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Environmentally friendly synthesis of silver nanoparticles using Indonesian medicinal plants extract: scale-up study and characterization

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ABSTRACT: This study is a further scale-up trial from the previously reported process optimization study on the green synthesis of silver nanoparticles (AgNPs) using Indonesian medicinal plant extracts. The present study utilized *Phyllanthus niruri* (PN) herb extracts, *Orthosiphon stamineus* (OS) leaf extracts, and *Curcuma longa* (CL) rhizome extracts as reducing agents to synthesize AgNPs on a larger scale. Upscaling increased the synthesis volume (from 100 mL to 1000 mL) and the centrifugation volume (from 1 mL to 40 mL) at various durations at 70°C for 60 min. The results showed that the minimum centrifugation duration to obtain optimal AgNPs was 5 minutes. The formation AgNPs were then characterized using Fourier transform infrared spectroscopy (FTIR), scanning electron microscope (SEM), particle size and zeta potential analyzer, and X-ray diffractometer (XRD). The results showed the spherical silver nanoparticles with an average particle size (MI) of 169 nm (PN), 179.1 nm (OS), and 623 nm (CL), polydispersity index (PDI) value of 0.2202 (PN), 0.2579 (OS), and 0.00872 (CL) and the face-centered cubic (fcc) crystal structure. The green synthesis method using PN and OS extracts proved effective, environmentally friendly, safe, and cost-effective for the synthesis of AgNPs. The findings of this study have the potential to provide a green synthesis method for the production of AgNPs on a larger scale.

KEYWORDS: *Orthosiphon stamineus; Phyllanthus niruri; Curcuma longa;* scale-up; green synthesis; silver nanoparticles.

1. INTRODUCTION

Silver nanoparticles (AgNPs) have attracted attention, especially in the pharmaceutical field, as their anti-bacterial and anti-inflammatory properties can accelerate wound healing [1,2,3]. In addition, AgNPs can also be used as a solution for microbial resistance to antimicrobial agents [4,5]. Numerous techniques have been reported for synthesizing AgNPs, such as the chemical reduction of silver ions in aqueous solutions with or without stabilizing agents, thermal decomposition in organic solvents, chemical reduction and photoreduction in reverse micelles, microwave-assisted synthesis, and sonochemical method [3,6]. However, many of these methods require toxic and hazardous chemicals, which may damage the environment. Therefore, green synthesis of AgNPs is currently being developed by utilizing the bioreductive deposition of plant extracts that is environmentally friendly, cost-effective, easy to up-scale, do not require high temperatures and pressures, and are free from toxic chemicals [6,7,8].

There are commercially available silver nanoparticles (AgNPs) used in medical applications as antiseptics and disinfectants due to the ability to inhibit the growth of bacteria and other microorganisms. AgNPs are also incorporated into wound dressings, helping prevent infections and promote healing. AgNPs can be found in various consumer products such as toothpaste, face creams, medical bandages, disinfectants, and insecticides. Several medicinal plants have been used to synthesize silver nanoparticles for biomedical applications. Examples of plants that have been used successfully are *Shorea robusta*, *Taraxacum officinale*, *Musa paradisiaca*, *Azadirachta indica*, and *Ocimum sanctum* [9]. Indonesian medicinal plants that can be used in the synthesis of AgNPs are *Phyllanthus niruri* (PN), Orthosiphon stamineus (OS), and Curcuma longa (CL). The herbs of *Phyllanthus*, the leaves of *Orthosiphons*, and the rhizome of *Curcuma* contain flavonoids and terpenoids that can act as bioreductor, thereby able to reduce Ag^{+} to Ag^{0} and act as capping and stabilizing

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agents [10,11,12]. The synthesis of AgNPs using those three Indonesian medicinal extracts from herbs, leaves, and rhizomes as bioreductor on a small scale has been carried out before [12]. Based on our previous study, we found that among the three Indonesian medicinal plant extracts, *Phyllanthus niruri* (PN) exhibited the most potent antioxidant activity. Its IC_{50} value was the lowest on the DPPH scavenging method, followed by *Orthosiphon stamineus* (OS) and *Curcuma longa* (CL). Our findings suggest that PN at a concentration of 0.5% is a promising reducing and capping agent for the green synthesis of AgNPs, as it produced the highest yield among the three extracts.

