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Extract of Rhoeo discolor Leaf as a Reducing and Stabilizing Agent in the Synthesis of Silver Nanoparticles

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ABSTRACT. Silver nanoparticles (AgNPs) have been synthesized using *Rhoeo discolor* leaf extract as a reducing and stabilizing agent. AgNPs were synthesized by adding silver nitrate to the extract of *Rhoeo discolor* and incubating it in a boiling water bath. The change in color of the mixture from clear to yellow indicated AgNPs formation. The synthesized AgNPs were spherical and showed an absorption peak at around 410-420 nm. The size of particles was distributed from 3 to 18 nm with an average size of 8 ± 2 nm, and the polydispersity index was 0.185. The stability test showed that synthesized AgNPs were stable for three months of storage at ambient temperature. The extract of *Rhoeo discolor* were responsible for reducing silver ion and stabilizing the synthesized AgNPs.

Keywords: green synthesis, Rhoeo discolor, silver nanoparticles

INTRODUCTION

Silver nanoparticles have become the most promising product in the field of nanotechnology. Nanotechnology has taken advantage of the properties of silver nanoparticles for application in various fields, such as the textile industry, pharmacology, and electrical component technology. AgNPs have been applied in household products such as food storage containers, wet wipes, detergents, shampoos, and cosmetics (Raj et al., 2018). The of development current silver nanoparticle applications aims to diagnose chronic diseases to drug delivery matrices by ensuring their safety and effectiveness (Qidwai et al., 2018).

The physical, chemical, photochemical, and biological methods have been studied to synthesize AgNPs. Chemical methods have been frequently used in the synthesis of AgNPs. Silver ion, reducing agent, and capping or stabilizing agent should be available in the chemical methods. In some cases, the reducing agent simultaneously plays a role as a capping agent. The principle of the chemical method is reducing silver ions to a zero-valent state (Gusrizal et al., 2021).

Several conventional approaches in metal nanoparticle synthesis are expensive, deadly, and nonenvironmental. To overcome these problems, natural sources such as bacteria, fungi, biopolymers, and plants are suitable for synthesizing AgNPs. The utilization of plant material is categorized as the biological method, primarily founded on the principle of the chemical method. In biological methods, molecules derived from living organisms act as reducing and stabilizing agents. Biological methods are considered eco-friendly and economical (Sharma et al., 2022).

Plant extract is commonly used in the biological synthesis of AgNPs since it does not require special treatment like isolation, culture, and culture maintenance. The synthesis tends to be quicker than microorganisms, economical, and easier. The product is also more environmentally friendly (Sharma et al., 2022; Shreyash et al., 2021). Plant extract contains various categories of metabolites that have been utilized in the synthesis of metal nanoparticles. Metabolites produced by plant species, including carbohydrates, alkaloids, terpenoids, phenolic compounds, and enzymes, are why plant extract is utilized in AgNP synthesis (Shreyash et al., 2021). The metabolites are believed to be essential in reducing silver ions and stabilizing the synthesized AgNPs. Hydroxyl, carboxylic, and amino functional groups are accountable for reducing metal ions and stabilizing them (Shah et al., 2015; Shreyash et al., 2021).

It has been reported that *Rhoeo discolor* shows a high content of secondary metabolites such as flavonoids, saponins, carotenoids, anthocyanins, terpenoids, ferulic acid, chlorogenic acid, vanillic acid, *p*-coumaric acid, steroidal compounds. Its phenolic-rich extract's antimicrobial activity has also been evaluated (García-Varela et al., 2015). *Rhoeo discolor* extract shows equivalent radical scavenging activity to α -tocopherol and is stronger than ascorbic acid (González-Avila et al., 2003).

Plant extracts that have antioxidant activity have been widely used to synthesize AgNPs (Srećković et al., 2023). The antioxidant activity of plant extracts is mainly due to phenolics and flavonoids compound. The antioxidant action of phenolic and flavonoids resides in their ability to donate electrons or hydrogen atoms. Keto-enol tautomeric transformation of flavonoids has been proposed to release hydrogen atoms and reduce silver ions (Ahmad et al., 2010; Shreyash et al., 2021).

This paper presents the use of *Rhoeo discolor* leaf extract to synthesize AgNPs. The choice of *Rhoeo discolor* leaf extract as a reducing agent in the synthesis of AgNPs was motivated by the presence of secondary metabolites and its antioxidant activity (García-Varela et al., 2015; González-Avila et al., 2003). The synthesis parameters such as pH, temperature and incubation time, and extract to silver ion concentration ratio were investigated. Characteristics and stability of synthesized AgNPs were also determined.

