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Pages: 1480-1487

Published online: 20 Jun 2019

Article

Development of polylactic acid (PLA) bio-composite films reinforced with bacterial cellulose nanocrystals (BCNC) without any surface modification

Endarto Y. Wardhono, Nufus Kanani, Alfirano & Rahmayetty

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The effect of monoethanolamine as stabilizing agent in *Uncaria gambir* Roxb. mediated synthesis of silver nanoparticles and its antibacterial activity

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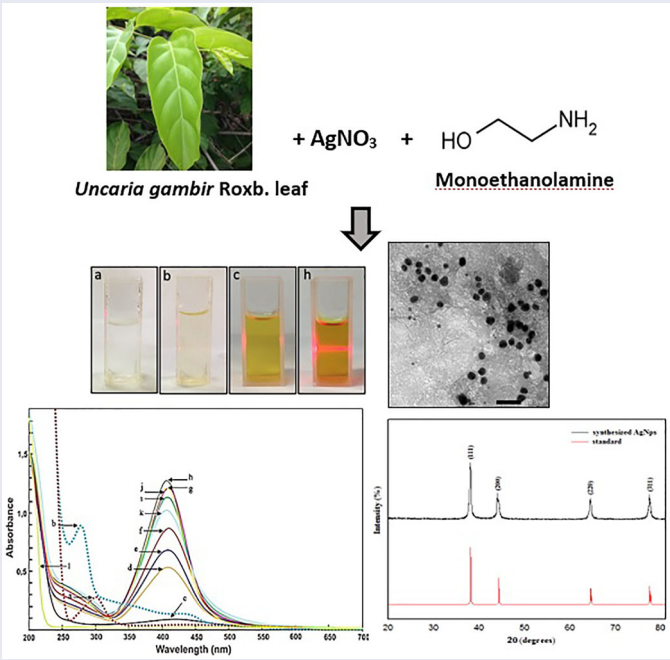
ABSTRACT

The development of an economic and eco-friendly approach of silver nanoparticles synthesis is an important issue due to its wide application, especially in biomedical applications. In present study, an eco-friendly method and size controlling is developed to synthesize silver nanoparticles using *Uncaria gambir* Roxb. and monoethanolamine (MEA) as bioreducing agent and stabilizing agent, respectively. The green synthesized silver nanoparticles are characterized using UV-Vis spectroscopy, X-ray diffraction (XRD), and transmission electron microscope (TEM). The silver nanoparticles are formed due to reducing of silver ion by catechin in *Uncaria gambir* Roxb. leaf extract which is visually recognized from the color change from pale yellow to grayish brown. The formation of silver nanoparticles is confirmed by UV-Vis spectroscopy analysis which provides SPR band range of 405–416 nm. The TEM analysis shows that spherical nanoparticles are formed with size range between 6-39 nm. The using of MEA with molar ratio to Ag^{+} 10/1 can maintain stability and reduce the particle size up to 40%. The result of XRD analysis shows 4 peaks referring to well-crystallized face-centred cubic silver nanoparticles. The prepared silver nanoparticle has exhibited good stability for more than 6 months. The synthesized silver nanoparticles exhibited good antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* strain with a diameter of inhibition zone up to 36 mm. The synthesized silver nanoparticles also showed better activity against *Escherichia coli* than that of *Staphylococcus aureus*. These results show a good potential of *Uncaria gambir* Roxb. mediated silver nanoparticles to be developed in biomedical application.

GRAPHICAL ABSTRACT



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Introduction

Nanoparticles have been gaining attention due to its excellent properties as compared to bulk materials like high conductivity, catalytic activity, chemical stability, high surface to volume ratio, etc.[1–3] These unique properties lead nanoparticles, especially metal nanoparticles, to be developed in various applications such as sensor,[4] electronic,[3] catalyst,[5–8] and especially as biomedical applications. Silver nanoparticles have been most advanced for nanomedicine, which for a long time had been emerged as an antibacterial agent and recently extended as anticancer as well.[2,9–14]

Various methods have been used to synthesize silver nanoparticles including laser ablation,[15] sonochemical,[16] microwave irradiation,[17,18] electrochemical,[19] and solvothermal.[20,21] The chemical reduction using inorganic bases is the most simple and rapid technique to fabricate colloidal silver nanoparticles.[3] Unfortunately, highly toxic chemicals used in the reduction method can contaminate the environment and affect the bio-application in the future.[22–24] Recent studies suggest a new 'green synthesis' approach, which uses extract of plants to form metal nanoparticles through a reduction process by active components contained in the plant. Examples include *Foeniculum vulgare*[25] *Gracilaria birdiae*,[26] *Tinospora cordifolia*,[27] *Tribulus terrestris*,[28] *Carica papaya*,[29] *Iresine herbstii*,[30] and olive leaves.[31]

