ORIGINAL ARTICLE

Green synthesis of gold nanoparticles from the aqueous extracts of *Sphagneticola trilobata* (L.) J.F Pruski as anti-breast cancer agents

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ABSTRACT

The invasive plant, *Sphagneticola trilobata* (L.) J. F. Pruski, has been known for its bioactivities and used to synthesize gold nanoparticles (AuNPs). Nonetheless, previous research has not directly compared the effectiveness of the plant parts in producing the AuNPs. The objective of this study was to compare the effectiveness of the flower and leaf of *S. trilobata* in synthesizing AuNPs. *S. trilobata* leaves and flowers were separately extracted using distilled water at 60°C for 30 min. The leaf and flower extracts were mixed with the HAuCl. $3H_2O$ and heated to 60°C for 30 min to yield AuNPs-ALSt and AuNPs-AFSt, respectively. AuNPs were also prepared using trisodium citrate ($Na_3C_6H_5O_7$) as a control. The resultant AuNPs were characterized using an ultraviolet-visible spectrophotometer, particle size analyzer, and scanning electron microscope. Antioxidant activity was evaluated based on 1-diphenyl-2-picrylhydrazyl (DPPH) inhibition and anticancer activity– 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide assay against MCF-7 cells. The AuNPs-ALSt and AuNPs-AFSt were revealed to have better stability and smaller particle diameters. AuNPs-ALSt and AuNPs-AFSt had average particle diameters of 11.86 ± 3.37 and 34.86 ± 23.56 nm, respectively. Agglomeration was predominantly observed in AuNPs synthesized using the flower or leaf extract as stipulated to be affected by the insufficient capping agent and intense hydrolytic reaction. AuNPs-AFSt had higher DPPH antioxidant activity than AuNPs-ALSt with half-maximal inhibitory concentrations of IC_{50} 123.44 and 168.83 ppm, respectively. Both AuNPs-ALSt and AuNPs-AFSt could inhibit 80% growth of the MCF-7; however, at lower concentrations, inhibitory effects were more pronounced in AuNPs-AFSt. Aqueous extracts of *S. trilobata* flowers and leaves could be used to synthesize AuNPs, whereas the former yielded AuNPs with higher biological activities.

Key words: Gold nanoparticle, green synthesis, invasive plant species, MCF-7, *Sphagneticola trilobata*

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INTRODUCTION

Sphagneticola trilobata (L.) J. F. Pruski has been reported in some studies to have therapeutic benefits such as anti-inflammatory, antioxidant, anticonvulsant, antiviral,

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antifungal, antibacterial, and anti-cancer properties.[1,2] In traditional medicine, the genus *Sphagneticola* has been used to treat wounds, ulcers, vaginal discharge, and gonorrhea.[2] Previous studies found that *S. trilobata* extract was active as an antioxidant, antibacterial, and cytotoxic agent.[3,4]

Previously, silver nanoparticles (AgNPs) with antibacterial activities had been successfully synthesized using an environmentally friendly approach based on *S. trilobata* flower extract.[5] *S. trilobata*-mediated synthesis of silver nanoparticles was reported to yield prominent antibacterial activity.[6] *S. trilobata* leaf extract had also been used as a bio-reductor for the AgNPs synthesis, where the nanoparticles were active as antibacterial and antioxidant agents with cytotoxic activity against Hep G2 hepatocellular carcinoma cancer cells.[7] In terms of gold nanoparticles (AuNPs), the synthesis was carried out using *Wedelia trilobata* extract.[8] The resultant AuNPs were found active in inhibiting the growth of HCT-15 colon cancer cells.[8]

Although there has been extensive study on this plant potential as a bio-reductant, none of the reported studies have investigated a direct comparison between the leaf and flower. Therefore, this study aimed to compare the AuNPs synthesized using the leaf and flower of *S. trilobata* extracts. The activity against MCF-7 breast cancer cell lines was also investigated. It is worth noting that breast cancer is the predominant cases of all other cancers in Indonesia.[9] This comparative analysis is expected to contribute significantly to determining which plant part of *S. trilobata* is the most effective bio-reductant.

