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Submission date: 19-May-2023 12:43PM (UTC+0800)
Submission ID: 2096814448
File name: 2023-C116_Fast_Microwave_IJC.pdf (530.21K)
Word count: 5037
Character count: 27567

Fast Microwave-Assisted Green Synthesis of Silver Nanoparticles Using Low Concentration of Seminyak (*Champeria* sp.) Leaf Extract

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Received: October 21, 2022
Accepted: February 22, 2023

DOI: 10.22146/ijc.78545

Abstract: *Silver nanoparticles (AgNPs) are fascinating materials for biomedical applications thanks to their strong antibacterial activity and biocompatibility. This study applied the green synthesis method using 0.5 wt.% Seminyak leaf extract and assisted with one min microwave irradiation to enhance AgNPs formation. Extremely small sizes AgNPs with an average particle size of 9.1 ± 4.1 nm and spherical shapes were obtained. The synthesized AgNPs displayed potent antibacterial activity against* Escherichia coli *and* Staphylococcus aureus *bacteria with a zone of inhibition of 12.3 ± 0.1 and 13.7 ± 0.7 mm, respectively. The MTT assay results demonstrated that the cells' viability of the obtained AgNPs was 88.5 ± 7.0 %, implying biocompatibility for biomedical applications.*

Keywords: *antibacterial materials; green synthesis; microwave irradiation; Seminyak leaf extract; silver nanoparticles*

■ INTRODUCTION

Currently, the demand for anti-infection implants has risen over time as an attractive approach to solving the implant-related infection problem during implantation. Many materials have been attempted to prevent implant-related infection, including peptides [1], chitosan [2], and silver nanoparticles [3]. Among those options, silver nanoparticles (AgNPs) emerged as one promising material as a filler for anti-infection implants, thanks to their potent antibacterial properties and excellent biocompatibility [4]. In addition, AgNPs also possess high electrical conductivity, making them potentially utilized as a filler for electroactive scaffold fabrication [3]. However, the utilization of hazardous reducing agents such as sodium borohydride ($NaBH_4$) and hydrazine (N_2H_4) during the fabrication of AgNPs has raised concerns for biomedical applications because of their toxicity [5].

In this context, AgNPs preparation through a green synthesis approach using plant extract is an appealing strategy because it offers non-toxic, cost-effective, and environmentally friendly methods [6]. Extracts from plants contain biomolecules such as proteins, amino acids, flavonoids, and polyphenols that act as reducing agents for AgNPs formation and as capping agents to prevent agglomeration of nanoparticles [6]. Various plant extracts such as butterfly pea flower extract (*Clitoria ternatea*) [4], Cilembu sweet potato (*Ipomoea batatas* L. var. Rancing) [3], and *Ferula ovina* Boiss. [7] have been explored to produce AgNPs with excellent antibacterial activity and extremely small nanoparticle sizes below 17 nm. Although many plants are already exploited for the green synthesis of AgNPs, there are plenty of unexplored plants that might offer unique characteristics. One of them is the Seminyak (*Champeria* sp.), an endemic plant in Riau province, Indonesia. Seminyak plant, particularly the leaf part, is utilized for the local diet and as an alternative medicine for headaches and stomachaches thanks to its excellent medicinal properties. Moreover, Seminyak leaf, as one of the plants from the genus *Champeria*, contains high concentrations of secondary metabolites such as flavonoids, phenolic, lutein, phytol, and β-carotene, which can be potentially utilized to reduce Ag^+ to produce AgNPs [8-9].