Uncaria gambir Roxb. is one of commodities in West Sumatera Indonesia as industrial plants which has high economic value. It has been used for a long time as plywood and wood particles. In addition, the leaves have been used as textile dyes and traditional medicine for burns. It is also proceeded as tea to smooth the process of digestion in the stomach and intestines, to heal headaches, diarrhea, dysentery, sore skin, and tanners.[32] *Uncaria gambir* Roxb. contains some polyphenol compounds predominantly catechin, which is expected to reduce Ag^+ to Ag^0 nanoparticles.[33,34]

In addition, it has been reported that nanoparticle size significantly impacts the performance in the biomedical application due to the surface reaction and specific surface area.[35] The size can be controlled using a stabilizing agent which could be long chain amine, polymer, ligand, surfactant, and dendrimer.[36] In a previous study, we synthesized silver nanoparticles by precipitation method using *Uncaria gambir* Roxb. leaf extract as bioreducing agent. Colloidal silver nanoparticles with a diameter of 10 to 30 nm are successfully formed in isopropanol solvent.[34] However, the using of aqueous solvent will be attractive and necessary for an easier process of synthesis. The using of capping agent such as alkanolamine compound in silver nanoparticles synthesis was reported by Jia et al.[36] In the study, triethanolamine (TEA) was used as a reducing agent to obtain silver nanoparticle with a size of 40 nm in the presence of polyvinylpyrrolidone (PVP) and polyethylene glycol (PEG) as stabilizing agents. To the best of our knowledge, this is the first report of using monoethanolamine (MEA) as a stabilizing agent in green synthesis of aqueous colloidal silver nanoparticles to control the size. The using of monoethanolamine has advantages in future applications

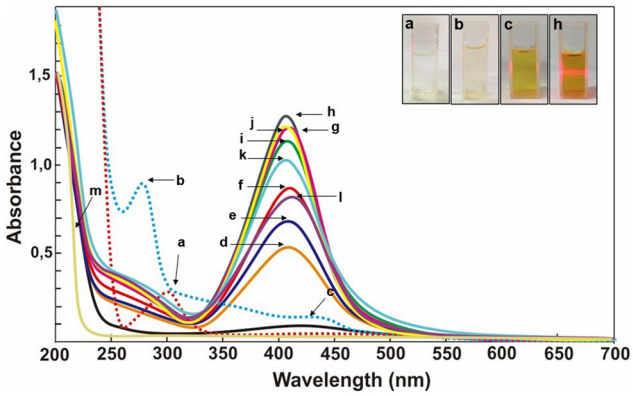

Figure 1. UV-Vis absorption spectrum of (a) silver nitrate, (b) *Uncaria gambir* Roxb. leaf extract, AgNps at reaction time of (c) 0, (d) 0.5, (e)1, (f) 2, (g) 4, (h) 24, (i) 48, (j) 168, (k) 720 hours, (l) 6 months, (m) mixture of Ag + and MEA. The inset shows the laser beam radiation.

such as bioconjunction application for cancer cell targeting.[37] In addition, the molar ratio of MEA/Ag^+ is varied to investigate the effect of concentration on the properties of nanoparticles. The antibacterial activity of fabricated silver nanoparticles is investigated against *Staphylococcus aureus* (gram-positive bacteria) and *Escherichia coli* (gram-negative bacteria).

Materials and methods

Preparation of Uncaria gambir Roxb. leaf extract

Uncaria gambir Roxb. leaves were collected from Experimental Garden, Faculty of Agriculture, Andalas University. The fresh leaves were thoroughly washed several times to clean from dust and unnecessary materials. The clean leaves were shade-dried at room temperature for approximately 5 days. The dried leaves were ground into fine powder, stored in an airtight container and placed protected from sunlight. The aqueous extract of *Uncaria gambir* Roxb. leaves was prepared by dissolving 5 grams of fine powder into 50 mL of sterile distilled water, then was stirred for 2 hours at a temperature of 65 °C. The mixture then was cooled and filtered to obtain the extract, then stored in a refrigerator at a temperature of 4 °C for future use.