EXPERIMENTAL SECTION Materials

Silver nitrate (GR for analysis, CAS number 7761-88-8, Merck), methanol (GR for analysis, CAS number 67-56-1, Merck), and sodium hydroxide (GR for analysis, CAS number 1310-73-2, Merck) were used without further purification. Fresh *Rhoeo discolor* was collected from Pontianak, Indonesia.

Instrumentation

UV-Visible spectrophotometer (Shimadzu UV-2600) was used to monitor the formation of AgNPs and to collect the surface plasmon resonance spectra. Fourier transform infrared (FTIR) spectrophotometer (Shimadzu 8201) was used to identify the functional group in the *Rhoeo discolor* leaf extract and synthesized AgNPs. The image of synthesized AgNPs was determined using a transmission electron microscope (TEM) (JEOL JEM-1400). A Microtrac Nanotrac Wave II/Q/Zeta was used to determine the polydispersity index and zeta potential of synthesized AgNPs. Centrifuge TOMY MX307 was used to separate synthesized AgNP colloids.

Preparation of Extract

Fresh *Rhoeo discolor* leaves were collected, airdried, and powdered with a mechanical grinder. The leaf powder (500 g) was macerated with 1 L of methanol for 3×24 hours. The solvent was replaced every day and evaporated by a rotary evaporator. Before being used to synthesize AgNPs, the extract was dissolved in distilled water.

Synthesis of AgNPs

The procedure of AgNP synthesis was adopted from the previous study (Octavianus et al., 2022). AgNPs were synthesized by reduction of silver nitrate with *Rhoeo discolor* leaf extract. The synthesis was carried out by mixing 5 mL of silver nitrate solution $(2 \times 10^{-4} \text{ M})$ with 5 mL extract $(4 \times 10^{-3} \% \text{ w/v})$ in a flask. Before being mixed with silver nitrate, the pH of the extract was adjusted to 12. The flask was then heated in a boiling water bath for one hour. After cooling in the tap water, synthesized AgNPs were measured using a UV-visible spectrophotometer. Several synthesis parameters were investigated, such as variation of pH, temperature and incubation time, and the extract to silver ion concentration ratio to optimize the synthesis condition parameters.

Characterization of AgNPs

To characterize the synthesized AgNPs, we adopted the previously published study (Gusrizal et al., 2017; Octavianus et al., 2022). The AgNPs formation was monitored by a UV-visible spectrophotometer in the wavelength range 300-700 nm with a 1 cm quartz cuvette. The sample for TEM analysis was prepared by immersing a copper grid in synthesized AgNPs colloid and drying it at room temperature. In taking the image, the 120 KV accelerating voltage was used. The dry sample for FTIR analysis was prepared by centrifugation of AgNPs colloid at 15,000 rpm for 20 minutes and dried in an oven at 45 °C for six hours. Dried AgNPs were scanned by the KBr plate method in the 4000-400 cm⁻¹.

Stability of AgNPs

We adopt the previously published study to determine the stability of synthesized AgNPs (Gusrizal et al., 2018). The synthesized AgNPs were stored in a bottle in a dark room at ambient temperature for three months. A UV-visible spectrophotometer was used to monitor the change of surface plasmon resonance spectra, including absorption peak intensity, the position of the maximum peak (λ_{max}), and the full-width half maximum (FWHM) of the peak at a certain time.

RESULTS AND DISCUSSION

Rhoeo discolor Extract

The dried Rhoeo discolor leaf (500 g) was extracted by maceration with methanol (3×1 L) and resulted in 4.6 g of dry extract. The extract component was analyzed by phytochemical screening (Harborne, 1984) and showed a positive result for alkaloid, tannin, and flavonoid and a negative result for terpenoid, steroid, and saponin.

