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Synthesis of silver (Ag) nano/micro-particles via green process using *Andrographis paniculata* leaf extract as a bio-reducing agent

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Abstract

In this work silver nano/micro-particles have been synthesized using *sambiloto* (*Andrographis paniculata*) plant extract as a bio-reducing agent. The effects of different plant extract concentrations, $AgNO_3$ precursor concentrations, and reaction time on the synthesized silver nano/micro-particles were investigated. The silver nano/micro-particles samples were then analyzed using UV-Vis spectrophotometer (UV-Vis), X-Ray Diffractometer (XRD), Field Emission Scanning Electron Microscopy (FESEM), Particle Size Analyzer (PSA), and Fourier Transform Infra-Red (FTIR) spectroscopy. The UV-Vis absorbance spectrum of the colloid silver nano/micro-particles exhibited that all samples had absorbance peaks at a wavelength around 450 nm, confirming the formation of silver nano/micro-particles. It was also found that the UV-Vis absorbance peak of the silver nano/micro-particles inversely increased with decreasing $AgNO_3$ solution concentration. Whereas, the higher the *sambiloto* extract concentration the higher the UV-Vis absorbance peaks. The UV-Vis absorbance peak increased with increasing synthesis time, suggesting that silver nano/micro-particles became more prominent. The UV-Vis absorbance peaks of the silver nano/micro-particles were about 0.0462, 0.0637, 0.0729, and 0.0936 at reaction time of 5, 10, 20, and 40 min, respectively. The XRD analysis result confirmed that the synthesized silver nano/micro-particles were in the form of nanocrystals with a face-centered cubic centered without any impurities. Additionally, the FESEM images showed that the silver nano/micro-particles had the primary particle size of 150-300 nm. There was the formation of some secondary particles with the size of about 0.7-1.5µm due to the agglomeration of primary particles. The particle size distribution analysis further confirmed the presence of primary and secondary particles. Meanwhile, the FTIR analysis confirmed the presence of four main peaks, linked to functional groups in the *sambiloto* extract and involved in the creation of silver nano/micro-particles.

Keywords: Silver nano/micro-particles; *Andrographis paniculata*; bio-reducing agent; SEM; XRD; UV-Vis; FTIR

1. Introduction

Nanotechnology is a very promising field of science and technology that has rapidly developed over the years and considered by many to be the next industrial evolution [1,2]. Nanotechnology-based products have also been applied in many fields including materials, energy, electronics, construction, pharmaceuticals, environmental, agriculture, and daily consumer and household products such as food and cosmetics [1–4]. Many other technology industries have been benefited from nanotechnology innovation and its impact is expected to increase significantly in future [2,3]. Nanotechnology based products can be classified into several categories such as nanomaterials (e.g. nanoparticles, nanofibers, nanotubes, nanofilms, and nanocomposites.), nanodevices, and nanosensor. [2,5–7]. Materials in nanometer size also called nanomaterials or nanoparticles are notably to have some unique chemical, physical, and biological properties that are far different than their normal sizes or bulk states [8]. Among the interesting nanoparticles are noble metal nanoparticles. Noble metals like gold and metal have been used since the ancient Egyptian civilization as the symbol of wealth and superiority even until now. These noble metals are commonly more expensive than other metals [9,10]. Among the noble metal nanoparticles, silver nanoparticles have been widely used by many researchers due to their enhanced antimicrobial, high thermal, electrical, and optical properties

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[8]. Silver nanoparticles have been utilized in many applications such as sensors (e.g, chemiluminescence, colorimetric, and fluorescence sensors) due to their surface plasmon characteristics. Hence, they can be applied to detect some pollutants like heavy metals in the environment [11,12]. Additionally, due to their cytotoxicity against cancer cells, silver nanoparticles can also be used for early cancer diagnostic and treatment [8]. They have been applied in catalysis, optoelectronics, pharmaceuticals, and cosmetics as well [13].