In general, water was used as a solvent in the green synthesis approach to extract useful organic compounds from plants to avoid the utilization of toxic organic solvents. However, the concentration of organic compounds in the obtained extract solution is sometimes too scarce for AgNPs formation due to its low concentration in the original plant or its low solubility in water. A higher extract concentration can be used to obtain the necessary quantity of reducing agents for AgNPs formation, but this strategy might not be sustainable. A sustainable and green strategy to combat this problem is by combining plant extract with microwave irradiation. Microwave irradiation has been studied extensively in the synthesis of AgNPs and succeeded in providing rapid initial heating, which increasing the reaction rate [10]. Francis et al. [11] successfully fabricated tiny AgNPs with robust antibacterial properties after combining *Elephantopus scaber* leaf extract and 4 min of microwave irradiation. In addition, it requires less energy than conventional heating methods [12] and is suitable for obtaining monodispersed nanoparticles with higher crystallinity in a short reaction time [11].

In this study, AgNPs were synthesized using 0.5 wt.% of Seminyak leaf extract and 1 min of microwave irradiation time. To the best of our knowledge, the utilization of Seminyak leaf extract together with AgNPs fabrication strategy to employ low extract concentration and short microwave irradiation time has not been explored yet. Thus, we expected this work could offer a rapid, sustainable, and safe synthesis method in AgNPs preparation for biomedical applications.

■ EXPERIMENTAL SECTION

Materials

Silver nitrate ($AgNO_3$), sodium hydroxide (NaOH), sodium chloride (NaCl), sodium dodecyl sulfate (SDS), and methanol with p.a. grade were acquired from Merck, Germany, without further modification. Biological reagents such as culture media containing Roswell Park Memorial Institute-1640 (RPMI-1640) 89%, Pen-Strep 1%, Fetal Bovine Serum (FBS) 10%, fungizone, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent, phosphate buffer saline (PBS), ciprofloxacin, Mueller Hinton Agar (MHA) media, and Formazans dyes were procured from Oxoid. Alcohol 70%, sterile distilled water, and demineralized water were purchased from Brataco Chemical, Indonesia. Seminyak leaf was obtained from the market in Riau, Indonesia. *Escherichia coli* (*E. coli*, ATCC 25922), *Staphylococcus aureus* (*S. aureus*, ATCC 25923), and cell line culture BHK-21 were supplied by Tropical Diseases Diagnostic Center, Universitas Airlangga.

Instrumentation

UV-Vis spectrophotometry (Hewlett Packard Agilent Technologies, 8453 Series) was carried out to confirm the existence of AgNPs in the sample by observing their surface plasmon resonance (SPR). The

functional groups of the extract and AgNPs were investigated by Fourier Transformation Infrared Spectroscopy (FTIR, Prestige 21 Shimadzu). X-ray diffraction (XRD, Bruker D8 Advance, using Cu Kα, λ = 1.54 Å) was performed to analyze the phase structure and crystallinity of the AgNPs. Dynamic Light Scattering (DLS, Beckman Coulter, Delsa Nano C Particle Analyser) was carried out to investigate the particle size and distribution of AgNPs. The stability of AgNPs was measured by Zeta Potential (Zetasizer Nano, Malvern Pananalytical Ltd). Morphological and particle size of the AgNPs were observed using Transmission Electron Microscopy (TEM, Hitachi HT77000 Series).

Procedure

Extract preparation

Seminyak leaf extract (hereinafter denoted as extract) was prepared based on the previous procedure with slight modifications [13]. Briefly, 1 g of dried leaf powders were mixed with 100 mL of demineralized water in a glass beaker, then heated and stirred at 100 °C for 30 min. The mixture was then filtered to ensure that the extract was free from any impurities. The extract was gradually added with NaOH until the pH of the solution reached 8 which is a favorable pH for AgNPs formation [3].

Fabrication of AgNPs

Three samples were used in this study, as shown in Table 1. Sample A was fabricated by combining 200 mM $AgNO_3$ and extract solution in a 1:1 ratio without the presence of microwave irradiation. Sample B was assembled by 1 min microwave irradiation (Panasonic NN GT35 1000 W, Japan) of 100 mM $AgNO_3$ solution without the presence of extract. Sample C was synthesized by mixing extract solution with 200 mM $AgNO_3$ in a 1:1 ratio and irradiated by microwave for 1 min. Samples A and B were observed after storing the samples for one day because of their slow transformation. While sample C was observed immediately after irradiation due to its fast transformation.