Synthesis of Silver Nanoparticles

Silver nanoparticles were synthesized by mixing a precursor silver nitrate with the extract of *Rhoeo* discolor with a volume ratio of 1:1. The *Rhoeo* discolor leaf extract 4×10^{-3} % w/v was adjusted to pH 12 by the addition of sodium hydroxide. In a flask, 5 mL of extract was mixed with 5 mL silver nitrate 2×10^{-4} M and heated in a boiling water bath. The color of the mixture changed from colorless to yellow. The measurement with a UV-visible

spectrophotometer showed that the absorption peak appeared at 410-420 nm. It confirmed the formation of AgNPs. The formation of yellow color and absorption peak is due to the surface plasmon resonance of AgNPs. The intensity of spectra is directly proportional to the concentration of AgNPs formed in the synthesis. The higher the concentration of synthesized AgNPs, the higher intensity of the peak (Pinto et al., 2010),

The result is similar to those obtained by several researchers using plant extract as a reducing agent. The AgNPs synthesized using aqueous Melia azedarach leaf extract showed an absorption peak of 400 nm (Jebril et al., 2020). AgNPs synthesized using aqueous leaf extract of *Cucumis prophetarum* showed an absorption peak at 420 nm (Hemlata et al., 2020). The λ_{max} slightly varied around 400 nm from different experiments resulting from the reaction condition, such as reducing and capping agents used in the synthesis (Ajitha et al., 2016; Rycenga et al., 2011).

Effect of pH on the synthesis of AgNPs

Many researchers have demonstrated that pH plays a role in the synthesis of nanoparticles using biological methods (Shreyash et al., 2021). Because no formation of AgNPs was detected while using the pHunadjusted extract in the synthesis of AgNPs, the pH of *Rhoeo discolor* leaf extract should be adjusted to basic condition by adding sodium hydroxide. The pH of the extract used to synthesize AgNPs was 10, 11, and 12. **Figure 1** shows the effect of extract pH on forming AgNPs. The intensity of spectra increases by increasing the extract pH.

The amount of AgNPs produced, indicated by the intensity of spectra, varied at the different pH of *Rhoeo discolor* leaf extract used in the synthesis. Based on **Figure 1**, the synthesis using the extract with pH 12 resulted in the maximum result. The addition of

sodium hydroxide to the extract causes the deprotonation of the hydroxyl group in the chemical constituent of the extract. The deprotonation of hydroxyl groups leads to the release of anion that can be oxidized by silver ions, forming the reduced silver as AgNPs (Ahmad et al., 2010; Shreyash et al., 2021). Under excessive sodium hydroxide, the formation of AgNPs was not detected. The alkaline solution forms silver hydroxide precipitates, making silver ions unavailable for reduction. The pH of the extract is thus a critical factor in the synthesis of AgNPs.

Effect of incubation temperature

The effect of incubation temperature in the synthesis of AgNPs was studied by carrying out the reaction at room temperature, heating in a water bath at 45 °C, 75 °C, and 95 °C. Figure 2 shows the intensity of surface plasmon resonance spectra of AgNPs increases by increasing the temperature of the reaction. There is a direct proportionality between temperature and yields of synthesized AgNPs. These results are in accordance with the previously reported study (Mittal et al., 2012; Sathishkumar et al., 2010).

Effect of incubation time

The incubation time for the reaction of *Rhoeo discolor* leaf extract with silver nitrate was determined by heating the mixture at intervals of 15 minutes, 30 minutes, 60 minutes, and 120 minutes. **Figure 3** shows the absorption intensity of the surface plasmon resonance spectra of AgNPs synthesized at different heating incubation times. The intensity of spectra increases by increasing the incubation from 15 to 60 minutes, indicating that the AgNPs continuously formed during the reaction. However, spectra intensity slightly decreases if the incubation time is prolonged to 120 minutes. The optimum incubation time for the synthesis is 60 minutes.

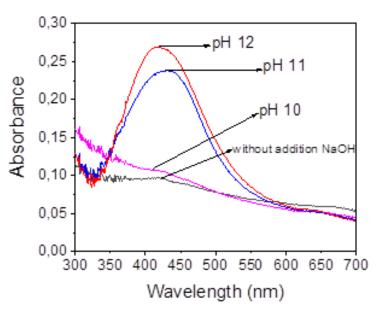


Figure 1 Surface plasmon resonance spectra of AgNPs synthesized with different pH of *Rhoeo discolor* leaf extract

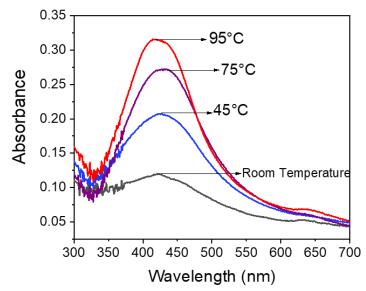


Figure 2 Surface plasmon resonance spectra of AgNPs synthesized with different incubation temperatures