There are two categories of methods to produce silver nanoparticles, top-bottom (physical method) and bottom-up (chemical and biological methods). Physical methods include evaporation-condensation, electrical irradiation, or gamma irradiation. Chemical method, meanwhile, includes electrochemical, chemical reduction, microemulsion, and solvothermal. Whereas, biological method includes bacteria, yeast, algae, fungi, and plant extract [13–15]. The latter is also called as biosynthesis. This method has been widely used and studied by many researchers due to being more environmentally friendly and less harmful compared to the chemical method [16]. Fig. 1 shows a schematic illustration of the biosynthesis of silver nanoparticles. This work focused on the synthesis of silver nanoparticles using biological method or biosynthesis especially using the plant extract. The utilization of plant extract for the biosynthesis of silver nanoparticles becomes more attractive to many researchers than other biological matters such as fungi, yeast, algae, and bacteria. It is because the plants (mostly the leaf part) are found easier to obtain and extract, low cost, and safe for humans [17, 18].

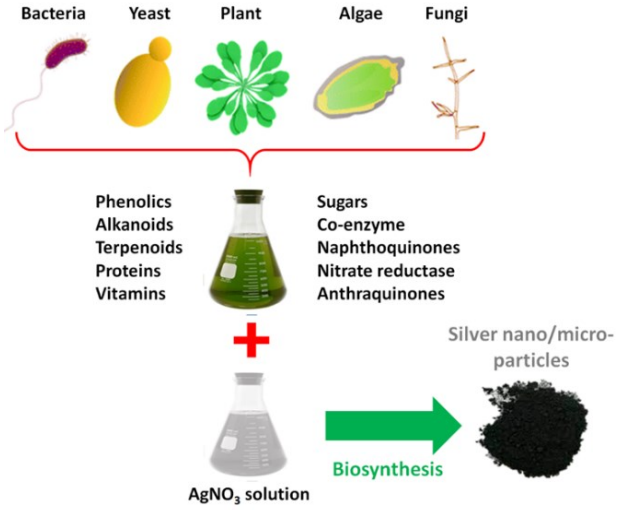

Fig. 1. Schematic graphic of biosynthesis procedure of silver nano/micro-particles using biological methods. Adopted from literature [14]

Hemlatta et al. [19] studied the production of silver nano-particles using leaf extract of *Cucumis prophetarum* and found that the synthesized silver nanoparticles had a good antimicrobial activity against bacteria and potential antiproliferative activity against some cancer cells. There are many literature that studied the biosynthesis of silver nanoparticles using different types of plant extract, such as *Cocous nucifera* [20], *Aloe vera* [21], *Musa paradisiacal* [22], *Moringa oleifera* [23], *Citrus sinensis* [24], *Nelumbo nucifera* [25]. Nevertheless, there are still many plant extracts that have not been studied yet or literature discussing about them, so far, are still rare.

Andrographis paniculate, usually called as *sambiloto* by the local people of Indonesia, is one of the famous herbal plants. There have been a few literature studying the biosynthesis of silver nanoparticles using *sambiloto* leaf extract. This study conducted the synthesis of silver nano/micro-particles using *sambiloto* leaf extract. It also investigated the effects of various $AgNO_3$ concentrations, *sambiloto* leaf extract concentrations, and synthesis time on silver nano/micro-particles production.

2. Materials and Methods

2.1. Materials and equipment

Obtained from a local chemical store, $AgNO_3$ was the main precursor used to synthesize silver nano/micro-particles. Fresh *Andrographis paniculata* leaf, usually called *sambiloto* by local people, was obtained from a local place in Yogyakarta city, Indonesia. Some villagers in Yogyakarta city commonly use this plant as a raw material for herbal medicine. To synthesize the silver nano/micro-particles, we used common labwares such as Erlenmeyer flask, glass beaker, thermometer, magnetic stirrer/hot plate, filter paper, oven, measuring glass, pipette, and funnel. Fig. 2 shows the photographs of dried *sambiloto* leaf, extract *sambiloto* leaf, and synthesized silver nano/micro-particles.

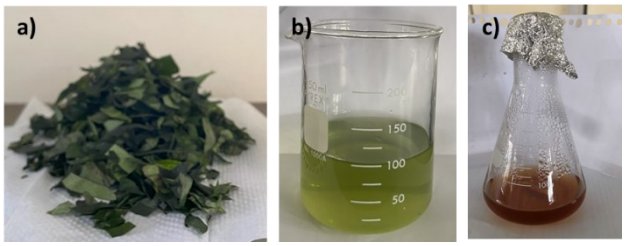

Fig. 2. Photographs of (a) dried *sambiloto* plant extract, (b) *sambiloto* extract, (c) synthesized silver nano/micro-particles