Biological characterizations of AgNPs

The antibacterial test was conducted using the disc diffusion method to evaluate the antibacterial activity of AgNPs against *E. coli* and *S. aureus*. The bacteria were inoculated on MHA media by streaking the swab three times over the entire agar surface. The sample-impregnated disk was placed on the inoculated MHA media and incubated at 37 °C for 24 h. Zone of inhibition (ZOI) was determined by measuring the bright zone around the disk, which is an indicator of the occurrence of antibacterial inhibition, using a caliper.

Table 1. Summary of samples in this experiment

No	Sample name	$AgNO_3$	Extract	Microwave
1	Extract	×	✓	×
2	Sample A	✓	✓	×
3	Sample B	✓	×	✓
4	Sample C	✓	✓	✓

The MTT assay was performed to investigate the cytotoxicity of the synthesized AgNPs against the BHK-21 cell line. This experiment was conducted by preparing BHK-21 cell culture in a 96-wells microplate with a density of 2×10^5 in 100 µL of culture media and splitting it in half. Each sample was repeated six times. The microplate was incubated for 20 h at 37 °C. Then, the microplate was removed from the incubation apparatus, added 5 mg/mL MTT reagent in 25 µL of PBS for each well, and incubated again for 4 h. Afterward, 50 µL of dimethyl sulfoxide (DMSO) solution was added to each well and incubated for another 10 min. The spectrophotometer was used to calculate the optical density value of formazan, which is the reduced form of MTT in active cells, at 540 nm [14]. The percentage of living cells was calculated using Eq. (1) as follow:

$$\text{Percentage of living cells} = \frac{\text{treatment} + \text{media}}{\text{cell} + \text{media}} \times 100$$

■ RESULTS AND DISCUSSION

Preliminary Observation of the Samples

Visual observation results

Early determination of AgNPs formation in the sample can be recognized from the color change of the solution to dark brown once the $AgNO_3$ solution is mixed with the extract solution [15]. Visual observation of the prepared samples can be observed in Fig. 1. The appearance of the extract (Fig. 1(a)) was transparent pale yellow. This appearance changed to brownish-yellow after

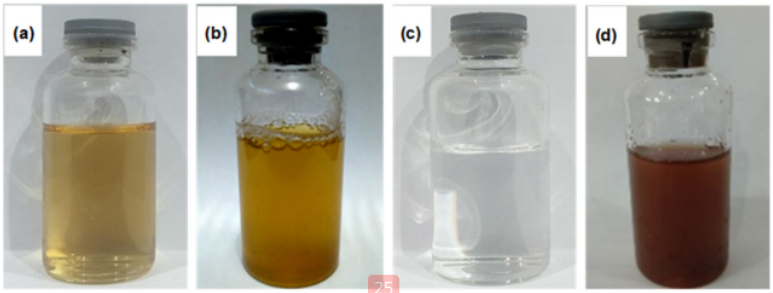

Fig 1. Visual observation of (a) extract, (b) sample A, (c) sample B, and (d) sample C

the addition of $AgNO_3$ solution and storing it for one day (sample A; Fig 1(b)).

Meanwhile, the color change was not observed in sample B (Fig. 1(c)) suggesting that solely 1 min microwave irradiation might not be enough to reduce silver ions (Ag^+). In addition, the absence of extract in sample B leads to the slower formation of AgNPs due to the lack of reducing agents in the solution. Interestingly, an immediate color transformation from pale yellow to dark brown was noticed in sample C (Fig. 1(d)). This color switch can be implied as the formation of AgNPs in the aqueous solution due to electron energy levels alteration and electrons excitation, indicating the reduction of Ag^+ into Ag^0 [16]. The combination between extract and quick microwave irradiation might induce the fast formation of AgNPs in the sample. Microwave irradiation offers rapid initial heating, which directs to enhanced reaction kinetics and reaction rates [17]. To confirm these findings, further characterization using UV-Vis spectrophotometry has been conducted.