Synthesis of colloidal silver nanoparticles

The prepared *Uncaria gambir* Roxb. leaf extract (4%, 1 mL) was added to 48 mL of 1 mM $AgNO_3$ (Merck, Analytical grade) while stirring at a constant rate at room temperature. It was then followed by adding 1 mL of MEA (Merck, Analytical grade) with concentration of 10 mM, 20 mM, and 30 mM, separately. In this study, the molar ratio of $AgNO_3$/MEA was varied to be 1:10, 1:20, 1:30, and without MEA as a comparison in order to investigate the effect of the stabilizing agent concentration on nanoparticles properties. The samples were coded as AgNps-MEA1, AgNps-MEA2, AgNps-MEA3, and AgNps0, respectively. The stability of formed silver nanoparticles was periodically monitored by measuring the wavelength and absorbance using UV-Vis

Table 1. Absorbance and wavelength of *Uncaria gambir* Roxb. mediated silver nanoparticles.

	Wavelength (nm)				Absorbance (a.u)			
Reaction Time	AgNps-MEA1	AgNps-MEA2	AgNps-MEA3	AgNps0	AgNps-MEA1	AgNps-MEA2	AgNps-MEA3	AgNps0
0 hr	416.07	0	0	429	0.088	0	0	0.011
0,5 hr	409.19	412.76	410	434	0.560	0.286	0.234	0.017
1 hr	409.83	415.46	414	437	0.705	0.316	0.285	0.022
2 hr	411.11	419.73	419	435	0.967	0.426	0.331	0.023
4 hr	409.97	423.55	426	441	1.267	0.539	0.456	0.020
24 hr	406.98	422.12	438	434	1.341	0.721	0.630	0.036
48 hr	406.97	426.43	432	443	1.187	0.940	0.859	0.065
168 hr	406.91	423.34	436	437	1.267	0.651	0.661	0.036
720 hr	405.90	415.00	446	441	1.028	0.634	0.411	0.022
6 months	418.00	422.00	450	452	0.821	0.622	0.503	0.013

spectroscopy (Thermo Scientific Evolution 201 UV-Vis) in a wavelength range of 200–700 nm. The sample was placed in a seal bottle in vigorous condition for future measurements.

Characterization of silver nanoparticles

Transmission electron microscope (TEM) images of synthesized colloidal AgNps were obtained with a JEOL-4000 operated at 120 kV. X-ray diffraction (XRD) analysis was carried out using Shimadzu XRD-7000S X-Ray diffractometer with CuK radiation ($\lambda = 1.5406$ Å), operated at a voltage of 30 kV and current of 30 mA.

Antibacterial activity test

Antibacterial activity was tested against *Staphylococcus aureus*, and *Escherichia coli* using agar diffusion method in Agricultural Engineering Faculty, Andalas University, Padang, Indonesia. Both bacteria were grown in nutrient agar (NA) and allowed to grow for 24 h at 37 °C. Suspended-bacteria was transferred to the 100 mL of NA medium then poured to the petri dish. Sterile cotton with nanoparticles sample (concentration of 100 µg/mL) were placed on well. Water and amoxicillin were used as negative and positive control, respectively. After incubating the samples for 24 hours in a temperature of 37 °C, the inhibition zone was measured. The test was conducted in duplicate.

Results and discussion

UV-Vis spectroscopy analysis

The addition of silver nitrate into the mixture stirring of *Uncaria gambir* Roxb. leaf extract and MEA immediately shows color changes from pale yellow to brownish yellow and finally grayish brown. The color changes indicate the formation of silver nanoparticles, attributed to surface plasmon resonance phenomenon (SPR).[23,36,38] It is determined further by using UV-Vis spectroscopy. Figure 1 shows that absorption bands of *Uncaria gambir* leaf extract and $AgNO_3$ in 279 and 301 nm, respectively, are shifted to an absorption band of silver nanoparticles in approximately 400 nm, immediately after the reaction. It strongly suggested that the silver nanoparticles are successfully formed due to the reduction of silver nitrate by the active compound in *Uncaria gambir* Roxb. leaf extract. Hydroxide group plays an important role in this reaction by reducing Ag^+ to Ag^0 through nucleation and growth process, forming silver nanoparticles, while the hydroxide groups are oxidized to ketone. Similar results in synthesizing silver nanoparticles using *Uncaria gambir* were reported by Arief et al.[33,34] It is also observed that the SPR intensity increased gradually with the increase of time reaction up to 24 hours, which shows a continuous formation of silver nanoparticles during the reaction.