2.2. Synthesis of silver nano/micro-particles

To prepare *sambiloto* extract, the following steps were conducted. First, fresh *sambiloto* leaves were cleaned using tap water and washed using distilled water. The *sambiloto* leaf extract was dried at room temperature for a day, and then the dried *sambiloto* leaves were cut into small pieces. About 200 mL of distilled water was boiled and agitated using a hot magnetic stirrer. After boiling, the temperature of the hot magnetic stirrer was decreased to 60-70°C. Subsequently, small pieces of dried *sambiloto* leaves (about 20 g) were put into the hot distilled water and stirred for about 1 hour. It was then continued by cooling the *sambiloto* extract before being filtered by Whatman filter paper. This concentrated *sambiloto* extract was put in the bottle glass and in the refrigerator for further use. The concentrated *sambiloto* extract was then diluted to 0.5 % and 1 % w/v. These two extract concentrations were then labeled as Extract-A and Extract-B.

2.3. Preparation of sambiloto extract

The following steps were conducted to synthesize silver

nano/micro-particles. First, about 50 mL solutions of silver nitrate ($AgNO_3$) with different concentrations (i.e., 0.0125 M, 0.025 M, 0.05 M, and 0.075 M) were prepared in an Erlenmeyer flask. Afterward, the Erlenmeyer flask was put on the hot magnetic stirrer, and the temperature was set at about 50°C while being stirred with a magnetic stirrer. While waiting for the temperature to reach the desired temperature, the concentrated *sambiloto* extracts (i.e., Extract-A and Extract-B) were taken from the refrigerator and stirred until being homogeneous. About 10 mL of both extracts were subsequently added into the silver nitrate solution (in an Erlenmeyer flask) and continuously stirred for 1 h. Table 1 shows the experimental design of the synthesis of silver nano/micro-particles. Further, about 5 mL of the synthesized colloidal silver nano/micro-particles (A-0.025) was taken from the Erlenmeyer flask in each 5, 10, 20, and 40 min and analyzed using a UV-Vis spectrophotometer. Fig. 3 shows the schematic procedure of the synthesis of silver nano/micro-particles via the "green" method using *sambiloto* leaf extract as a reducing agent. After finishing (60 min), the synthesized colloidal silver nano/micro-particles were then centrifuged at 9000 rpm for 10 min to separate the silver nano/micro-particles from the colloid. Afterward, the sediment of silver nano/micro-particles was washed using DI water and ethanol and centrifuged again. The final sediment was dried in the oven at 80°C overnight.

Table 1. Experimental design of synthesis of silver nano/micro-particles

Extract conc. (wt%)	$AgNO_3$ conc. (M)	Sample name
	0.0125	A-0.0125
0.5 % w/v	0.025	A-0.025
(Extract-A)	0.05	A-0.05
	0.075	A-0.075
	0.0125	B-0.0125
1 % w/v	0.025	B-0.025
(Extract-B)	0.05	B-0.05
	0.075	B-0.075

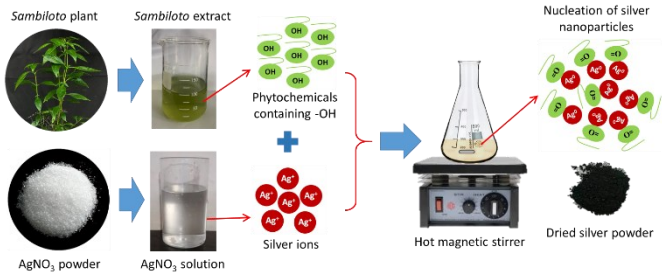

Fig. 3. Schematic procedure of biosynthesis of silver nano/micro-particles

2.4. Characterization of silver nano/micro-particles

The synthesized silver nano/micro-particles samples were analyzed using UV-Vis spectrophotometer (UV-Vis), X-Ray Diffractometer (XRD), Field Emission Scanning Electron Microscopy (FESEM), Particle Size Analyzer (PSA), and Fourier Transform Infra-Red (FTIR) spectroscopy. The FESEM analysis was done at a magnification of 20,000 X and 40,000 X. For the UV-Vis spectroscopy analysis, the UV-Vis analysis wavelength was at 350-600 nm. Whereas, for the FTIR, the wavenumber range was at 4000-500 cm^{-1}. The particle size analysis was done using patented Polarization Intensity Differential Scattering (PIDS) technology which allowed the analysis of powdered particles from 40 nm up to 2µm in diameter. Furthermore, the XRD analysis was conducted to confirm the nanocrystal structure of silver nano/micro-particles produced. The XRD analysis was done at a 2-theta scan range of 30-80º, scan rate of 10.0 deg/min, and scan step of 0.01. For the XRD analysis, only one sample was taken for the analysis due to the limited amount of powder produced.