UV-Vis spectrophotometry results

UV-Vis spectrophotometry was performed to investigate the presence of phytoconstituents in the extract and to detect the presence of AgNPs in solution due to their SPR characteristic, which typically occurred at 400 to 500 nm [18]. In Fig. 2, the extract showed two peaks at 285 and 324 nm, implying the existence of phenolic compounds which are known as active components that contributed to reducing Ag^+ in AgNPs formation [19] and also one of the secondary metabolites that existed in *Champeria* family [8-9].

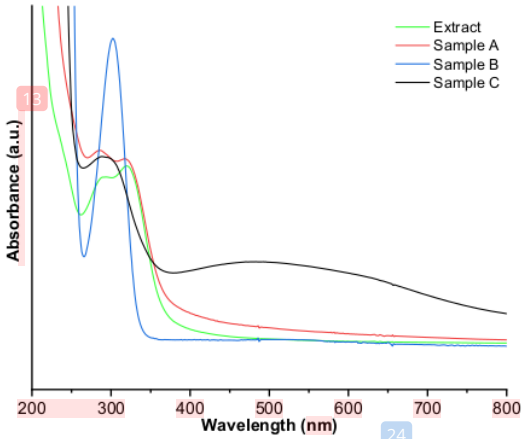

Fig 2. UV-Vis spectra of extract (green), sample A (red), sample B (blue), and sample C (black)

Characteristics of the AgNPs' SPR peak were not observed in sample A, suggesting Ag^+ reduction using the extract and AgNPs formation might not occur even after one day. In addition, peaks observed from sample A indicate similarities with the extract peaks. This might be a suggestion that no reduction process occurred in the solution. Meanwhile, sample B spectrum only demonstrates a sharp peak at 300 nm, which is a characteristic of $AgNO_3$ [20]. This result implies that 1 min microwave irradiation without the presence of reducing agents from the extract might not be enough to produce AgNPs. Intriguingly, sample C showed the presence of a broad peak at 400–700 nm, with the highest point at 483 nm, which is correlated with the AgNPs' SPR peak. This phenomenon suggests that the

extract can provide the necessary reducing agents while the cooperation of microwave irradiation offers uniform heating to the sample that will enhance the formation rate of AgNPs [21]. It is also noted that peak at 324 nm in sample C is not observed, indicating that the phenolic compound from the extract acted as a reducing agent and successfully reduced Ag^{+} into Ag^{0}. Further characterizations to investigate the presence of AgNPs and their biological characteristic was performed on sample C.

Materials Characterization of Sample C

Several characterization methods, such as FTIR, XRD, TEM, DLS, and zeta potential were performed on sample C. The results of these characterizations can be seen in Fig. 3, respectively.

FTIR results

The comparison of FTIR spectra of sample C and extract is presented in Fig. 3(a). It was observed that both spectra have similar peaks, implying that the phytochemical compounds in the extract might be responsible as capping agents to prevent the agglomeration of AgNPs. The emergence of notable peaks at wavenumber 3425 cm^{-1} can be designated as the O–H stretching group of polyphenols or flavonoids [11]. In addition, two peaks were observed at 2918 and 2850 cm^{-1} in the extract, which is related to the C–H stretching functional group of alkane [22]. Two peaks related to the C=O stretching of the amide proteins and flavonoids were detected at 1639 and 1409 cm^{-1} [23]. The peak at 1159 cm^{-1} can be ascribed to C–O stretching functional groups and the presence of a group of flavonoids and terpenoids that are widely found in the leaves [24] and also were found in *Champeria* family [8-9].

XRD result

Fig. 3(b) demonstrated the XRD pattern of sample C with five peaks at 2θ values of 38.12°, 44.3°, 64.44°, 77.38°, and 81.66° that were designated to lattice planes of (111), (200), (220), (311), and (222), respectively. This pattern indicates that the synthesized silver metal in sample C was comprised of face-centered cubic (FCC) lattice and matched with JCPDS No. 00-004-0783 [3]. From this XRD pattern, it is clear that the AgNPs formed using the extract were in the form of high crystalline [25]. Furthermore, the most intense diffraction peak in the lattice plane (111) proves that the obtained AgNPs are dominant in the (111) direction [26].