Toxicity and activity tests are required to develop further AgNPs synthesized using PN, OS, and CL into pharmaceutical dosage forms. These steps need a large amount of AgNPs sample. Therefore, it is necessary to increase the scale of synthesis. However, it is crucial to investigate the feasibility of scaling up the process to apply this method on a larger scale. In this study, we aimed to characterize the scale-up trial of the green synthesis of AgNPs using the same three Indonesian medicinal plant extracts as reducing agents. By increasing the synthesis and centrifugation volumes, we aimed to determine the feasibility of producing AgNPs using a larger-scale process. The results of this study can provide valuable insights into the development of green synthesis methods for nanotechnology applications. In this current research, the up-scaling trial was done by increasing the synthesis volume (from 100 mL to 1000 mL) and also the centrifugation volume (from 1 mL to 40 mL) compared to the previous study [12].

However, increasing the centrifugation volume can prolong the sedimentation path and increase the centrifugation duration needed to obtain optimal AgNPs [8]. Therefore, optimizing the centrifugation duration was necessary to avoid incomplete separation of AgNPs with a short or unwanted agglomerate with a long centrifugation duration. The centrifugation duration was optimized at four different durations (5, 10, 15, and 20 minutes), with 15 minutes chosen as it had been previously applied on a small scale [12]. The formed AgNPs on a large scale were then characterized using an FTIR, SEM, particle size and zeta potential analyzer, and X-ray diffraction (XRD).

2. RESULTS and DISCUSSION

The present study aimed to upscale the eco-friendly synthesis of AgNPs using extracts from *Phyllanthus niruri* (PN), *Orthosiphon stamineus* (OS), and *Curcuma longa* (CL). These plant extracts were previously optimized and known to contain bioactive compounds with excellent reducing and stabilizing properties. We characterized the physical characteristics of the plant extracts and evaluated the efficiency of the green synthesis method in producing AgNPs on a larger scale.

2.1. Physical characteristics of the PN, OS, and CL extract

The parent extract of PN and OS at the concentration of 10% showed dark-green color, while CL was dark-yellow. This dark-green color in PN and the leaves of OS is attributed to the chlorophyll content whereas the dark yellow color in CL is a result of its c curcuminoids. The sample extract of PN (0.50%) and OS (0.125%) were light green, whereas CL (0.50%) was light yellow. These sample extracts were used to synthesize AgNPs on a larger scale.

2.2. Synthesis of AgNPs

The green synthesis process was scaled up by increasing the synthesis volume from 100 mL to 1000 mL or by a factor of 10. The synthesis process was closely monitored, and a color change was observed, indicating the successful formation of AgNPs. For *Phyllanthus niruri* (PN), the color changed from dark green to reddish brown; for *Orthosiphon stamineus* (OS), the color changed from dark green to brownish; and for *Curcuma longa* (CL), the color changed from yellow to reddish brown. Additionally, the formation of AgNPs was further confirmed by a peak at 400-450 nm in the UV-Vis spectra.

The physical characteristics of the plant extracts used to synthesize AgNPs are crucial to the success of the green synthesis method. The dark-green color of the parent extracts indicates the presence of various phytochemicals, which can act as reducing agents for the synthesis of AgNPs [13]. *Phyllanthus niruri* (PN) contains several bioactive compounds, including lignans, flavonoids, terpenoids, polyphenols, and alkaloids, that can serve as effective bioreductors. *Orthosiphon stamineus* (OS) contains bioactive compounds, such as polymethoxylated flavonoids, phenylpropanoids (caffeic acid derivatives), and terpenoids (predominantly diterpenoids and triterpenoids). Bioactives from *Curcuma longa* (CL) that can be used as bioreductors include curcuminoids (dimethoxycurcumin, dihydrocurcumin, and tetrahydrobisdemethoxycurcumin), terpenes (monoterpenes and sesquiterpenes), and phytosterols. The color of the parent extracts used for the synthesis of AgNPs can vary depending on the chemical content of the extract. The color change of the parent extract may not always be a reliable indication of the successful formation of AgNPs. The synthesis of AgNPs

involves a complex process that depends on various factors, such as the concentration of the extract, the concentration of the silver ions, and the reaction time. The successful formation of AgNPs should be confirmed through characterization techniques such as UV-Vis spectroscopy, particle size analyzer (PSA), and scanning electron microscopy (SEM), which can provide information on the size, shape, and morphology of the synthesized AgNPs