MATERIALS AND METHODS

Materials

Materials used in this study included $HAuCl_4.3H_2O$, trisodium citrate ($Na_3C_6H_5O_7$), ascorbic acid, 1-diphenyl-2-picrylhydrazyl (DPPH), dimethyl sulfoxide, sulfuric acid, chloroform, methanol ethanol, phosphate-buffered saline (PBS), Dulbecco's Modified Eagle Medium, and 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT). All materials were procured from Sigma-Aldrich with analytical grades. The plant sample, namely, *S. trilobata* (L.) J. F Pruski, was collected in Langsa, Aceh, Indonesia.

Methods

Extraction of Sphagneticola trilobata leaves and flowers

The collected samples were air-dried for 10 days at room temperature. Thereafter, the sample (10 g) was immersed in distilled water (100 mL) and heated to 60°C for 30 min. The filtrate was collected and stored at 4°C until further use. Each extract was subjected to qualitative phytochemical screenings for the presence of alkaloids, saponins, steroids, phenols, flavonoids, and tannins, following the procedure in previous reports.[8,10]

Chemical and green synthesis of gold nanoparticle

The precursor solution was firstly prepared by diluting $HAuCl.3H_2O$ (0.5 g) into distilled water until the concentration reached 394 ppm, as suggested by a previous study.[11] AuNPs were chemically synthesized by adding a 100 mL (10^5 ppm) solution of $Na_3C_6H_5O_7$ to a 500 mL precursor solution. The color change was observed per milliliter of dropped $Na_3C_6H_5O_7$ solution. AuNPs obtained from using $Na_3C_6H_5O_7$ were denoted as AuNPs-Citrate. Similarly, in the green synthesis, 10 mL of each *S. trilobata* leaf and flower extract was added to 100 mL precursor solution, respectively. Each mixture was heated to 60°C for 30 min before being centrifuged at 6000 rpm for 15 min. The resultant AuNPs were air-dried at room temperature. The AuNPs obtained from the leaf and flower extracts were denoted as AuNPs-ALSt and AuNPs-AFSt, respectively.

Characterization of gold nanoparticles

Apparent changes of color due to the formation of nanoparticles were observed visually and photographed. The maximum wavelength and particle size were determined by an Orion AquaMate 8100 ultraviolet-visible (UV-Vis) spectrophotometer and particle size analyzer (PSA) (Zetasizer Nano ZS, Malvern). The morphology was characterized by Jeol JSM-6510 LA scanning electron microscope (SEM).

1-diphenyl-2-picrylhydrazyl assay

Antioxidant activity determination of the samples was carried out using the DPPH assay in accordance with the suggestion from a previous study.[4] The leaf and flower extracts were prepared with a concentration variation ranging from 25 to 400 ppm, respectively. DPPH powder was dissolved in methanol until the concentration reached 0.4 mM, and 1 mL of the solution was further added to a test tube containing 5 mL extract in the respective concentration. After 30-min incubation at room temperature, the mixture was measured for its UV-Vis absorbance at 517 nm.

3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide assay

The resultant AuNPs were tested for its activity in inhibiting MCF-7 breast adenocarcinoma cells using the MTT assay based on protocols reported previously.[12] The vehicle for both control and AuNPs-exposed cells was PBS. Cells were cultured in a RPMI-1640 medium (10 mL) and the number of cells in each well was kept under 5000 cells/100 µL media. The AuNPs with respective concentrations were added to the cell culture and incubated for 48 h (37°C; 5% CO_2). The MTT was added to the cell culture before re-incubated for another 4 h (37°C; 5% CO_2) and subsequently added with ethanol-dissolved formazan crystal for optical density reading at 565 nm using an ELISA microplate reader.