3. Results and Discussion

3.1. UV-Vis analysis

To check the presence or formation of silver nano/micro-particles in the colloid after the synthesis process, a UV-Vis spectroscopy analysis was conducted. About 5 mL of synthesized colloid was taken for the analysis. Fig. 4 shows the UV-Vis analysis of silver nano/micro-particles prepared at different concentrations of $AgNO_3$ solutions (i.e., 0.0125 M, 0.025 M, 0.05 M, and 0.075 M) and *sambiloto* leaf extract concentrations (i.e. A = 0.5 wt%, and B = 1 wt%). Based on the literature, the presence of silver particles in the colloid can be detected at the UV-Vis wavelength range of 400- 500 nm [26]. In this work, the UV-Vis analysis was conducted at the wavelength range of 350-600 nm. As seen in Fig. 4, the peak UV-Vis spectrum of all silver nano/micro-particles samples was at the wavelength of around 450 nm, confirming the presence of silver nano/micro-particles in the synthesized colloid. Additionally, there was a similar trend in the UV-Vis spectrum of silver nano/micro-particles prepared using *sambiloto* extract concentration of 0.5 % and 1 % w/v, as shown in Fig. 4(a) and 4(b), respectively.

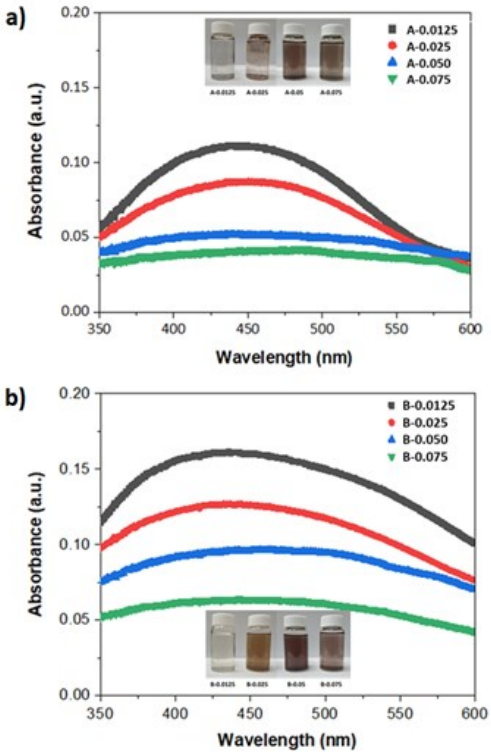

Fig. 4. UV-Vis analysis of silver nano/micro-particles synthesized at different concentrations of $AgNO_3$ solutions and *sambiloto* leaf extract concentrations

As noticed in both of the Figures, as the $AgNO_3$ solution zconcentration decreased, the UV-Vis peak spectrum inversely increased. The highest UV-Vis peak spectrum was noticed in the sample with the lowest $AgNO_3$ concentration of 0.0125 M with the absorbance values of 0.1115 and 0.1615 for silver nano/micro-particles samples of A-0.0125 and B-0.0125 M, respectively. Additionally, as observed in the inset of Fig. 4, the turbidity of colloidal silver nano/micro-particles prepared at higher $AgNO_3$ solution concentration was also higher or denser. Qualitatively, it can be suggested that the higher $AgNO_3$ solution concentrations, the more silver nano/micro-particles produced. Table 2 shows the summary of UV-Vis peak absorbance of all the silver nano/micro-particles samples. It was found that with decreasing $AgNO_3$ solution concentration, the UV-Vis absorbance peak of the silver nano/micro-particles inversely increased. The similar results can also be found in other literature [27–29].