The color changes observed during the synthesis process and after the addition of NaOH indicate the reduction of Ag^+ ions to AgNPs. The color change from yellow to reddish-brown observed in the CL extract may be due to curcumin, which can act as a reducing agent for Ag^+ ions [14]. The peak observed in the UV-Vis spectra at 435 nm is characteristic of the surface plasmon resonance (SPR) of AgNPs [15]. The maximum wavelength and absorbance of the solution during the synthesis process can be used to determine the size and concentration of the synthesized AgNPs.

After the AgNPs are formed, 40 mL of the solution mixture is centrifuged at 2,500 rpm for 15 minutes. This step was conducted to remove undesired components [12]. Then, the supernatant obtained was centrifuged at 10,000 rpm at various durations (0, 10, 20, 30 for PN; 5, 10, 15, 20 minutes for OS; 5, 10, 15, 30, 45 for CL). Finally, the absorbance of the supernatant was measured at the maximum wavelength of 400-450 nm using a UV-Vis spectrophotometer.

The study aimed to optimize the upscale green synthesis of silver nanoparticles (AgNPs) using extracts from *Phyllanthus niruri* (PN), *Orthosiphon stamineus* (OS), and *Curcuma longa* (CL). In this regard, the color of the supernatant from each centrifugation duration was evaluated and found to be not significantly different from that of the sample extract, indicating that the synthesized AgNPs had precipitated entirely in just 5 minutes. This was further supported by the UV-Vis spectra, which showed no significant difference in absorbance at the λmax= 435 nm at four different durations in 5, 10, 15, 20 minutes (OS); 5, 15, 30, and 45 minutes (PN, CL). Therefore, it can be concluded that 5 min is the minimum centrifugation duration required for the complete precipitation of AgNPs.

The synthesis of AgNPs on a large scale with a centrifugation duration of 5 minutes resulted in 49 mg of AgNPs PN, 2.5 mg of AgNPs OS, and 32.89 mg of AgNPs CL or with the yield of 0.98% (w/w) (PN), 0.20% (w/w) (OS), and 0.66% (w/w) (CL). Compared to the previous study conducted on a small scale, the results of this current study were not in line with the prediction. The low yield may have been due to agglomeration prior to centrifugation at 2,500 rpm. On a small scale, the entire synthesis product can be directly centrifuged at 2,500 rpm, while on a larger scale, the synthesis product needs to stand for around 20 minutes before centrifugation. This time is required for weighing the instruments used on a large scale, such as the centrifuge tube and its contents. Unfortunately, the agglomerates formed before centrifugation at 2,500 rpm resulted in minimal AgNP residue that could be collected through the centrifugation process at 10,000 rpm [4]. In addition, due to the limitation of centrifuge capacity, the centrifugation (2,500 rpm) was carried out in several steps. The time needed to wait for each step's centrifugation also made AgNPs agglomerates before centrifugation was carried out. The agglomerates formed before centrifugation were then removed in the centrifugation process at 2,500 rpm, causing minimal AgNP residue that can be collected through the centrifugation process at 10,000 rpm. The agglomeration process that occurred before centrifugation at 2,500 rpm could be caused by the addition of 0.2 M NaOH, which was still too much, indicating the need for future optimization of the NaOH volume to increase the synthesized AgNPs amount.

2.3. Characteristic of AgNPs

Figure 1 shows that PN and OS AgNPs analyzed using SEM showed a spherical shape with sizes ranging from 100-700 nm, and CL AgNPs showed sizes ranging from 200-400 nm.