RESULTS AND DISCUSSIONS

Chemical and green synthesis of gold nanoparticles

Apparent changes of color of the precursor solution indicating the formation of AuNPs are presented in Figure 1. For example, the clear-yellow precursor solution when reacted with $Na_3C_6H_5O_7$ resulted in a color change into clear gray. Meanwhile, nanoparticle synthesis using the *S. trilobata* extracts resulted in dark purple solutions. The apparent color changes observed in this present study are similar to those reported previously.[8] In another study using a different plant extract (*Camellia sinensis*), the color changed into a reddish tinge.[13] The reduction reaction from Au^3 + to Au^0 is the basic principle of AuNP synthesis using $HAuCl_4$ as the precursor.[14]

Ultraviolet-visible spectra and phytochemical profile of gold nanoparticles

The UV-Vis spectra of AuNPs citrate, AuNPs-ALSt, and AuNPs-AFSt are presented in Figure 2a-c. The formation of surface plasmon resonance by the nanoparticle can be clearly observed at 300 nm for AuNPs-Citrate. As for the AuNPs-ALSt and AuNPs-AFSt, the spectral peak was broadened (300-650 nm), probably caused by the different formation of AuNPs species and the presence of secondary metabolites in the extract. The spectral peaks for the surface plasmon resonance of the AuNPs were similar to those reported previously, ranging from 350 to 600 nm.[8,11,13,15]

Herein, the observation of the UV-Vis spectra was performed for 7 days to observe the stability of AuNPs formation. We found that the plant extract could stabilize the AuNPs even until the seventh day, which was not observed in AuNPs produced by the chemical route using $Na_3C_6H_5O_7$. This finding is in line with a previous study suggesting the secondary metabolites in the extract could act as nanoparticle stabilizers.[16] Secondary metabolites contributing to the stability effects include alkaloids, triterpenoids, tannins, and flavonoids.[17] In the present

Figure 1: Gold nanoparticle solutions using trisodium citrate (a), aqueous extract of *Sphagneticola trilobata* leaves (b), aqueous extract of *Sphagneticola trilobata* flowers (c). AuNPs: Gold nanoparticles, *S. trilobata*: *Sphagneticola trilobata*