Table 2. UV-Vis peak absorbance of silver nano/micro-particles samples

Extract conc. (wt%)	$AgNO_3$ conc. (M)	Sample name	UV-Vis peak absorbance (a.u.)
	0.0125	A-0.0125	0.1115
0.5 % w/v	0.025	A-0.025	0.0876
(Extract-A)	0.05	A-0.05	0.0520
	0.075	A-0.075	0.0424
	0.0125	B-0.0125	0.1615
1 % w/v	0.025	B-0.025	0.1271
(Extract-B)	0.05	B-0.05	0.0967
	0.075	B-0.075	0.0642

Additionally, to investigate the effect of *sambiloto* leaf extract concentration on the silver nano/micro-particles synthesis, the UV-Vis spectrum of silver nano/micro-particles synthesized by the two extract concentrations, 0.5 % w/v (Extract-A) and 1.0 % w/v (Extract-B) were plotted and compared for all the four $AgNO_3$ solution concentrations, as shown in Fig. 5. As noticed, all the Figures show the similar trend in which the UV-Vis absorbance peaks of all Extract-A samples were higher than those of the Extract-B samples. It can be qualitatively concluded that the higher the use of extract concentration, the higher the amount of silver nano/micro-particles produced. This result is similar to the literature [30,31]. The values of each absorbance peaks are summarized in Table 2.

Furthermore, the effect of synthesis time on the silver nano/micro-particles formation was also studied. Fig. 6 shows the evolution of the UV-Vis spectrum of silver nano/micro-particles as the reaction time increased (i.e., 5, 10, 20, 40 min). As expected, with the increasing reaction time, the UV-Vis absorbance peak also increased, suggesting that the presence of silver nano/micro-particles became more prominent. This result was in accordance with the literature [29, 30]. The UV-Vis absorbance peaks of the silver nano/micro-particles were about 0.0462, 0.0637, 0.0729, and 0.0936 at reaction time of 5, 10, 20, and 40 min, respectively.

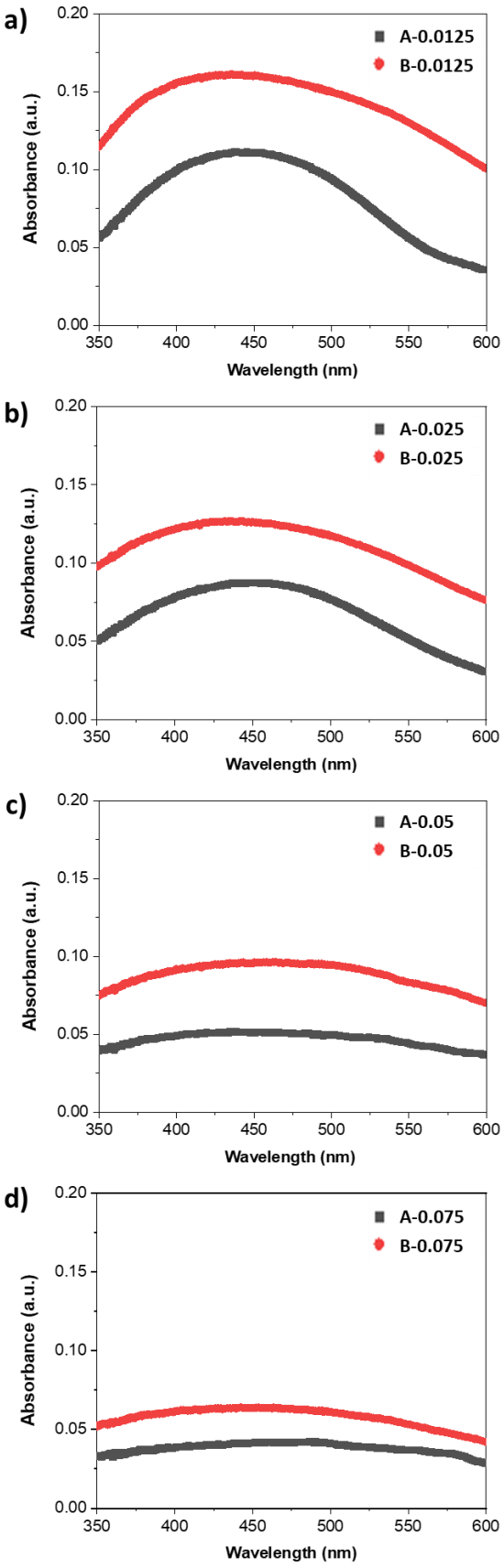

Fig. 5. Comparison of UV-Vis spectrum of silver nano/micro-particles synthesized by the two extract concentrations, 0.5 % w/v (Extract-A) and 1.0 % w/v (Extract-B)

