Characteristics and antibacterial activity of green synthesized silver nanoparticles using red spinach \((\textit{Amaranthus Tricolor} \ \text{L.})\) leaf extract

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ABSTRACT
In this study, the green synthesis of silver nanoparticles is conducted using red spinach \((\textit{Amaranthus tricolor})\) leaf extract. This study focuses on the synthesis optimization by investigating the effect of red spinach leaf extract volume percentage on the characteristics of silver nanoparticle AgNPs size distribution and antibacterial activity for \(\text{Escherichia coli}\). AgNPs characterization was performed by using UV-Visible spectrophotometer, particle size analysis, transmission electron microscope, energy dispersive-x-ray spectrometry, Fourier-transform infrared spectrometer and x-ray diffraction techniques. The experimental results indicate that AgNPs were successfully synthesized, and the particle size is affected by red spinach \((A. \ tricolor)\) leaf extract volume percentage. At the range of 2–20%, the lower volume percentage produces AgNPs with uniform size (40.45 nm, polydispersity index = 0.063 nm) and spherical form. The smallest size of AgNPs demonstrates the highest antibacterial activity.

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Introduction

Green synthesis of nanoparticles becomes an interesting topic in recent years (1). In the perspective of replacement of hazardous and non-renewable chemicals, utilization of plant extract as a reductor in the synthesis of metal nanoparticles is an emerging method that is widely developed (2). The basic principle of the synthesis is the capability of flavonoid and alkaloid to reduce metal ion precursor. There are some metal and metal oxide nanoparticles synthesized using plant extract reductor, such as silver nanoparticles (AgNPs) (3), gold nanoparticles (AuNPs) (4,5), zinc oxide (ZnONPs) (6) and platinum nanoparticles (PtNPs) (7). Among these nanoparticles, green synthesis of AgNPs is interesting since many studies reported the characteristics of synthesized nanoparticles as function of kind of plant extract, composition, and method of synthesis (8). Green synthesized AgNPs has been reported to have antibacterial, antifungal, anticancer and antioxidant activities. As many other nanoparticles, the activity, physical and chemical properties of AgNPs are affected by its form and shape, which are affected by the plant extract used, composition and method of synthesis (9). Over the years, numerous studies on the biosynthesis of AgNPs using plant extracts were reported. Some of the previous studies reported the green synthesis of AgNPs using Lantana camara (10), Parkia speciosa Hask pod (11), apple extract (12), Buddleja globosa hope (13) and Azadirachta indica (14), with characteristics form, particle size and chemical/biological activity tabulated in Table 1. Some investigations demonstrated the biological/chemical activity of AgNPs, which is closely related with the physicochemical characteristics of AgNPs.

From several investigations listed in Table 1, it is found that while using plant extract for AgNO\(_3\) synthesis, molar ratio is an important parameter for the particle size formed, instead of the method applied for the nanoparticles formation. Exploration on other plant extracts for
AgNPs synthesis is still an interesting topic. Due to the previous studies reporting high potency of red spinach (Amaranthus Tricolor L.) to provide secondary metabolite compounds, this study considers the use of A. Tricolor L. for AgNPs synthesis. To our knowledge, there is no report on the utilization of A. Tricolor L. extract as a reducing agent in the synthesis. This study focuses on the synthesis optimization and evaluation of antioxidative and antibacterial activities of the prepared AgNPs.

### Materials and methods

#### Materials

All the reagents in this study are of analytical grade and used without further purification. Silver nitrate (AgNO₃) and ethanol were purchased from Merck-Millipore (Germany) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) was supplied by Sigma-Aldrich (Singapore). Red spinach (A. Tricolor L) was obtained from the local market in Sleman District, Yogyakarta, Indonesia. Red spinach extract (RSE) was prepared by the maceration method (Figure 1). Dry red spinach leaves of about 10 g were immersed in ethanol:water (1:1) solvent overnight. RSE was collected by filtering the solution from the mixture.

#### Synthesis of AgNPs

The synthesis of AgNPs was performed by mixing AgNO₃·10⁻³ M solution with RSE at varied volume percentage of 2%, 4%, 6%, 8%, and 20%. All mixtures were heated at 70° C for 2 h to accelerate reduction reaction of Ag⁺ to Ag⁰ (21). From the varied RSE volume percentages, the obtained AgNPs were encoded as AgNPs-x with x = 2, 4, 6, 8, and 20, respectively. The confirmation of reduction was performed by UV–Visible spectroscopy analysis. Further analysis of the AgNPs was evaluated by particle size analyzer, Fourier-transform infrared microscope and transmission electron microscope (TEM). HORIBA particle size analyzer with dynamic light scattering system and JEOL TEM instrument operated at 120 kV were employed for these analyses. For FTIR analysis, Perkin–Elmer spectrophotometer instrument was utilized, and to make sure the single phase of Ag has been obtained from the synthesis, x-ray diffraction (XRD) analysis was performed using Shimadzu X6000 instrument operating with Ni-filtered CuKα as a radiation source.