TEM result

The TEM image in Fig. 3(c) shows the morphology of nanoparticles with a spherical shape that is well dispersed in sample C. The particle size of AgNPs was also measured using ImageJ and followed the Gaussian trend as demonstrated in Fig. 3(d). Moreover, it was revealed that the synthesized AgNPs have an average particle size of 9.1 ± 4.1 nm, which was comparable with our previous AgNPs that were prepared by extract of Cilembu sweet potatoes (9.95 ± 3.69 nm) [3], and relatively smaller than the previous research using another leaf-mediated extract where the average particle size was in the range between 12 to 30 nm [27]. This phenomenon can be accredited to a high concentration of secondary metabolites such as flavonoids, phenolic, lutein, phytol, and β-carotene in Seminyak leaf extract, leading to rapid reaction times which correspond to extensive nucleation sites and smaller nanoparticles. The smaller particle size of AgNPs leads to higher antibacterial activity and spherical shapes have been recognized to exhibit improved antibacterial properties because they can provide a larger surface-to-volume ratio [28].

DLS and zeta potential results

DLS measurement was conducted to investigate the average particle size of the obtained AgNPs and their particles distribution. Fig. 3(e) displays that the average particle size of the synthesized AgNPs is 145.5 nm with a moderate polydispersity index (PI; 0.469). It was notable that the DLS tends to generate bigger average particles size than the TEM because the former quantifies the hydrodynamic diameter, which is the combined diameter of the core nanoparticles and the outer capping layer [29]. The stability of AgNPs in the sample can be predicted by performing zeta potential measurement. Fig. 3(f) exhibited that the zeta potential value of the synthesized AgNPs was 0.28 mV, which is more neutral than our previous AgNPs that were prepared by extract of Cilembu sweet potatoes (–41.0 mV) [3]. Since the zeta potential value of the synthesized AgNPs was in the range