The inset picture in Figure 1 shows the laser beam radiation of samples which is performed to demonstrate the Tyndall effect of dispersed colloidal silver nanoparticles. $AgNO_3$ (a) and *Uncaria gambir* Roxb. leaf extract (b) do not perform light line through the solution. After mixing (c), a light line is observed due to the light dispersion of colloidal silver nanoparticle in the reaction. The intensity increases after 24 hours (h), confirming the increase of nanoparticles number formed in the reaction. After 6 months, the peak of metallic silver nanoparticles is still observed in wavelength of 418 nm with an absorbance value of 0.821 a.u. This result indicates the stability of synthesized silver nanoparticles for the time up to 6 months. The absorbance and wavelength value are given in Table 1.

In UV-Vis spectroscopy study, metal nanoparticles provide an absorption band based on SPR phenomenon which is strongly influenced by the size and shape of the nanoparticles.[39,40] In this study, absorption bands are observed at wavelength range of 405–416 nm (Table 1) which strongly indicates the formation of spherical silver nanoparticles. Some similar results are previously reported that colloidal spherical AgNps absorb light in approximately 400–450 nm. Soshnikova et al.[41] reported the preparation of silver nanoparticles due to bioreduction using cardamom fruits which are indicated by SPR absorption peak at 428 nm. Singh et al.[27] synthesized silver nanoparticles using *Tinospora cordifolia* and form absorption band at 420–425 nm. Additionally, green synthesis of silver nanoparticles was reported by Yan-yu et al.[42] using G*inkgo biloba*, Gopinath et al.[28] using *Tribulus terrestris* fruit, Choudhary et al.[43] and Khalil et al. [31] using olive leaves, obtained silver nanoparticles with SPR band at 450 nm, 435 nm, and 440–458 nm, respectively. These results are supported further by TEM analysis.

The results of absorbance measurement (Table 1) show that the absorbance decreases with the increasing of MEA concentration. This result shows that the concentration of MEA affects the number of silver nanoparticles formed in

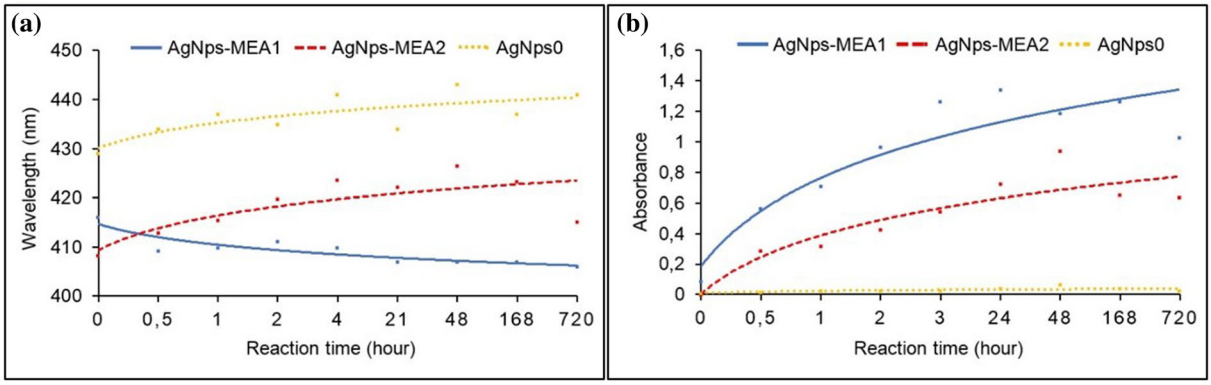

Figure 2. (a) Wavelength of MEA stabilized AgNps in variated concentration and (b) Absorbance of MEA stabilized AgNps in variated concentration.