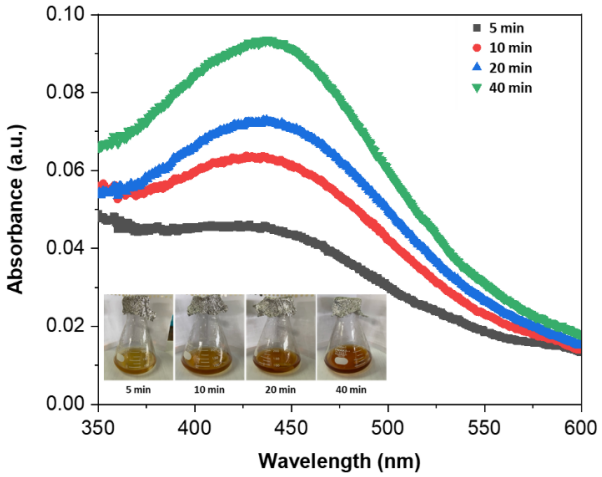

Fig. 6. UV-Vis analysis of silver nano/micro-particles prepared at different reaction time (i.e. 5, 10, 20, 40 min)

3.2. X-ray diffractometer (XRD) analysis

The XRD analysis was conducted to confirm the nanocrystallinity of the powdered silver nano/micro-particles synthesized using *sambiloto* leaf extract. The XRD analysis was conducted at the 2-theta range of 30-80º. Fig. 7 shows a typical XRD spectra of the produced silver nano/micro-particles (Sample B-0.050). As noticed, the XRD pattern of the synthesized silver nano/micro-particles sample was similar to the XRD standard pattern of PDF#04-0783 [32].

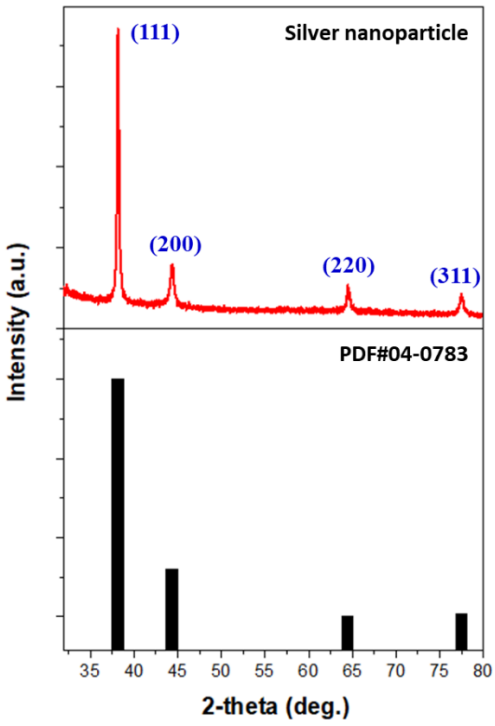

Fig. 7. XRD spectra of the silver nano/micro-particles

As noticed in the Figure, the XRD peaks of the silver nano/micro-particles were at 2-theta values of 38. 16°, 44.38°, 64.51°, and 77.45°, which corresponded to (111), (200), (220), and (311) Bragg's planes, respectively [33,34]. Other researchers have found similar results using different bio-based active materials for the biosynthesis of silver nano/micro-particles [33–37]. This evidence showed that the silver nano/micro-particles were in the form of nanocrystals with face-centered cubic centers. Additionally, the XRD spectra of the silver nano/micro-particles showed no additional peaks. Thus, it can be concluded that the use of *sambiloto* leaf extract as a reducing agent could successfully synthesize a pure phase cubic nanocrystal structure of silver nano/micro-particles.

3.3. Field emission scanning electron microscopy (FESEM)

The FESEM analysis was also conducted to study the morphology of the synthesized silver nano/micro-particles. Fig. 8 shows the FESEM images of the silver nano/micro-particles sample of B-0.050, prepared using Extract-B and $AgNO_3$ solution concentration of 0.050 M. The FESEM analysis was conducted at two magnifications of 20,000 X and 40,000 X. As noticed, the silver nano/micro-particles had a spherical-like shape with the primary particle size in the range of approximately 150-300 nm. The shape and size of the primary silver nano/micro-particles, however, were not uniform. There was also some agglomerations of primary particles that formed some secondary particles with a size of about 0.7-1.5µm. The big size of silver nano/micro-particles was likely caused by the high concentration of $AgNO_3$ solution. It has been confirmed by the UV-Vis analysis results explained in the previous paragraph. It was suggested that the higher the $AgNO_3$ solution concentration, the bigger the size of the silver nano/micro-particles synthesized [38]. Nevertheless, the FESEM images of the rest of the samples were not available in this study.