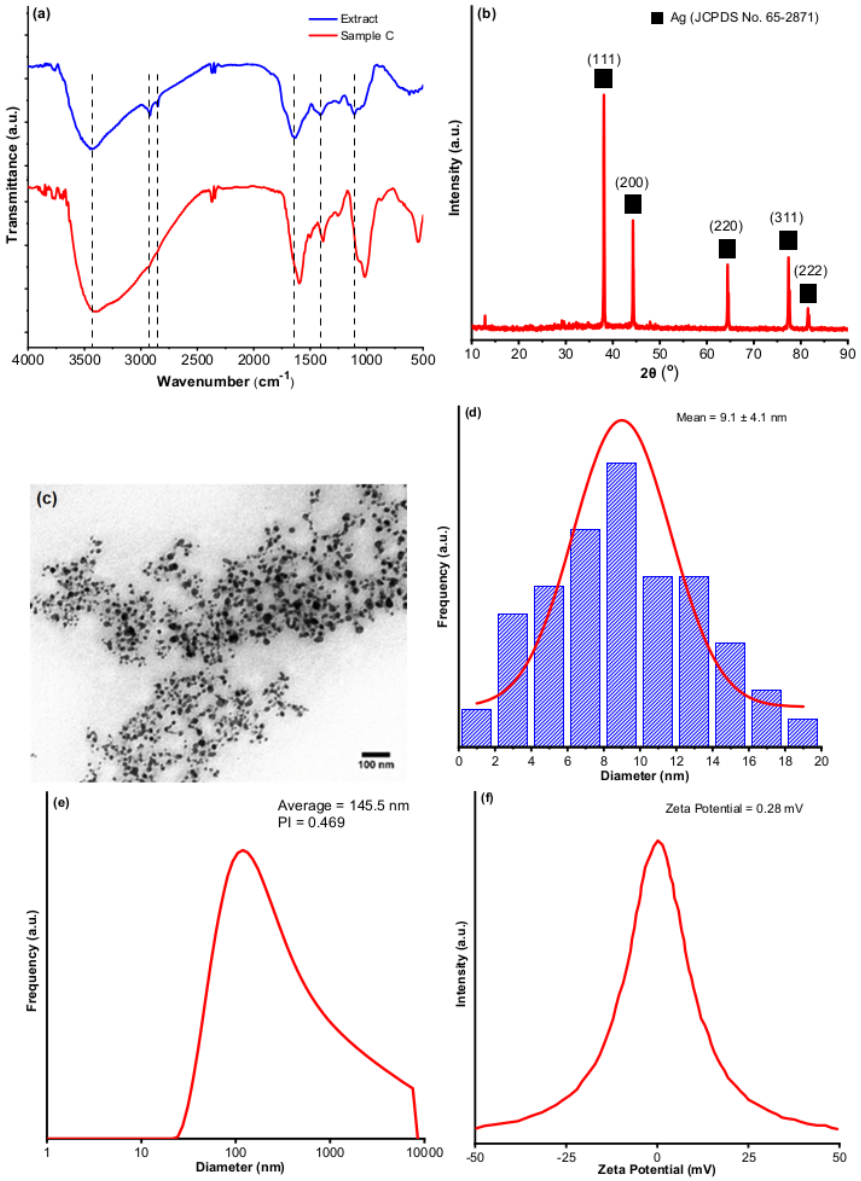

Fig 3. Further characterization results of sample C: (a) FTIR spectra (in comparison with extract, (b) XRD, (c) TEM, (d) particle distribution based on TEM image, (e) DLS, and (f) zeta potential results

of 0 ± 3 mV, the synthesized AgNPs are susceptible to agglomeration and might form big particles after some time [26]. Therefore, it is reasonable that the DLS result of the obtained AgNPs (145.5 nm, with PI 0.469) is relatively bigger and less uniform than the DLS result of our previous work (105.5 ± 12.6 nm, with PI 0.29 [3]).

Biological Characterization of Sample C

Antibacterial test results

Representative images of antibacterial test results of samples and a summary of the measured samples' ZOI were shown in Fig. 4 and Table 2 respectively. Antibacterial test results showed that ZOI of the extract was not observed against *E. coli* (Fig. 4(a)) and *S. aureus* (Fig. 4(b)), suggesting that the extract did not have any antibacterial activity against both bacteria [30]. On the other hand, sample C which contains AgNPs showed antibacterial activity against *E. coli* (Fig. 4(c)) and *S. aureus* (Fig. 4(d)) with ZOI of 12.3 ± 0.1 and 13.7 ± 0.7 mm, respectively (Table 2). According to the fact that the ZOI of sample C is in the range of 10–20 mm, sample C can be considered to have strong antibacterial activities against both bacteria [31].

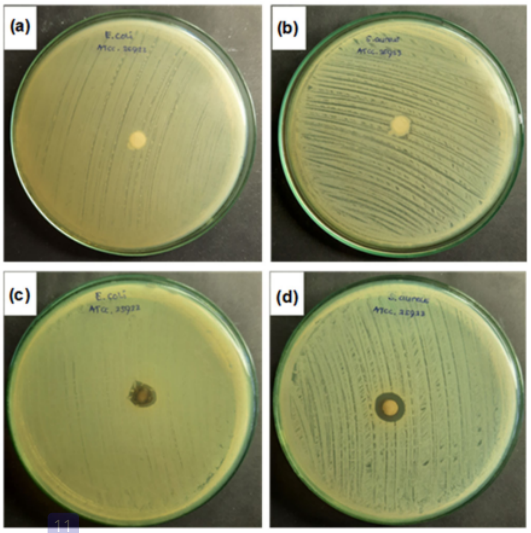

Fig 4. Antibacterial activity of the extract against (a) *E. coli*, (b) *S. aureus*, and sample C against (c) *E. coli*, (d) *S. aureus*