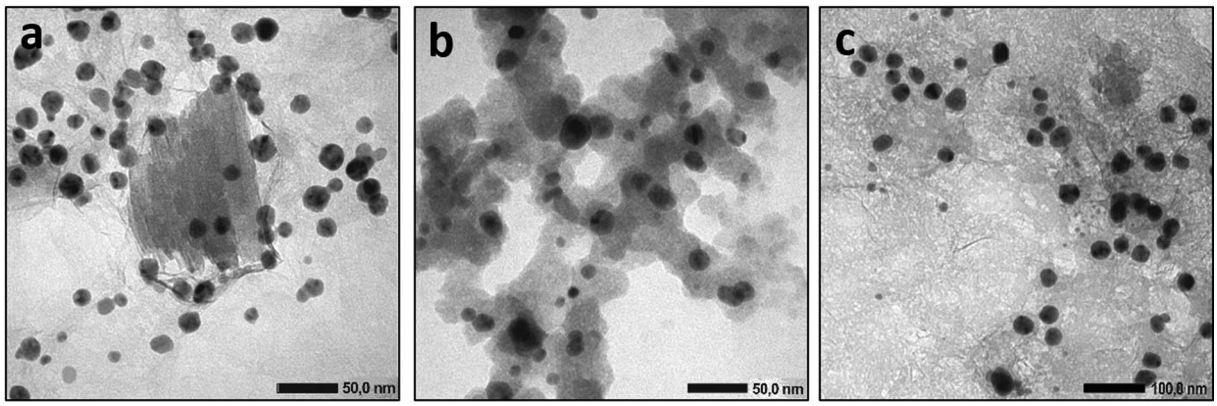

Figure 3. (a) TEM images of AgNps-MEA1, (b) TEM images of AgNps-MEA3, (c) TEM images of AgNps0.

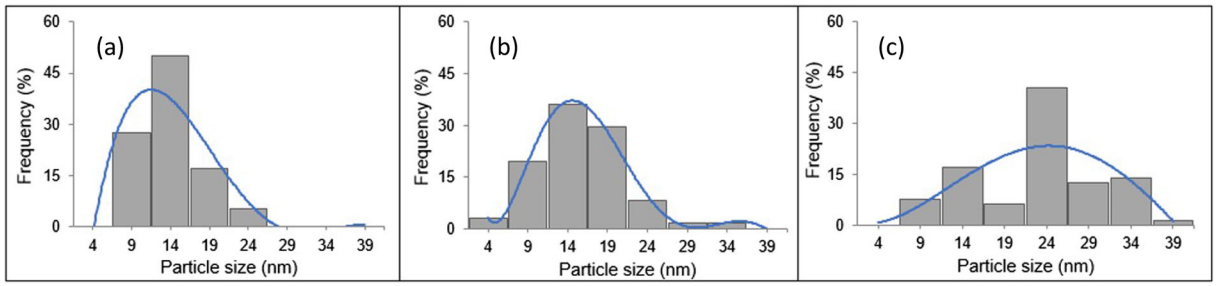

Figure 4. Particle size distribution of MEA stabilized nanoparticles (a) AgNps-MEA1, (b) AgNps-MEA3 and (c) AgNps0.

the reaction. This is probably due to the interaction occurred between extract and the excess MEA, which may block the reduction of Ag^{+} by the extract. As a result, the number of silver nanoparticles formed in the reaction decrease.

Figure 2 shows a blueshift of AgNps-MEA1 which indicates the reducing of particle size as the increasing of time reaction. Besides, it was observed that the absorbance value of AgNps-MEA1 increases as the increase of reaction time which indicates the stability of colloidal silver nanoparticles. These results suggest that the using of MEA in appropriate concentration can perform a stabilization mechanism to control the growth, hence control the size. It is further supported by TEM analysis result. In addition, the reaction between MEA and $AgNO_3$ do not provide any specific SPR band, which shows that in this study MEA only act as a stabilizing agent instead of bioreducing agent.

TEM analysis

The TEM analysis image (Figure 3) shows that *Uncaria gambir* Roxb. leaf extract synthesized AgNps are spherical in shape with diameter range of 6–41 nm. The particles average size of AgNps-MEA1; Ag-Nps-MEA3; and AgNps0 are 14; 15; and 23 nm, respectively. It was observed that the particle size increased along with redshift of wavelength in UV-Vis spectroscopy analysis, as described earlier. It is an indication that there is a strong relation between SPR absorption band and particle size. Based on Mie Theory, the size of spherical nanoparticles can be estimated from the wavelength values in UV-Vis spectroscopy analysis, where the increasing (red-shifted) of wavelength indicates the increase of particle size.[44] In addition, the particle size distribution of silver nanoparticles is calculated using ImageJ software. The results show that an increase of MEA concentration led to a broader size range (Figure 4). The size range of AgNps-MEA1, AgNps-MEA2,