Meanwhile, PSA found that the synthesized PN AgNPs had an MI of 169 nm and a PDI value of 0.2202. OS AgNPs had an MI of 179.1 nm, PDI value of 0.2579, and zeta potential of 39.9 mV. CL AgNPs had an MI of 623.0 nm and a PDI value of 0.00872 (Figure 2 and Table 1). Based on the measurement results by PSA, it can be seen that the size of the AgNPs obtained from the synthesis on a large scale was smaller than the AgNPs obtained in the previous study PN, OS, CL [12]. However, the synthesized AgNPs still had a polydisperse system when viewed from the PDI value obtained.

The particles measured by PSA tend to be larger than those measured by SEM. This tendency is possibly due to PSA's working principle, which measures the particles' hydrodynamic diameter, thus causing the size obtained to be larger [12]. However, in this study, the measurement results by SEM appeared to be slightly larger than the measurement results by PSA, presumably caused by the aggregation due to solvent evaporation during sample preparation [4].

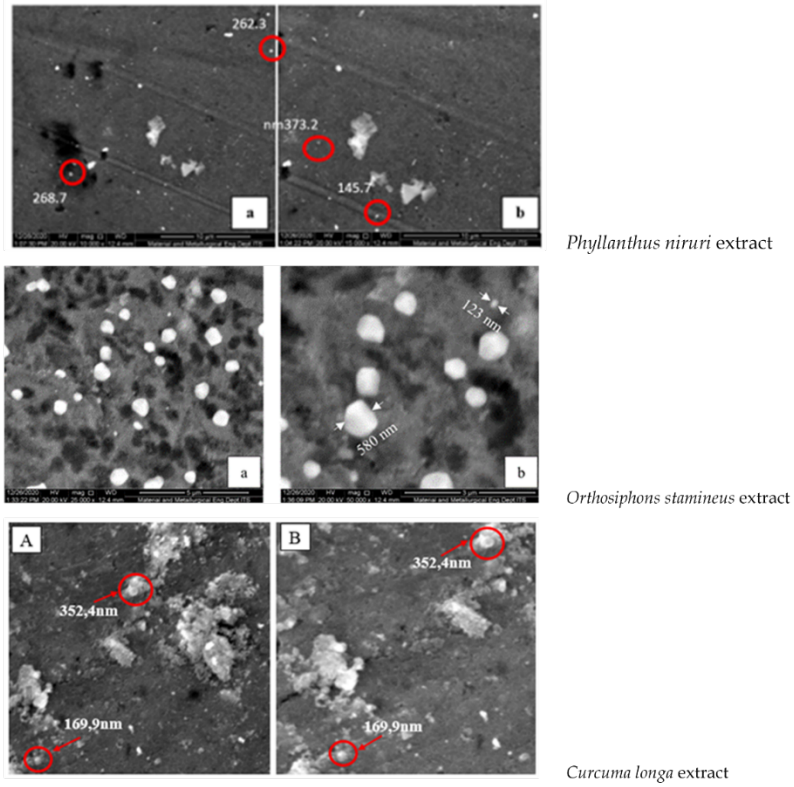

Figure 1. SEM profiles of synthesized AgNPs at a magnification of 25,000x (a) and 50,000x (b) for OS extract, and 10,000x (a) and 15,000x (b) for PN and CL extracts. The SEM profiles show the presence of silver nanoparticles. The plant extracts used in the synthesis have been eliminated.

Table 1. Yields, Particle Mean Diameters (MI), and Polydispersity Index values of the three Indonesian medicinal plants on a small scale and after scaling up.

Results	*Phyllanthus niruri*		*Orthosiphon stamineus*		*Curcuma longa*	
	Small scale	Upscale	Small scale	Upscale	Small scale	Large scale
Yields (%)	15.76	0.98	1.27	0.20	2.46	0.66
MI (nm)	715.0	169	729.0	179.1	918.0	623.0
PDI value	0.0085	0.2202	0.1716	0.2579	0.6040	0.00872

According to ISO 22.412:2017, monodisperse samples generally have a PDI value of <0.05, while polydisperse samples generally have a PDI value of >0.7 [16]. This polydisperse system is also visible in the UV-Vis spectra, characterized by broad peaks. The cause of this polydisperse system is presumably attributed to variations in the growth rate of individual AgNPs during the nucleation step [4].