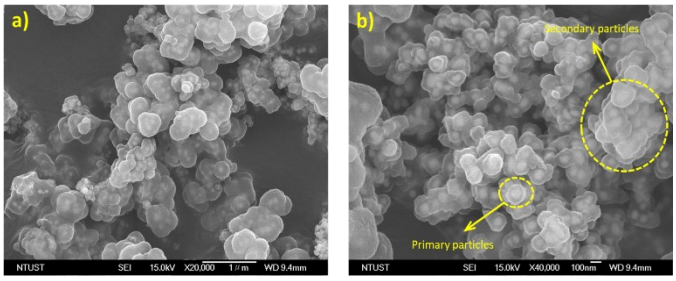

Fig. 8. SEM image of silver nano/micro-particles with different magnification, a) 20,000X and b) 40,000X (Sample B-0.050)

3.4. Particle size distribution (PSD) analysis

Particle size distribution (PSD) analysis was also conducted to further study about the particle size distribution of the silver nano/micro-particles. Fig. 9 shows the particle size distribution of the silver nano/micro-particles at two different *sambiloto* extract concentrations (i.e., Extract-A and Extract-B) and $AgNO_3$ solution concentrations (0.050 and 0.075 M). As noticed from the Figure, the particle size of the silver nano/micro-particles synthesized using higher *sambiloto* extract concentration (i.e., 1% w/v or Extract-B) provided smaller particle size than the one prepared at lower *sambiloto* extract concentration (i.e., 0.5% w/v or Extract-A). This result was in accordance with the UV-Vis analysis results (see Fig. 5). Moreover, as observed from the particle size distribution histogram, the percentage number of silver nano/micro-particles prepared using the same *sambiloto* extract concentration at two different $AgNO_3$ solution concentrations

(i.e. A-0.050 vs A-0.075 and B-0.050 vs B-0.050) were quite similar. These results were in accordance with the UV-Vis analysis results (see Fig. 4), where the UV-Vis spectrum curve and absorbance peaks of A-0.050 vs. A-0.075 and B-0.050 vs. B-0.075 were almost similar.

Additionally, as observed in Fig. 9(a), there were two particle size distribution histograms. Whereas, in Fig. 9(b), there were three particle size distribution histograms. The presence of secondary and tertiary histogram was likely due to the presence of secondary and tertiary silver nano/micro-particles, which were formed due to the agglomeration of primary silver nano/micro-particles. This finding was consistent with the FESEM images (see Fig. 8), which revealed the presence of primary and secondary particles due to agglomeration. Consequently, there were also more than one median of particle size, usually called as d_{50}. The d_{50} of silver nano/micro-particles prepared using Extract-A were 680 nm and 1.45 µm, whereas Extract-B were 220 nm; 700 nm; and 1.6 µm.