Table 2. Summary of the antibacterial test results from the disc diffusion method

No.	Sample name	Type of bacteria	ZOI (mm)
1	Extract	*E. coli*	0
		S. aureus	0
2	Sample C	*E. coli*	12.3 ± 0.1
		S. aureus	13.7 ± 0.7

Table 3. Cell viability of BHK-21 cells for extract and sample C

No	Sample name	Cell viability (%)
1	Control cell	100.0 ± 4.9
2	Extract	84.4 ± 2.2
3	Sample C	88.5 ± 7.0

These strong antibacterial activities of AgNPs in sample C could be correlated with their small particle size (9.1 ± 4.1 nm), providing high numbers of silver atoms on the surface and releasing Ag^+ to kill bacteria cells [28].

MTT assay results

The cell viability of the extract and AgNPs in sample C was determined by MTT assay after 1 day in BHK-21 cells culture. Table 3 suggests that both samples were non-toxic because the percentage of cell viability is above 60% (approximately 84.4% for extract and 88.5% for sample C respectively) [32]. Cell viability of AgNPs is lower than control cells because AgNPs could induce mitochondrial dysfunction by producing reactive oxygen species (ROS), causing damage to cell membranes, proteins, and DNA leading to cell apoptosis [33]. However, in this research both extract and sample C were non-toxic. Thus, it can be concluded that the obtained AgNPs were biocompatible for biomedical applications.

■ CONCLUSION

Green synthesis method in AgNPs formation using a low concentration of Seminyak leaf extract (0.5 wt.%) and incorporation with short microwave irradiation time (1 min) led to a rapid synthesis process and successfully prepared AgNPs with the extremely small size of 9.1 ± 4.1 nm. The synthesized AgNPs demonstrate notable antibacterial properties against *E. coli* and *S. aureus* with ZOI of 12.3 ± 0.1 and 13.7 ± 0.7 mm, respectively. The cytotoxicity of AgNPs was very minimal on BHK-21 cells, resulting in high cell viability. These findings suggest the potential of microwave-Seminyak-mediated AgNPs for various biomedical applications such as drug carriers, promotion of wound repair and bone healing, biosensors, and antibacterial scaffold. In the future, the stability, and biocompatibility

of the AgNPs in the human body should be observed as these properties are critical for their prospective applications.

■ ACKNOWLEDGMENTS

This research was funded by The Research, Community Service and Innovation Program (P3MI) for Electronic Materials Physics Research Group, Faculty of Mathematical and Natural Sciences, Institut Teknologi Bandung, 2021.

■ AUTHOR CONTRIBUTIONS

Conceptualization, Arie Wibowo, and Muhammad Bagas Ananda; Methodology, Arie Wibowo, Anggraini Barlian, Prihartini Widiyanti, and Muhammad Bagas Ananda; Data acquisition, Muhammad Bagas Ananda, Tami Bachrurozy, Fathan Aditya Sanjaya, Helmi Majid Ar Rasyid, Fitriyatul Qulub, and Prihartini Widiyanti; Data analysis, Arie Wibowo, Anggraini Barlian, Prihartini Widiyanti, Tami Bachrurozy, and Muhammad Bagas Ananda; Funding acquisition, Akfiny Hasdi Aimon; Project administration, Arie Wibowo, and Akfiny Hasdi Aimon; Resources, Arie Wibowo, and Akfiny Hasdi Aimon; Supervision, Arie Wibowo, and Anggraini Barlian; Writing—original draft, Muhammad Bagas Ananda, Arie Wibowo, Fitriyatul Qulub, and Prihartini Widiyanti; Writing—review & editing, Anggraini Barlian, Akfiny Hasdi Aimon, Tami Bachrurozy, Fathan Aditya Sanjaya, and Helmi Majid Ar Rasyid. All authors have read and agreed to the published version of the manuscript.

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