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High antibacterial properties of green synthesized gold nanoparticles using *Uncaria gambir* Roxb. leaf extract and triethanolamine

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ABSTRACT

The development of antibacterial agents is increasingly gaining much attention due to the expansion activity of multi-drug resistant bacteria in causing many diseases. Metallic nanoparticles especially gold nanoparticles in the field of nanotechnology is one of the new and significant materials to be developed as antibacterial agents. Gold nanoparticles have been successfully synthesized using *Uncaria gambir* Roxb. leaf extract as a bioreducing agent and triethanolamine as a capping agent, through reduction of Au^{+3} by flavonoid compound in the leaf extract. The effect of triethanolamine and hydrogen tetrachloroaurate (III) acid concentration on the stability and size of nanoparticles was studied. The presence of triethanolamine 1% as a capping agent successfully maintained the stability of colloidal gold nanoparticles with a concentration of hydrogen tetrachloroaurate (III) acid at 100 ppm. X-Ray Diffraction analysis showed peak patterns corresponding to the metallic gold standard with a crystallite size of 32.52 nm. Transmission Electron Microscope analysis showed that particles were spherical, hexagonal, and triangular, where the smallest particle size was 7 nm. Synthesized gold nanoparticles showed great antibacterial activity against *E. coli* and *S. aureus*. The stability and antibacterial activity of synthesized gold nanoparticles showed good potential to be developed in biomedical applications.

INTRODUCTION

Pathogenic bacteria are bacteria that cause diseases in humans through water, food, and clothes. Some medical treatments, predominantly drugs, are used and developed to cure some bacteria-caused diseases. However, instead of curing the diseases, nowadays, people prefer to prevent the bacteria infection by combining certain antibacterial agents into things such as food containers, cosmetics, clothes, and surgery kits (Abbasi *et al.*, 2016). For this purpose, antibacterial agent development is currently gaining some attention. In this point, nanotechnology offers a promising approach to develop and modify the excellent properties of metal nanoparticles for biomedical application goals (Franci *et al.*, 2015).

Nanotechnology is the study of the synthesis, strategy, and structure manipulation of materials in the size of 1–100 nm (Iravani, 2011; Moodley *et al.*, 2018). Recently, the study of nanotechnology has drawn a lot of attention due to the excellent properties which are fundamentally different in chemical, physical, and biological aspects in the bulk materials (Heiligtag and Niederberger, 2013). One of the most developed fields is metallic nanoparticles which have been widely applied in medical application, such as in body tissue repair, gene delivery, drug delivery, immunodiagnostic, optic, food industry, environment, and imaging (Ahmed and Ikram, 2015; He *et al.*, 2019; Kim *et al.*, 2012; Marza *et al.*, 2019). The application is based on the potential of the metallic nanoparticles to act as antibacterial agents due to the nanometer size and high surface to volume ratio (Khalil *et al.*, 2013). Among all the metals, silver and gold are the most developed because of their high potential as antibacterial agents (Dykman and Khlebtsov, 2012). Besides, it is due to the high ability to functionalization, nontoxicity, ease of detection, and photothermal activity (Dizaj *et al.*, 2014). Some studies proposed that the antibacterial activity mechanism of metal nanoparticles relates to toxic-free metal ion which arises from the surface of nanoparticles and infiltrated into bacteria cell. These toxic metal ions were then accumulated in the cell membrane and disturb

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the metabolism of the cell, leading to cell death. In addition, this is might be due to the nano size of the metal particles for easy entrance into the cell (Dizaj *et al.*, 2014; Labanni *et al.*, 2019; Parham *et al.*, 2016).

Various methods can be used to fabricate gold nanoparticles, such as reduction method (Heidari *et al.*, 2014), hydrothermal (Tippayawat *et al.*, 2016), microwave (Ngo *et al.*, 2016), and laser irradiation (Gonzalez-Rubio *et al.*, 2016), and many other. Among the methods, chemical reduction is the most favorable because of its simplicity and low-energy requirements (Elia *et al.*, 2014). However, the utilization of hazardous chemical reagents can be toxic and threaten the environment and living organisms. Hence, an environmental green synthesis method is currently developed by the researchers to reduce the dangerous impacts. In this method, some types of living organisms are used as bioreducing agents, such as plants, algae, and fungi, whose active compounds were used (Shah *et al.*, 2015).