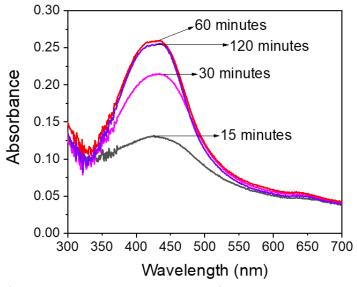


Figure 3 Surface plasmon resonance spectra of AgNPs synthesized with different incubation time

Effect of silver nitrate concentration

The effect of silver nitrate concentration was determined by carrying out the reaction using the different initial concentrations of silver nitrate. The concentration of silver nitrate varied from 0.5 \times 10 $^{\text{-4}}$ to 1.0 imes 10⁻³ M, and the concentration of the extract was 1×10^{-2} % w/v. Figure 4 shows that the intensity of spectra increases by increasing the concentration of silver nitrate to 8×10^{-4} M, indicating that the extract as a reducing agent is still available for forming AgNPs. The intensity of spectra at 410 nm decreases, accompanied by increasing the intensity of spectra at the longer wavelength for the reaction using silver nitrate 1×10^{-3} M. It indicates that the population of small-size nanoparticles decreases due to aggregation. A similar result also is found when synthesis uses Coleus aromaticus. Synthesis is efficient if the concentration of silver nitrate is less than 1 mM. A higher concentration of silver nitrate generates the aggregation of AgNPs (Vanaja et al., 2013).

Effect of extract concentration

In the synthesis of AgNPs, the *Rhoeo discolor* leaf extract act as a reducing agent without an additional capping agent. To understand the effect of extract concentration, the synthesis was carried out using the extract concentration varied from 2×10^{-3} to 2×10^{-2} % w/v. **Figure 5** shows the surface plasmon resonance spectra of synthesized AgNPs. The synthesis using the concentration of extract 4×10^{-3} % w/v yields the maximum result. The peak intensity slightly decreases by increasing the concentration of extract upper 4×10^{-3} % w/v. The higher concentration of extract negative in larger particles, indicated by an increase in spectra intensity at the longer wavelength.

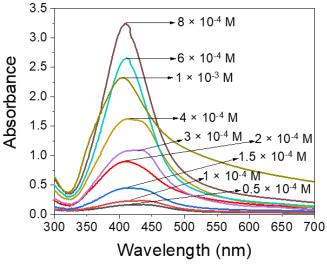


Figure 4 Surface plasmon resonance spectra of AgNPs synthesized with different initial concentrations of silver nitrate

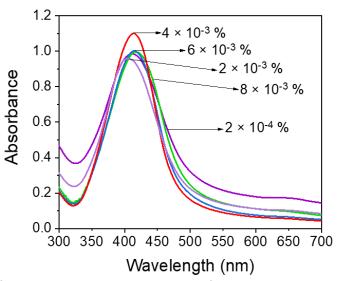


Figure 5 Surface plasmon resonance spectra of AgNPs synthesized with different concentrations of *Rhoeo discolor* leaf extract

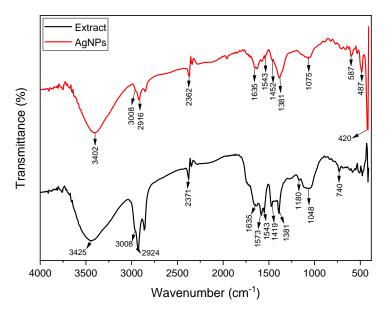


Figure 6 FTIR spectra of Rhoeo discolor leaf extract and synthesized AgNPs

Characterization of Synthesized AgNPs

The properties and characteristics of synthesized AgNPs can be affected by the synthesis parameter such as pH, the concentration of extract and metal salt, temperature, and reaction time (Sharma et al., 2022). It is essential to optimize the condition for the synthesis. For characterization, AgNPs were synthesized by adding 5 mL silver nitrate 2×10^{-4} M with 5 mL pH 12 adjusted extract 4×10^{-3} % w/v. The mixture was heated at 95 °C in a water bath. After synthesis, the synthesized AgNPs were cooled in tap water.

FTIR Analysis

FTIR spectra of extract and synthesized AgNPs are presented in **Figure 6**, and the prominent absorption bands of the extract are tabulated in **Table 1**. The FTIR spectra of *Rhoeo discolor* leaf extract is similar to previously published data (Chunduri & Shah, 2016). The absorption band confirms the existence of flavonoids, tannins, and alkaloids in the *Rhoeo discolor* leaf extract as the result of phytochemical screening.

