besar. The reaction was observed by a colour change after 48 h incubation. The end of the reaction showed that the silver nanoparticle formed had brownish yellow and reddish-brown solution. Phytochemical analysis showed that AgNP-C. gigantea leaves extract contains alkaloids, steroids, tannins, phenolics dan saponins. However, AgNP-C. gigantea flowers extract contains alkaloids, terpenoids, steroids, flavonoids, tannins, phenolics, and saponins. The concentration of AgNO$_3$ as a reagent affects the UV-Vis characterization.

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