The zeta potential of the synthesized AgNPs was 39.9 mV. Nanoparticles are stable if they have a zeta potential greater than +/- 30 mV, while nanoparticles with a zeta potential less than +/- 30 mV tend to aggregate and flocculate due to van der Waals forces [11]. Therefore, the AgNPs obtained from the synthesis on a large scale can be said to be stable.

Subsequent FTIR characterization showed that the synthesized AgNP peaks were similar to those in the sample extract (Figure 3). The observed spectra indeed represent a combination of both the plant extracts and the minor AgNPs. We have added explanation as follows:

The presence of minor AgNPs synthesized during the green synthesis process can contribute to the spectral features that seem to overlap with those of the plant extracts. The characteristic absorption bands in the spectra of the extracts may be partly concealed by the spectral signals from the formed AgNPs.

This overlap can occur due to the relatively small size of the AgNPs formed during the green synthesis, resulting in less intense and broader peaks that might coincide with the peaks of the plant extracts. Consequently, it can be challenging to distinguish between the contributions of the extracts and the AgNPs in the spectral data. Careful analysis and deconvolution techniques may be required to tease out these contributions and provide a clearer understanding of the composite spectra. The O-H bond was seen in the

synthesized AgNPs and the sample extract, which was characterized by broad peaks at the wavenumbers of 3337.83 and 3341.55 cm^{-1} (PN), 3335.97 and 3341.56 cm^{-1} (OS), 3337.83 cm^{-1} (CL) respectively.

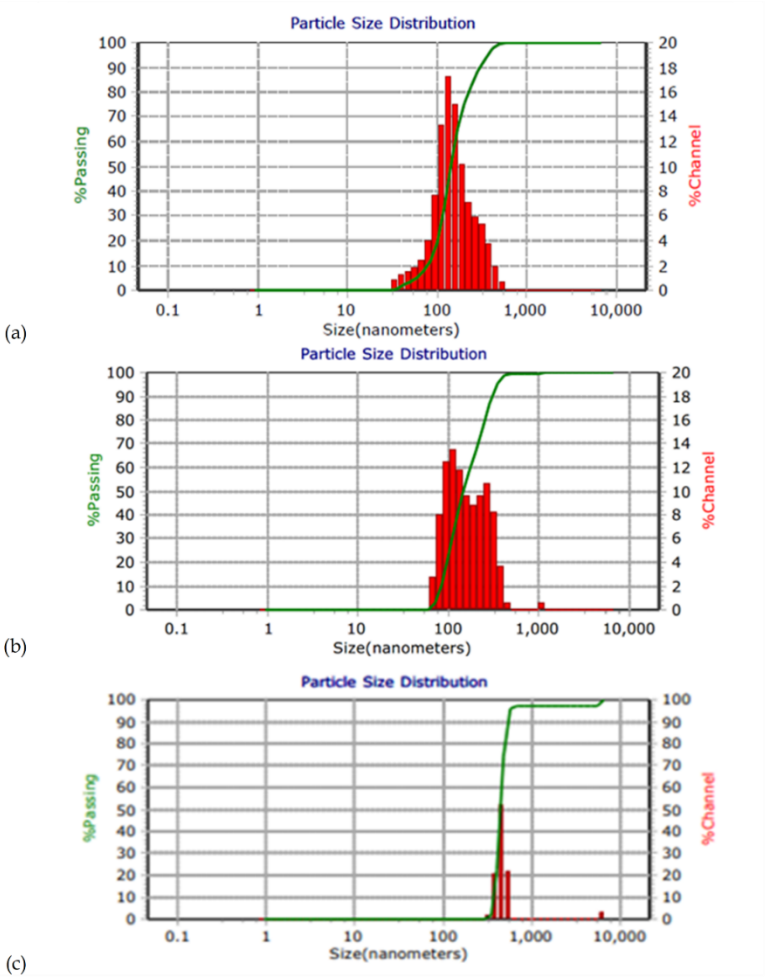

Figure 2. Size distribution of AgNPs measured with the particle size analyzer (a) PN, (b) OS, (c) CL.