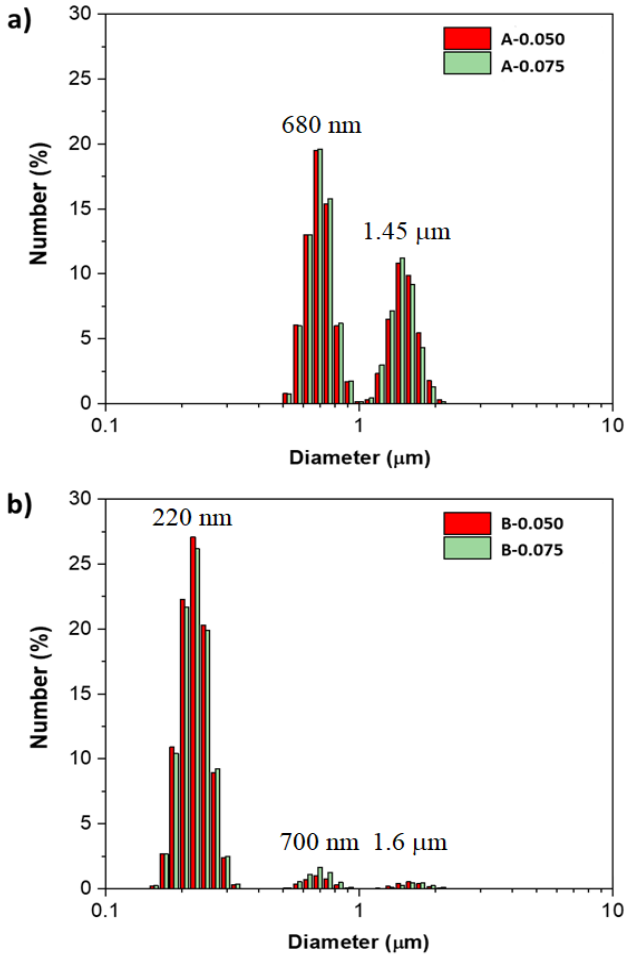

Fig. 9. Particle size distribution of silver nano/micro-particles prepared by two extract concentrations (i.e. Extract-A and Extract-B) and two $AgNO_3$ solution concentrations (i.e. 0.050 and 0.075 M)

3.5. Fourier transform infra-red (FTIR) spectroscopy

Furthermore, an FTIR analysis was also conducted to investigate the possible chemical or functional groups present on the surface of the synthesized silver nano/micro-particles. Fig. 9 shows the FTIR spectrum of silver nano/micro-particles (sample B-0.050) with four main peaks at positions of 3423, 1645, 1380, and 1072 cm^{-1}. The intense broadband at 3423 cm−1 could be related to the O−H stretching mode, whereas the band at 1645 cm−1 was the characteristic of amid (NH)–C=O and C=C stretching vibration. The characteristic of N–H stretching was at a wavenumber of 1341.01 cm^{-1} [39]. Additionally, the bands at around 1072 cm^{-1} were related to primary amine C–N stretching vibrations [34, 40]. These four main peaks were attributed to the functional groups belonging to *sambiloto* extract, involved in the formation of silver nano/micro-particles [41].

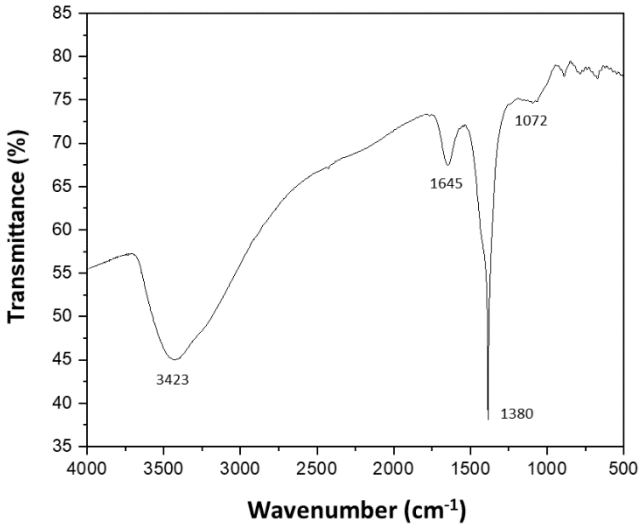

Fig. 10. The FTIR spectrum of silver nano/micro-particles (Sample B-0.050)

4. Conclusions

Silver nano/micro-particles have been successfully synthesized via "green" method using *Andrographis paniculate* (*sambiloto*) leaf extract. The UV-Vis analysis results showed that all synthesized silver nano/micro-particles had absorbance peaks at the wavelength around 450 nm, which confirmed the presence or formation of silver nano/micro-particles in the synthesized colloid silver nano/micro-particles. It was also found that with decreasing $AgNO_3$ solution concentration, the UV-Vis absorbance peak of the silver nano/micro-particles inversely increased. Whereas, the higher the *sambiloto* extract concentration, the higher the UV-Vis absorbance peaks. Moreover, with increasing reaction time, the UV-Vis absorbance peak also increased, suggesting the presence of silver nano/micro-particles became more prominent. Furthermore, the XRD analysis results confirmed that the synthesized silver nano/micro-particles were in the form of nanocrystals with face-centered cubic centered without any impurities. Additionally, FESEM images showed that the silver nano/micro-particles had a spherical-like shape with the primary particle size in the range of approximately 150-300 nm. The shape and size of the primary particles, in this case, were not uniform. There were also some agglomerations of primary particles forming some secondary particles with the size of about 0.7-1.5µm. This result was confirmed by the particle size distribution analysis. Furthermore, according to the FTIR analysis, there were four main peaks that were caused by functional groups in the *sambiloto* extract, involved in making the silver nano/micro-particles.

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