FTIR spectra of synthesized AgNPs are similar to FTIR spectra of extract, indicating the extract is attached to the surface of AgNPs. The change of FTIR at 1180 cm⁻¹ indicates that the hydroxyl functional group might perform a function of reducing silver ions. The absorption band at 587, 487, and 420 cm⁻¹ in the AgNP spectra presented in **Figure 6** indicates the presence of silver metal (Hussain et al., 2015; Loo et al., 2012).

TEM Image

Figure 7 shows the TEM image and particle size distribution of synthesized AgNPs. The image confirms that the *Rhoeo discolor* leaf extract can reduce the silver ions to form the particles in a nanoscale with a spherical shape. The size of particles distributes from 3 to 18 nm with an average of 8 nm. Dynamic light scattering measurement showed the polydispersity index of synthesized AgNPs was 0.1846 indicating good uniformity of synthesized AgNPs, and the particles were spherical with potential zeta -7.7 mV.

The shape of synthesized AgNPs also is confirmed by the surface plasmon resonance spectra showing the λ_{max} at 413 nm. At that wavelength, the shape of particles is spherical (Stamplecoskie & Scaiano, 2010). The potential zeta value indicates that the particles have a negative charge owing to the extract capping the particles.

Stability of AgNPs

The stability of synthesized AgNPs was observed from the change of surface plasmon resonance spectra during the storage. The parameter observed was the λ_{max} , the intensity of the absorption peak, and FWHM (Gusrizal et al., 2018). **Table 2** shows the surface plasmon resonance spectra profiles of synthesized AgNPs during storage for three months.

 Table 1 Absorption bands of Rhoeo discolor leaf extract

Wave number (cm ⁻¹)		Interpretation	
This study	Chunduri & Shah, 2016		
3425	3420	O-H stretching	
3008	3010	C-H stretching of alkene	
2924	2930	C-H stretching of alkanes	
1728	1740	C=O stretching	
1635	1616	C=C aromatic	
1573		N-H bending	
1381	1350	350 C-N stretching	
1180		C-O stretching of secondary alcohol	
1048	1028	C-O stretching of primary alcohol	

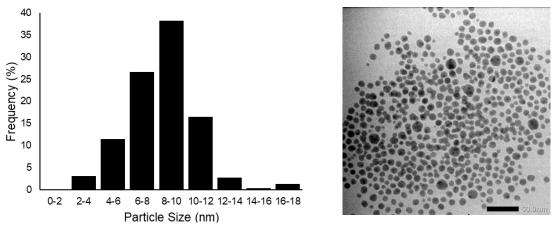


Figure 7 Size distribution and TEM image of synthesized AgNPs (scale bar = 50 nm)

Duration of	λ_{max}	Peak Intensity	FWHM
storage	(nm)	(Absorbance)	(nm)
After synthesis	413	1.283	94
1 day	413	1.278	93
2 days	412	1.265	94
3 days	412	1.250	93
1 week	412	1.248	93
2 weeks	412	1.213	94
3 weeks	413	1.201	94
1 month	413	1.088	95
2 months	413	1.099	94
3 months	413	1.149	95

 Table 2 Profile of surface plasmon resonance spectra of synthesized AgNPs during the storage

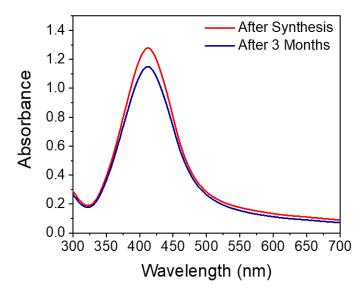


Figure 8 Surface plasmon resonance spectra of synthesized AgNPs after 3 months of storage

Table 2 shows no significant change in λ_{max} and FWHM over the observation period. However, the intensity of the peak slightly decreases, as shown in **Figure 8**. The decrease in peak intensity might indicate the dissolution of AgNPs. The observation shows that the chemical constituents of the extract could protect the synthesized AgNPs from aggregation. It was supported by no increasing peak intensity at a longer wavelength (Tejamaya et al., 2012). The negative charge of the particles owing to the chemical constituent of extract capping the particles produces negative-negative repulsion that protects the particles aggregation.

CONCLUSIONS

In this study, we demonstrated the rapid and simple method for the synthesis of AgNPs using *Rhoeo discolor* leaf extract as a reducing agent. The average particle size of synthesized AgNPs was 8 ± 2 nm and stable for 3 months of storage under ambient temperature. The chemical constituents in the extract were responsible for the synthesis of AgNPs by simultaneously playing a role as a reducing and stabilizing agent.

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