The O-H bonds are thought to originate from the phenolic compounds in the ethanol extract of PN, OS, and CL extract, which function as a capping and stabilizing agent in AgNPs. Figure 3 illustrates that the intensity of the OH groups associated with synthesized AgNPs is more pronounced compared to the OH groups in PN, OS, and CL extracts. The elevated intensity suggests that there are still some trace amounts of the extract's component associated with AgNPs. In fact, OS itself contains various phenolic compounds, such as rosmarinic acid, 3'-hydroxy-5,6,7,4'-tetramethoxyflavone, sinensetin, and eupatorin [12,17,18]. The –OH group indicates the presence of polyphenols and flavonoids in the *Phyllanthus niruri* ethanol extract. These flavonoids and polyphenols act as bioreductors in the synthesis of AgNPs. The CL analysis using FT-IR on the ethanol extract of *Curcuma longa* and AgNPsAg showed absorption at wave number 3337.83103 cm^{-1}, indicating the presence of OH groups, and OH groups indicating the presence of a stabilizing agent.

The last characterization by XRD (Figure 4) showed that the synthesized AgNPs had a face-centered cubic (fcc) crystal structure after being compared with the standard diffractogram pattern from JCPDS-ICDD no. 04-0783.

The XRD patterns of the synthesized AgNPs OS measured at an angle of 2θ showed characteristic peaks at PN 38.1607°, 44.2352°, 64.4756°, 77.5834°, and 81.6913° (PN), 38.0782°, 44.0559°, 64.3831°, 77.3699°, and 81.6228° (OS), 38.0174°, 44.0147°, 67.2978°, 76.5931° and 81.5549° (CL) according to JCPDS-ICDD no. 04-0783. Besides the peaks mentioned above, there are other peaks in OS at 27.6973, 32.1121, and 54.7475; PN 27.7478, 32.1749 and 54.764; CL 26.6095, 27.6760, 32.0865, 54.6736, 57.3878 and 67.2978 which are suspected to be the peaks of Ag_2O.

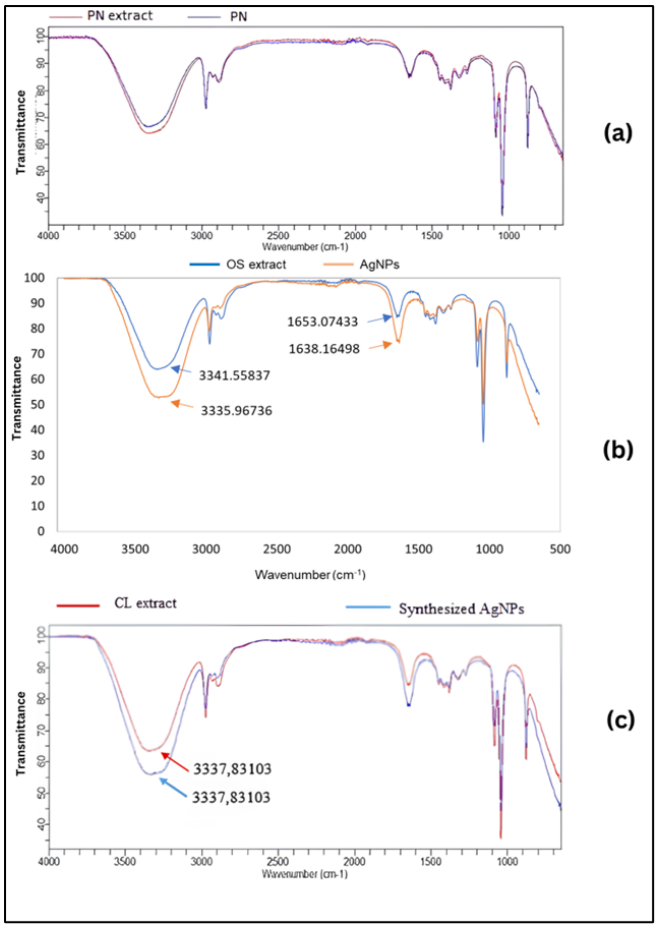

Figure 3. FTIR spectra of the extracts and the synthesized AgNPs by (a) PN (b) OS (c) CL extracts before purification.

In summary, this study successfully optimized the physical characteristics of PN, OS, and CL extracts for the green synthesis of AgNPs. The observed color changes and UV-Vis spectra confirmed the formation of AgNPs, showcasing the potential of green synthesis methods using plant extracts for large-scale production of AgNPs. However, before conducting in vitro and in vivo tests for developing various dosage forms, it is essential to optimize the volume of NaOH and adjust the synthesis volume to the available centrifugation equipment capacity.