Uncaria gambir (U. gambir) Roxb. is a species of flowering plant found in West Sumatera. For a long time, it has been used in materials such as tanner base material for dyeing, pesticide, glue plywood, and especially as medicine to heal illnesses due to the content of catechin, which are classified as flavonoid compounds, up to 33% (Fauza, 2014). In this study, *U. gambir* Roxb. leaf extract was used as a bioreducing agent to synthesize gold nanoparticles. In our previous studies, we have successfully synthesized silver nanoparticles by employing *U. gambir* Robx. as a bioreducing agent by the hydrothermal method in water solvent (Arief *et al.*, 2015), in isopropanol solvent (Arief *et al.*, 2017), and also by reduction method in the presence of diethanolamine (Labanni *et al.*, 2018). To the best of our knowledge, this is the first report of the study on the synthesis of gold nanoparticles using *U. gambir* Roxb. as a bioreducing agent.

In addition, a stabilizer agent is necessary to be added to the reaction to control the growth and the size of the particles to form stable colloidal nanoparticles (Patel *et al.*, 2017). This control is substantial for the medical application since the antibacterial activity is strongly affected by size, shape, and concentration of the nanoparticles (Franci *et al.*, 2015). Hence, in this study, we use triethanolamine as a capping agent. The concentration of $HAuCl_4$ as a precursor was also varied to investigate the effect of concentration on the properties of gold nanoparticles.

MATERIALS AND METHODS

Materials

$HAuCl_4$ as the precursor and triethanolamine (TEA) as the capping agent in the synthesis were purchased from Merck. Fresh *U. gambir* Roxb. leaves were taken from Payakumbuh, West Sumatera, Indonesia. *Uncaria gambir* Roxb. was identified and deposited at Herbarium Universitas Andalas (ANDA) Padang, Indonesia with specimen number of 01AL-Arniati Labanni.

Preparation of Uncaria gambir Roxb. leaf extract

First, *U. gambir* Roxb. leaves were washed with tap water to remove dirt, and then were shade-dried for a week at room temperature. Furthermore, *gambir* leaves were mashed using a grinder to obtain a powder. The powder was then stored in a dark sealed container and protected to prevent damage and deterioration.

Plant extracts were obtained by weighing 10 g of plant powder, which were then added with 100 ml of distilled water and heated at 60°C for 2 hours. The mixture was then filtered to obtain the extract. The extract was stored in the refrigerator for future use.

Synthesis of gold nanoparticles

Every 25 ml of HAuCl4 solution with concentrations 20 and 100 ppm was mixed with 10 ml of 1% TEA and 15 ml of 6% *U. gambir* Roxb. extract to a total volume of 50 ml. These mixtures were then stirred for 6 hours at room temperature. The formation and growth of colloidal gold nanoparticles were monitored periodically using ultra violet – visible (UV-Vis) spectrophotometer (510000 SECOMAM). The colloidal gold nanoparticles without TEA as a capping agent were also prepared with the same precursor concentration. The TEA stabilized gold nanoparticles were then coded as AuNps-1 and AuNps-2 for using 20 and 100 ppm $HAuCl_4$, respectively. The unstabilized gold nanoparticles were coded as AuNps-3 and AuNps-4 for the concentration of 20 and 100 ppm $HAuCl_4$, respectively.

Characterization

The crystallinity of AuNps was studied using X-ray diffraction (XRD) Phillips X'pert Powder PAN analytical with radiation of CuK (λ = 1,5406 Å) operated at 30 kV and 30 A. The powder sample of AuNps was prepared by precipitating the colloidal AuNps. The obtained solid was separated from the filtrate, then washed with distilled water, and dried in a hot air oven to obtain the powder AuNps. The size distribution and image of colloidal AuNps shape were studied using transmission electron microscope (TEM) JEOL JEM 1400.

Antibacterial activity test

The as-synthesized *U. gambir* Roxb. mediated AuNps were tested against *Escherichia coli* and *Staphylococcus aureus* bacteria using the disc diffusion or Kirby Bauer method. Nutrient agar medium was used to cultivate bacteria. Fresh overnight bacteria culture was taken and 100 µl of inoculum was spread on nutrient agar (NA) agar plates. Sterile paper discs of 5 mm diameter (containing 50 µg/ml AuNps) were placed in each plate, along with positive and negative control. Positive and negative control used in the test were water and amoxicillin, respectively. After incubating for 24 hours at 37°C, the inhibition zone of the samples was measured. The test was conducted in duplicate, and all data reported are average values.

RESULTS AND DISCUSSION

UV-Vis spectrophotometer study

The formation of gold nanoparticles were then confirmed using the UV-Vis spectrophotometer at a wavelength of 515–570 nm based on surface plasmon resonance (SPR) phenomenon. The result of the UV-Vis spectrophotometer (Fig. 1) provides a spectrum of the *U. gambir* Roxb. extract, which shows a peak at 279 nm, referred to catechin. When reacted with HAuCl4, the absorption peak shifted to a wavelength of 534 nm, which confirmed the formation of gold nanoparticles, indicating that Au^{3+} ions have been successfully reduced to Au^0 by the content of catechin in *U. gambir* Roxb. leaf extract. This is in accordance with the previous synthesis by Yu *et al.* (2016) using *Citrus maxima* aqueous extract and Paul

et al. (2015) using *Pogestemon benghalensis* which showed the local surface plasmon resonance (LSPR) band of gold nanoparticles at 535–538 nm and 510–560 nm, respectively.