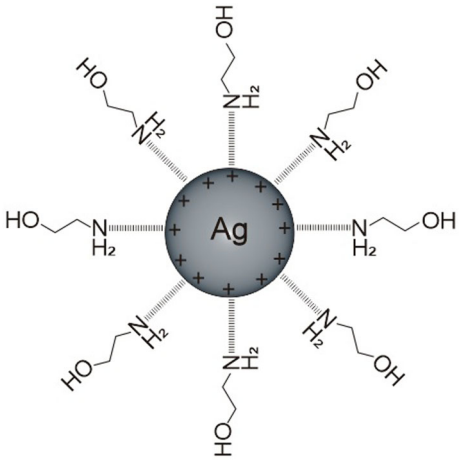

Figure 5. Proposed interaction of MEA with AgNps.

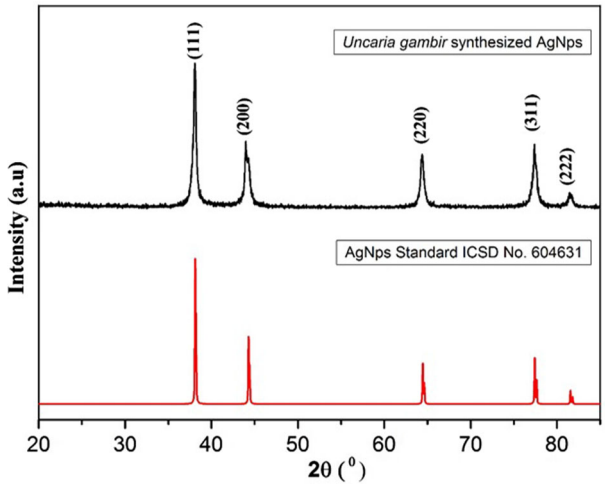

Figure 6. X-Ray diffraction of *Uncaria gambir Roxb.* mediated silver nanoparticles.

and AgNps0 of the particles are observed to be 6–24 nm, 1–36 nm, and 6–41 nm, respectively. The rise of MEA concentration led to an increase of particles diameter. It may be due to the excess amount of stabilizing which inhibits the interaction between Ag^+ and the active compound in *Uncaria gambir* Roxb. leaf extract. As a result, it reduces the possible reduction reaction. Deficiency of nucleation reactions causes the growth process dominated, and finally bigger sized particles are formed. A similar result is reported by Wu et al.[3] in synthesizing AgNps using triethylamine as reducing as well as stabilizing agent, where the raising of triethylamine concentration provides a larger particle diameter. We suggest that it is necessary to conduct a future study on the optimum concentration of stabilizing agent involved in preparing AgNps.

In addition, the results of TEM analysis also present a larger particle size of uncapped AgNps than MEA capped AgNps. The using of MEA as stabilizing agent can reduce the particle size up to 40%, which clearly suggests that stabilizing agent can control the growth of AgNps hence control the particle size. According to Yamamoto et al.,[45] amino group forms a protective monolayer on the surface of silver nanoparticle through Ag-N interactions. In this study, those inhibit electrostatic interaction between partial Ag, hence prevent aggregation as shown in Figure 5. Small sized nanoparticles can provide a greater surface area to volume ratio and establish the biomedical application.

XRD analysis

The X-ray diffraction is studied to confirm the crystalline structure of prepared AgNps. XRD pattern (Figure 6) shows five distinct peaks at 2θ 38.08° (111), 43,99° (200), 64.35° (220), 77.38° (311), and 81.49° (222), which referred to face-centred cubic structure of silver nanoparticles (ICSD No. 604631.[46] This result confirms the UV-Vis spectroscopy and TEM analysis result. Suitable patterns are reported by Singhal et al.[47] by synthesizing silver nanoparticles using *Ocimum sanctum* leaf extract and Mittal et al.[16] using *Potentilla fulgens* as bioreducing agent. Referring to the highest peak using Scherrer formula, the crystal size is estimated to be 5 nm. This result is in agreement with the TEM analysis result.

Antibacterial activity test

Silver nanoparticles are well known as antimicrobial agent against various pathogenic bacteria.[48–50] In order to evaluate the antibacterial activity of synthesized silver nanoparticles, 2 types of bacteria; *E. coli* (gram-negative) and *S. aureus* (gram-positive), were tested using agar diffusion method. Water and amoxicillin were used as a negative and positive control, respectively. The result (Figure 7) reveals the inhibition zone in AgNps0 and AgNps-MEA both against *E. coli* and *S. Aureus*. The diameters of inhibition zone are shown in Table 2. Test was performed in duplicate, and all the data reported are average values.