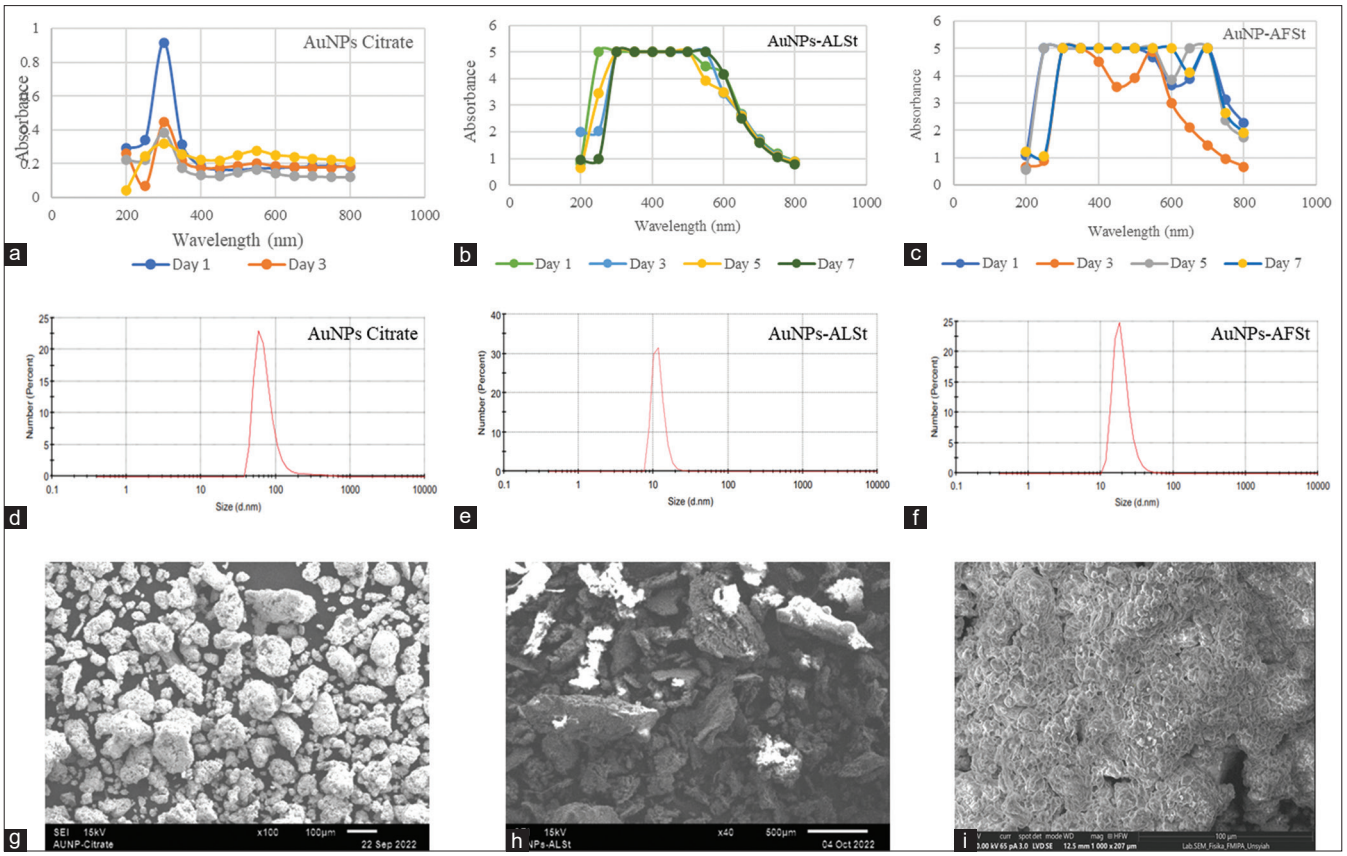

Figure 2: Ultraviolet-visible spectra (a-c) and size distribution (d-f) of gold nanoparticles (AuNPs)-citrate, AuNPs-ALSt, and AuNPs-AFSt. SEM images of AuNPs produced through chemical (g) and green synthesis (h and i) routes. AuNPs: Gold nanoparticles

study, those secondary metabolites were observed qualitatively in both the leaf and flower extracts [Table 1].

Particle size of gold nanoparticles

The particle size of AuNPs and its distribution are presented in Figure 2d-f. The average diameters were 77.51 ± 17.50, 11.86 ± 3.37, and 34.86 ± 23.56 nm for AuNPs-Citrate, AuNPs-ALSt, and AuNPs-AFSt, respectively. The smallest diameter was observed in AuNPs-ALSt, followed by the AuNPs-AFSt. In a previous study using *Muntingia calabura* leaf extract to synthesize AuNPs, the average diameter size was 78.2 nm.[18]

Surface morphology of gold nanoparticles

Surface morphologies of AuNPs retrieved from SEM analysis are presented in Figure 2g-i. The findings suggest the presence of high agglomeration and poly-dispersed particles, especially observed in AuNPs synthesized through green method (AuNPs-ALSt and AuNPs-AFSt). The agglomeration was attributed to an insufficient number of capping agents on the particle surface. In addition, the ratio of precursor solution to reductor or extract influenced the formation of aggregates. An increased incubation time and gold ion concentration intensify nucleation and agglomeration. Nanoparticle synthesis in a polar solvent like distilled water also induces aggregation by hydrolytic reaction.[7,19] Aggregation of AuNPs from plant extract was also reported in previous studies.[7,19,20]

Table 1: Qualitative phytochemical analysis of the chemical and green approach of gold nanoparticle synthesis

Secondary metabolites	AuNPs-citrate	AuNPs-ALSt	AuNPs-AFSt
Alkaloids			
Dragendorff	–	–	–
Mayer	–	–	–
Wagner	–	+++	+++
Steroids	–	–	–
Phenols	–	++	+++
Triterpenoids	–	+++	+++
Tannins	–	+++	++
Saponins	–	+	+++
Flavonoids	–	+	+