In spectrophotometry analysis, it was observed that the concentration and the utilization of capping agents strongly affect the optical properties of the gold nanoparticles. TEA capped AuNps both at HAuCl$_4$ concentration of 20 ppm (AuNps-1) and at a concentration of 100 ppm (AuNps-2), showing better stability than non-stabilized AuNps both at a concentration of 20 ppm (AuNps-3) and at a concentration of 100 ppm (AuNps-4). AuNps-1 and AuNps-2 did not show precipitation until the reaction time of 168 hours, while in AuNps-3 and AuNps-4, the precipitation was observed after 24-hour reaction time. The precipitation of the nanoparticles occurs due to the agglomeration. It is caused by the growth and the van der walls interaction among the nanoparticles (Sinha and Mukherjee, 2014). The use of a capping agent or stabilizer can minimize the interaction between nanoparticles, thus preventing the excess growth of nanoparticles which causes agglomeration (Chu *et al.*, 2018). In this study, results have shown that the use of TEA as a capping agent successfully prevented or delayed the occurrence of agglomeration and deposition of nanoparticles up to seven times.

In addition, the results exhibited that the concentration of the HAuCl$_4$ precursor can affect the speed of agglomeration and precipitation of nanoparticles. It was observed in the TEA-unstabilized gold nanoparticles where the AuNps-3 was precipitated before 24 hours and AuNps-4 was precipitated after 24 hours. In contrast, the TEA-stabilized AuNps-1 and AuNps-2 showed good stability and did not show precipitation until 168 hours. These results showed that TEA can maintain the stability of the gold nanoparticles even with 5× precursor concentration.

The formation of nanoparticles was also investigated using laser beam radiation based on the Tyndall effect. The inset picture in Figure 1 showed the laser beam radiation on AuNps-1 in the time reaction of 0, 2, and 24 hours. It was observed that light was dispersed in the colloid, which confirmed the scattering of the light by the particles. The color of the colloidal nanoparticles turned dense after some time, which confirmed that the number of colloidal nanoparticles increased continuously during the reaction.

The wavelength and absorbance values of *U. gambir* Roxb. mediated gold nanoparticles characterized by the UV-Vis spectrophotometer are presented in Table 1. It shows that the absorbance of the nanoparticles was increased with the increase of concentration from 20 to 100 ppm of the precursor, both in
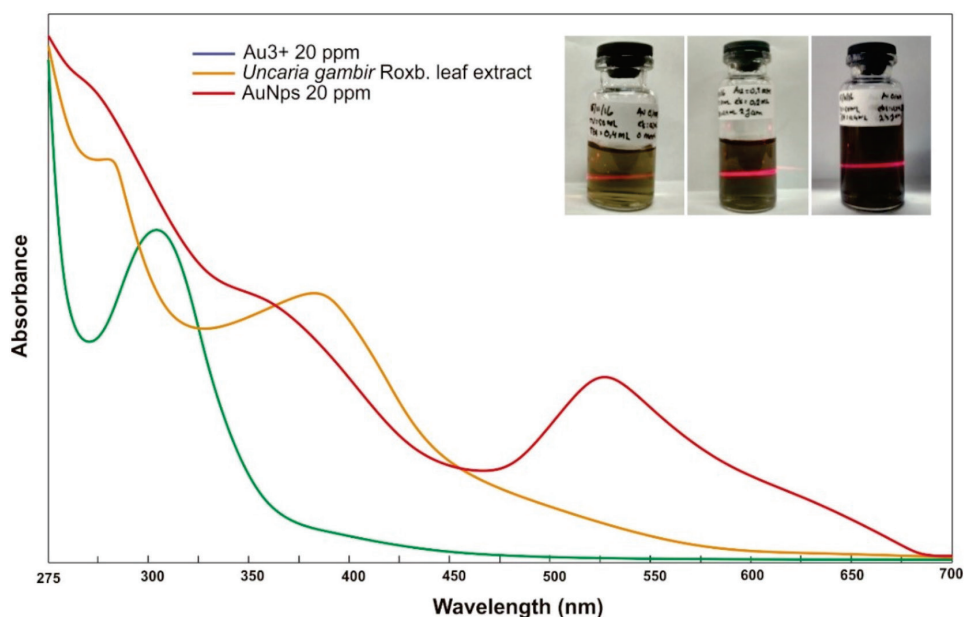

Figure 1. UV-Vis spectra before and after reaction. Inset shows the laser beam radiation after a. 0 hour, b. 2 hours, and c. 24 hours.