This study also sheds light on the challenges and limitations of synthesizing AgNPs on a large scale, such as the need to optimize the centrifugation duration for the complete precipitation of AgNPs. Further optimization of the synthesis process is necessary to increase AgNPs yield, which has significant implications for their potential applications in various fields, including medicine and industry.

3. CONCLUSION

Scale-up of the synthesis of silver nanoparticles using ethanol extract of *Phyllanthus niruri* herbs, *Orthosiphon stamineus*, and *Curcuma longa* rhizome has been carried out. The duration of centrifugation at the AgNPs collection step was optimized, and 5 min was concluded as the minimum centrifugation duration to obtain optimal AgNPs. The synthesized AgNPs on a large scale with a minimum duration of centrifugation produced the yield of 0,98% (PN), 0.20% (OS), 0,66% (CL)(w/w), with a spherical shape, MI size of 169 nm (PN); 179.1 nm (OS); 623.0 nm (CL). PDI value of 0.2202 (PN); 0.2579; (OS); 0.00872 (CL). Further optimization of the synthesis process is required to increase the yield of AgNPs, which has important implications for their potential applications in various fields, including medicine and industry.

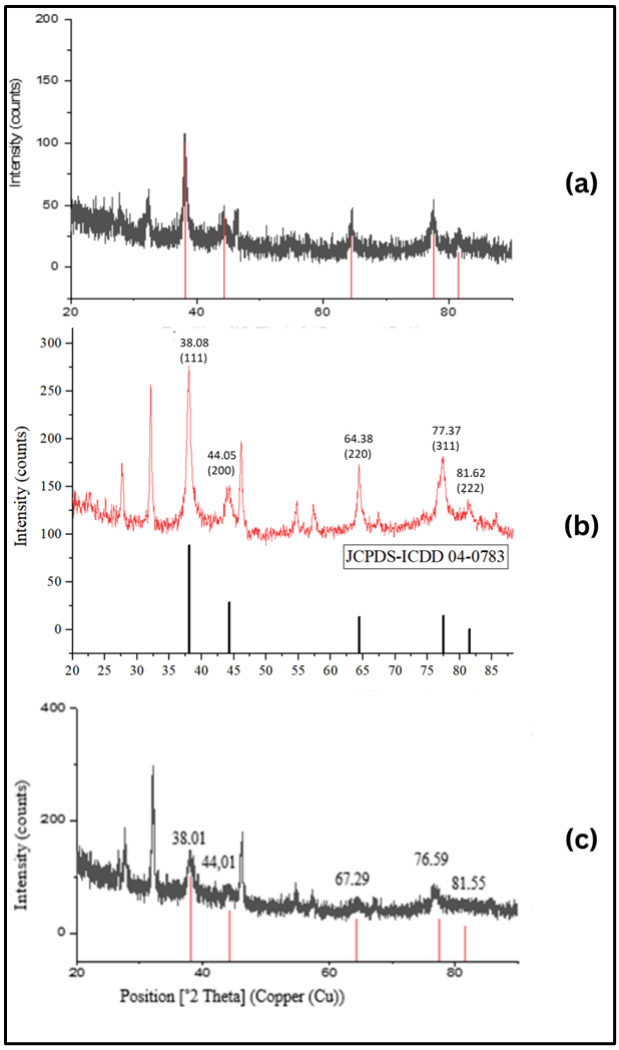

Figure 4. XRD pattern of synthesized AgNPs and standard AgNPs (a) PN (b) OS (c) CL.

4. MATERIALS AND METHODS

4.1. Plant materials and chemicals

Phyllanthus niruri herbs, *Orthosiphon stamineus* leaves, and *Curcuma longa* rhizomes were obtained from the Center for Research and Development of Medicinal Plants and Traditional Medicines (Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional - B2P2TOOT), Tawangmangu, Central Java, Indonesia. The leaves and herbs were authenticated by the Center of Information and Development of Traditional Medicine (Pusat Informasi dan Pengembangan Obat Tradisional - PIPOT), University of Surabaya, with certificate number 1434/DT/2021. Collected herbs, leaves, and rhizomes were dried to constant weight in the dark at room temperature. The chemicals used were silver nitrate (Merck, Darmstadt, Germany), sodium hydroxide (Merck, Darmstadt, Germany), ethanol (PT. Smart-Lab, Tangerang, Indonesia), and distilled water.