### Table 1. Some researches on biosynthesis of silver nanoparticles.

| Plant extract       | Particle size (nm) | Results                                                                 | References |
|---------------------|--------------------|------------------------------------------------------------------------|------------|
| Buddleja globosa    | 16                 | Biosynthesis in room temperature and low concentration of extract gives the low-cost and efficient method for silver nanoparticles synthesis | (13)       |
| Origanum vulgare L. | 11.27              | Increasing toxicity to various bacterial and fungal microorganisms including Shigella sonnei, Micrococcus luteus, Escherichia coli, Aspergillus flavus, Alternaria alternata, Paecilomyces variotii, Phialophora alba | (15)       |
| Eriobotrya japonica | 20                 | AgNPs were found to exhibit effective antibacterial activities against E. coli and Staphylococcus aureus | (16)       |
| Crocus sativus L.   | 12–20              | AgNPs had an inhibiting activity against E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Shigella flexneri and Bacillus subtilis | (17)       |
| Alpinia katsumadai  | 12.6               | AgNPs showed the antioxidant activity and the antibacterial activity against E. coli and S. aureus | (18)       |
| Asadirachta indica  | 34                 | AgNPs showed antibacterial activities against both Gram-positive (S. aureus) and Gram-negative (E. coli) microorganisms | (19)       |
| Parkia speciossa  | 104–160            | Particle size of AgNPs is affected by synthesis method: reflux, microwave and sonication | (13)       |
| Pod Convolvulus arvensis | 90.9 nm | Prepared AgNPs show the potency for the catalytic reduction of azo dyes in the presence of NaBH₄ as a reducing agent | (19)       |
| Artemisia vulgaris   | 25                 | Synthesized nanoparticles presented effective antibacterial activity against E. coli, S. aureus, P. aeruginosa, K. pneumoniae and Haemophilus influenzae | (20)       |

Figure 1. Red spinach (Aramanthus tricolor L) sample.
**Antibacterial activity test of AgNPs**

Antimicrobial activity of AgNPs was assessed for *Escherichia coli* inhibition using well-diffusion technique. Nutrient agar and nutrient broth were prepared by mixing agar and nutrient broth, respectively, and were then autoclaved at 121°C for 60 min for sterilization. The sterilized liquid media were then utilized for the inoculation of *E. coli* samples and were poured into the well of the prepared Petri plates followed by incubation at 37°C for 24 h to determine the inhibition of the growth of *E. coli*. The inhibition zone was calculated by the bacteria counter scanner HORIBA.

**Results and discussion**

Figure 2 shows UV–visible spectra of the synthesized AgNPs at different volumes of RSE. RSE shows some maximum wavelengths at the range of 220–350 nm and intense spectrum at 664 nm. These spectra are associated with the presence of anthocyanin and phenolic compounds in RSE, which is similar to the results presented by the previous study (22). All AgNPs exhibits the maximum wavelength in the range of 390–430 nm as indication of the surface plasmon resonance (SPR) absorption band. These SPR absorption bands are attributed to electronic resonance of UV–absorption band. These SPR absorption bands are attributed to the presence of either polyphenol or aromatic molecular structures from RSE. Since all AgNPs samples demonstrate the single SPR band, it is predicted that the nanoparticles are in a spherical form, and meanwhile, as there are two or more SPR bands, it correspond to the anisotropic molecules (23). From the varied RSE volume percentage, it is confirmed that the higher volume percentage of RSE gives the higher maximum wavelength with broader area. The most intense spectrum correlated with the formation of SPR appeared from AgNPs-2.

Particle size analysis was performed for ensuring the particle size distribution and particle size average. The distribution curves are presented in Figure 3, and the particle size average data are tabulated in Table 2. The uniform particle size was obtained by the RSE volume percentage of 2% (40.45 nm) with smaller polydispersity index (PI) value (PI = 0.063). The higher volume percentage of RSE, the higher particle size average and PI suggesting the widely distributed particle size. This phenomenon is in line with the UV–visible spectra and could attribute to the aggregation at higher RSE concentration to form higher particle size (24,25).