–: Absence, +: Presence, AuNPs: Gold nanoparticles

DPPH antioxidant activity of gold nanoparticles

The antioxidant activities of AuNPs are expressed as half-maximal inhibitory concentration (IC_{50}) presented in Table 2. The IC_{50s}, in ascending order, were 123.44, 168.83, and 253.74 ppm for AuNPs-AFSt, AuNP-Citrate, and AuNPs-ALSt, respectively. Hence, AuNPs-AFSt has the

Table 2: The inhibitory concentration 50s for the diphenyl-2-picrylhydrazyl antioxidant activities of the resultant gold nanoparticles

Sample	Equation	R^2	IC_{50} (ppm)
AuNPs-citrate	Y=0.0003x+0.6488	0.769	168.83
AuNPs-ALSt	Y=0.0002x+0.7408	0.746	253.74
AuNPs-AFSt	Y=0.0004x+0.626	0.730	123.44

AuNPs: Gold nanoparticles, IC_{50}: Inhibitory concentration 50

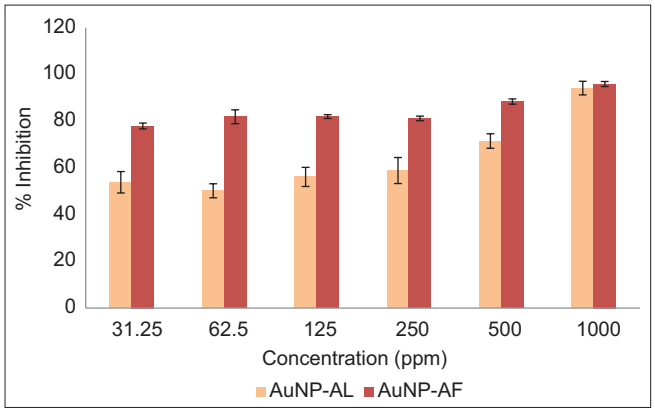

Figure 3: Inhibition activities of the AuNPs-ALSt and AuNPs-AFSt against MCF-7. AuNPs: Gold nanoparticles

highest antioxidant activity. As the IC_{50} fell within the range of 101-250 ppm, the antioxidant activities were considered moderate.[3] Previously, an IC_{50} of 1900 ppm was obtained for AuNPs synthesized through the green route.[11] This suggests that the AuNPS resulted herein is better compared to those obtained in a previous study.[11] The presence of bioactive phytoconstituents in the plant extract has been attributed to this antioxidant activity.[21]

Anticancer activity of gold nanoparticles

The anticancer potential of the resultant AuNPs herein was evaluated using MTT assay against MCF-7 cell lines, where the results are presented in Figure 3. Significant cytotoxicity against the cell lines was immediately observed even when the concentration was 31.25 ppm for both AuNPs-ALSt and AuNPs-ALFt, although the latter had higher inhibition activity. Higher inhibitions were always observed for AuNPs-ALFt when the concentration ranged from 31.25 to 500 ppm compared to AuNPs-ALSt. At 1000 ppm, however, both AuNPs-ALSt and AuNPs-ALFt equally contributed to 80% mortality. Similar anticancer activity of green-route-synthesized AuNPs had been witnessed in previous studies against HCT-15 human colon cell cancer.[10]

CONCLUSIONS

AuNPs had been successfully synthesized using leaf and flower extracts of *S. trilobata*. AuNPs obtained through the green route had better stability, smaller particle size, and higher antioxidant activity compared to those obtained chemically ($Na_3C_6H_5O_7$). Both flower and leaf extracts of *S. trilobata* had apparently similar stabilizing effects toward the AuNPs. In terms of particle size, smaller diameter average was obtained when the leaf extract was used. In terms of antioxidant and MCF-7 inhibitory activities, AuNPs obtained using the flower extract performed better. Therefore, when the aim was biological activity, AuNPs synthesized from the aqueous extract of the *S. trilobata* flowers are better than that of *S. trilobata* leaves. Confirmation through *in vivo* studies and multiple *in vitro* assays is still required to yield solid conclusions.

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Conflicts of interest

There are no conflicts of interest.

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