4.2. Preparation of *Phyllanthus niruri* herbs extracts, *Orthosiphon stamineus* leaves extracts, and *Curcuma longa* rhizomes extracts.

The dried PN herbs, OS leaves, and CL rhizome were crushed using a blender to a powder, and then each was sieved using a 20 Mesh Sieve. The sifted powder results were PN, OS, and CL crude drugs powder. Making PN, OS, and CL extract using the Ultrasound-Assisted Extraction (UAE) method. First, 10.0 grams of PN, OS, and CL crude drugs powder was weighed, then 50 mL of 80% ethanol solvent was added. Then extracted using an ultrasonic bath for 10 minutes. The extraction results were filtered using a Buchner funnel and put into a 100 mL volumetric flask. Then, the dregs obtained were added again with 50 mL of 80% ethanol solvent and filtered into the same volumetric flask. The entire filtrate was added with 80% ethanol until it reached a volume of 100.0 mL. The solution was used as the parent extract with a concentration of

10% (w/v). The results of the primary solution PN, OS, and CL were pipetted respectively 50.0 mL, 12.5 mL, and 50.0 mL, then added 80% ethanol until 1000.0 mL to obtain test extracts with concentrations of PN (0.50%), OS (0.125%), and CL (0.50%).

4.3. Synthesis of AgNPs using PN, OS, and CL extracts.

From 1000 mL of the sample extract, 10.0 mL was removed, then replaced by adding 10.0 mL of 100 mM $AgNO_3$ to obtain one mM $AgNO_3$ in the mixture. The synthesis process was carried out by constant stirring using a magnetic stirrer (Thermo Scientific Cimarec SP88857105, Barnstead Thermolyne, NH, USA) at 150 rpm (PN), 70 rpm (OS), 190 rpm (CL) for 30 minutes (PN, CL), 15 minutes (OS) at 30°C (PN, OS), and 60°C (CL) according to Kartini (2020). Then, 100 µL of 0.2 M NaOH was added at 0, 5, 10, 15 minutes and 0, 10, 20, and 30 minutes (PN, CL), and after the addition of NaOH, the color change of the solution and its UV-Vis spectrum (λ= 300-500 nm) were observed using a spectrophotometer (Shimadzu UV-1900, Tokyo, Japan). The color change from light-green to reddish-brown and the appearance of absorbance at 400-450 nm indicates the formation of AgNPs.

Next, 40 mL of the solution mixture containing AgNPs was centrifuged using Sorvall Biofuge Stratos Centrifuge (Thermo Scientific Nalgene, Rochester, NY, USA) for 15 minutes at 2,500 rpm to remove undesired components. After centrifugation, the supernatant was taken and centrifuged at 10,000 rpm at various durations (0, 10, 20, 30 for PN. 5, 10, 15, 20 minutes for OS. 5, 10, 15, 30, 45 for CL) to collect AgNPs. The supernatant produced for each centrifugation duration was then observed for its color and UV-Vis spectrum to determine the minimum duration required to precipitate the AgNPs. Finally, the results in pellets were washed several times using distilled water, then stored in a desiccator until reaching their constant weight to obtain dried AgNPs [19].

4.4. Characterization of AgNPs

In this study, the synthesized AgNPs were characterized using several techniques. These techniques included SEM (Hitachi Flexsem 100, Tokyo, Japan), which was used to observe the morphology of the particles, as well as a particle size and zeta potential analyzer (Microtrac Nanotrac Wave II, Haan, Germany) to determine the particle size, size distribution, and zeta potential. In addition, FTIR (Agilent Cary 630, Santa Clara, CA, USA) was used to identify the functional groups on the surface of the particles, and XRD (PANanalytical BV, Almelo, the Netherlands) was used to analyze the crystallinity of the synthesized AgNPs.

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