Figure 4 depicts the FTIR spectra of the AgNPs-2% and RSE. Prominent bands of absorbance observed at around 3300–3600 cm$^{-1}$ indicate the presence of (O–H) functional group in RSE sample. Other observed peaks at 1645.57, 2126.47 and 2940.99 cm$^{-1}$ denote –C = O, –O–C- and alkane (C–H), respectively. The peaks at $\nu \sim 1040$ cm$^{-1}$ and $\nu \sim 1380.5$ cm$^{-1}$ are attributed mainly due to C–C and C–N vibrations of the tetrapyrrrole ring of chlorophyll, which is associated with the UV–visible spectra. The identified absorption spectra are similar with the FTIR analysis from spinach extract (26). Either the absence of 2940.99 cm$^{-1}$ absorbance or the shift of 1645.57 cm$^{-1}$ in AgNPs spectrum is the indication of the Ag$^+$ bioreduction to Ag$^0$, as these bands are due to the secondary metabolite compounds such as anthocyanin chlorophyll or flavonoid in RSE. These data are similar to other studies on AgNPs biosynthesis (10).

The TEM image of the AgNPs-2% shown in Figure 5 expresses the spherical shape of nanoparticles of 5–40 nm. The data fit with the particle size distribution and UV–visible spectrum of AgNPs = 2%, indicating the range of size of nanoparticles. It is also seen that the particles are surrounded by a thin layer as capping organic material from the plant extract. The capping organic material is useful to stabilize the nanoparticles. The presence of organic material is also revealed by the energy dispersion X-ray spectra of AgNPs, which exhibits the presence of Ag, C and O. The C and O signals come from organic compounds of RSE as the capping AgNPs. There is no signal of N, indicating the absence of AgNO$_3$ due to the complete reduction of Ag$^+$ in the synthesis.

XRD measurement was performed to ensure the single material of AgNPs formed in the synthesis. Figure 6 shows the reflection spectra of filtered AgNPs-2%. The XRD pattern shows four intense peaks at 2\theta values ranging from 30 to 80. There are intense peaks at 2\theta values of 31.9, 45.31, 65.4 and 78.4 corresponding to (111), (200), (220) and (311) planes refer to the JCPDS, file no.04-078 for silver (27).
The antimicrobial property of AgNPs was investigated for E. coli colonies, and the results obtained are presented in Table 3. The inhibition zones of the samples are compared with amoxicillin as a positive control and water:ethanol (1:1) as a RSE solvent, and the obtained zone indicates the maximum antibacterial activity of AgNPs-2% is the highest among the prepared samples.

Table 2. Particle size data of synthesized AgNPs.

| Sample     | AgNPs-2 | AgNPs-4 | AgNPs-6 | AgNPs-8 | AgNPs-20 |
|------------|---------|---------|---------|---------|----------|
| Particle size | 40.45   | 98.30 nm| 128.63  | 2198.4 nm| 200.72 nm|
| PI         | 0.063   | 0.194   | 0.135   | 0.206   | 0.266    |

Figure 3. Particle size distribution of (a) AgNPs-2%, (b) AgNPs-4%, (c) AgNPs-6%, (d) AgNPs-8% and (d) AgNPs-20%.

Figure 4. FTIR spectra of synthesized AgNPs in comparison with RSE.

Figure 5. TEM image of synthesized AgNPs.
are formed by complete reduction of Ag+ from AgNO3 by a bioreductor. UV-visible spectrophotometry, FTIR spectroscopy, and XRD analyses demonstrate the antibacterial activity toward E. coli, with the trend that the smaller particle size results in the increase in antibacterial activity.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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**Table 3.** Inhibition zone of antibacterial activity test of synthesized AgNPs.

| Sample           | Measurement 1 (mm) | Measurement 2 (mm) |
|------------------|--------------------|--------------------|
| AgNPs-2%         | 11.8               | 10.5               |
| AgNPs-4%         | 7.8                | 10.0               |
| AgNPs-6%         | 7.8                | 8.6                |
| AgNPs-8%         | 7.8                | 7.8                |
| AgNPs-20%        | 7.8                | 8.3                |
| RSE              | 7.8                | 8.0                |
| Amoxicillin      | 19.6               | 19.3               |
| Water: ethanol (1:1) | 1.2               | 1.2               |

**Conclusion**

Silver nanoparticles (AgNPs) were successfully synthesized using red spinach (*A. tricolor*) leaf extract as a bioreductor. UV-visible spectrophotometry, FTIR spectroscopy, and XRD analyses demonstrate the antibacterial activity toward *E. coli*, with the trend that the smaller particle size results in the increase in antibacterial activity.

The antibacterial activity is lower at the higher RSE volume percentage, which is also in line with the increasing particle size average. The inhibition zones of AgNPs-6%, AgNPs-8% and AgNPs-20% are similar with the inhibition zone of RSE, which means that at different RSE percentages, the nanoparticles give no effect on the antibacterial activity. This observed data indicate the effect of particle size in that the smaller particle size gives more effective in the antibacterial activity. The smaller size contributes to the more effective penetration into bacteria cell membrane for further destruction of the sulfur- and phosphorus-containing complexes such as DNA and cell death.
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