It was observed that AgNps-MEA exhibits larger inhibition zone than AgNps0 both against *E. coli* and *S. aureus*. In the test against *E.* coli, AgNps-MEA even shows a larger inhibition zone than the positive control. This result clearly suggests that the addition of MEA as capping agent affects the antibacterial activity of AgNps. This relates to the size of silver nanoparticles, referred to the result of TEM analysis, where the stabilized and small-sized AgNps perform better antibacterial activity. In addition, inhibition zone against *E. coli* is observed larger than inhibition zone against *S. aureus*. These results suggest that *Uncaria gambir* Roxb. mediated silver nanoparticles have potential to be developed as antibacterial agent, specifically against gram-negative bacteria. This may be due to the lower permeability through membrane of gram-positive bacteria since gram-positive bacteria has peptidoglycan layer about 80 nm, 10 times thicker than in gram-negative bacteria. This thinner peptidoglycan layer of gram-negative bacteria leads AgNps to be more active against *E. coli* than *S. aureus*.[51,52] The small size and large surface area of silver nanoparticles can abridge it to be incorporated into the cells, causing the wall destruction, disrupting the metabolism, and leading to the cell death.[52,53] However, it is necessary to conduct a future study about the exact mechanism for the inhibition of bacteria of MEA stabilized silver nanoparticles.

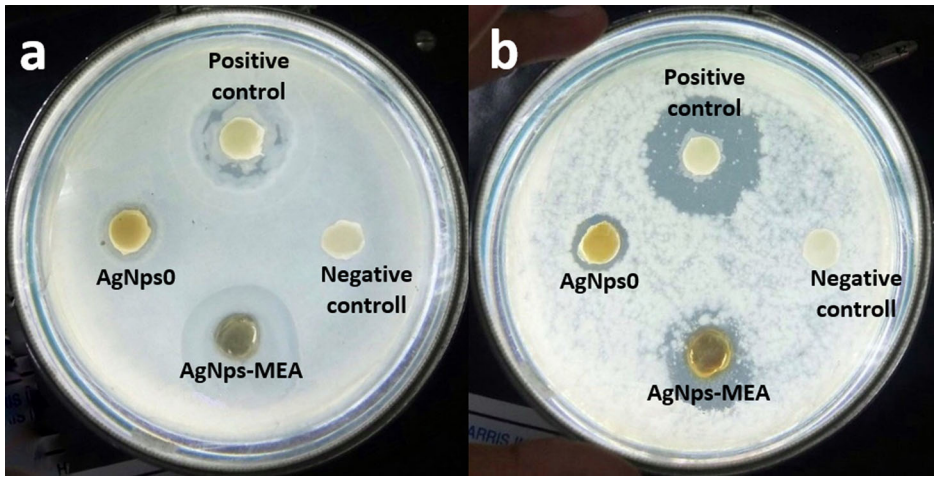

Figure 7. Antibacterial activity of silver nanoparticles against (a) *E. coli* and (b) *S. Aureus*.

Table 2. Diameter of inhibition zone of synthesized AgNps against *E. coli* and *S. aureus*.

	Inhibition zone (mm)[a]			
Bacterial strain	AgNps0	AgNps-MEA	(+) Control	(-) Control
E. coli	14	34	28	–
S. aureus	16	28	36	–

[a]Test was performed in duplicate and all data reported are average values.

Conclusion

Silver nanoparticles with particle size average of 6-41 nm had been successfully synthesis through bioreduction reaction using *Uncaria gambir* Roxb. leaf extract. The results of UV-Vis spectroscopy and TEM analysis show the formation of size-controlled nanoparticles, which is stable up to 6 months after reaction. The nanoparticle size can be reduced up to 40% with the presence of MEA in appropriate concentration as capping agent, due to control the growth of the nanoparticles. The XRD analysis confirms a formation of well-crystallized silver nanoparticles with FCC structure. Antibacterial activity test of synthesized silver nanoparticles showed better activity against *E. coli* than that of *S. aureus*. with diameter of inhibition zone up to 35 mm. These results show a good potential of *Uncaria gambir* Roxb. mediated silver nanoparticles to be developed in biomedical application.

Disclosure statement

The authors declare no conflict of interest.

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