Table 1. Absorbance and wavelength value of *U. gambir* Roxb. mediated colloidal gold nanoparticles.

Reaction Time (h)	Wavelength (nm)				Absorbance			
	AuNps-1	AuNps-2	AuNps-3	AuNps-4	AuNps-1	AuNps-2	AuNps-3	AuNps-4
0.5	-	-	-	545	-	-	-	1.688
1	-	-	515	543	-	-	0.338	1.939
2	541	555	515	538	0.271	1.158	0.339	2.055
4	541	560	515	538	0.318	1.241	0.346	2.258
6	544	565	515	538	0.331	1.447	0.352	2.295
24	534	570	520	535	0.426	0.577	0.376	2.355
168	534	-	522	534	0.407	-	0.402	2.313

TEA stabilized and unstabilized gold nanoparticles. This result suggested that the higher concentration of the precursor leads to the increase of nanoparticles formed in the reaction. In addition, the wavelength values showed similar results, where a higher concentration provides a higher wavelength value.

Crystallinity study

The crystalline nature of synthesized Au nanoparticles was further studied by XRD analysis. Figure 2 shows the XRD pattern of powdered Au nanoparticles. Five distinct peaks were observed at 38.22° (111), 44.41° (200), 64.62° (220), 77.73° (311), and 81.77° (222) which referred to a well crystalline face-centered cubic (FCC) gold nanoparticles, based on the International center for diffraction data (ICDD) standard 039065-2870. This result was in agreement with previous studies which used *Citrus maxima* (Yu *et al.*, 2016), *Pogestemon benghalensis* leaf extract (Paul *et al.,* 2015), *Stevia rebaudiana* leaf extract (Sadeghi *et al.*, 2015), and *Lantana camara* leaf extract (Dash *et al.*, 2015). Based on the Debye–Scherrer formula, Au crystallite size was estimated to be 32.5 nm which is slightly smaller than the crystallite size (36.28 nm) reported by Li *et al.* (2016) by studying the gold nanoparticles synthesized using extreme bacterium *Deinococcus radiodurans*.

Morphology study

The results of TEM analysis AuNPs-1, AuNps-2, AuNps-3, and AuNps-4, which have been synthesized, can be seen in Figure 3. It was observed that the synthesized gold nanoparticles have a variety of shapes such as hexagonal, triangular, and, predominately, round shapes. This TEM result showed that the use of TEA as a capping agent in various concentrations affected the particle size. The particle size distribution of AuNps-1, AuNps-2, AuNps-3, and AuNps-4 was obtained as seen in Figure 4a–4d, respectively.

The particle size ranges of AuNps-1, AuNps-2, AuNps-3, AuNps-4 were 13–82 nm, 4–16 nm, 10–56 nm, and 11–62 nm, respectively, with an average size of 29, 11, 23, and 31 nm, respectively. The TEA unstabilized gold nanoparticles showed that the increase of precursor concentration leads to an increase in size. Different from the TEA stabilized gold nanoparticles, the increase in concentration did not exhibit any effect on the particle size. These results suggested that TEA successfully maintained the particle size of gold nanoparticles even in larger concentration synthesis. The nitrogen element in TEA is expected to play a crucial role in the capping mechanism of gold nanoparticles. The interaction between positively surface charged gold nanoparticles and positively charged nitrogen forms a protective monolayer on the surface of gold nanoparticles. This protective monolayer prevents excessive interaction among the gold nanoparticles, hence preventing aggregation and controlling the growth of gold nanoparticles (Yamamoto *et al.*, 2006).

Antibacterial activity study of Uncaria gambir Roxb. mediated gold nanoparticles

Figure 5 shows well-defined zones of inhibition of gold nanoparticles against *S. aureus* (a) and *E. coli* (b), as a representative of gram-positive and gram-negative bacteria. The inhibition zone of the sample was shown in Table 2. The test was conducted in duplicate, and all the data reported are average values.

The inhibition zone of AuNps-1, AuNps-2, AuNps-3, and AuNps-4 were 6, 7, 6, and 6 mm against *S. aureus* and 8, 9, 7, 6, mm against *E. coli*, respectively. Whereas amoxicillin as a positive control was measured to be 4 mm against *S. aureus* and 5 nm against *E. coli*.

A slightly higher inhibition zone of AuNps was observed in the test against *E. coli* than the test against *S. aureus*. This is due to the difference in cell wall composition between positive strain and negative strain bacteria. The peptidoglycan layer in negative strain bacteria is thinner than that in positive strain bacteria, making AuNps to easily enter the cytoplasm. The detailed mechanism of the antibacterial activity of AuNps is currently studied and will be reported in the next paper.
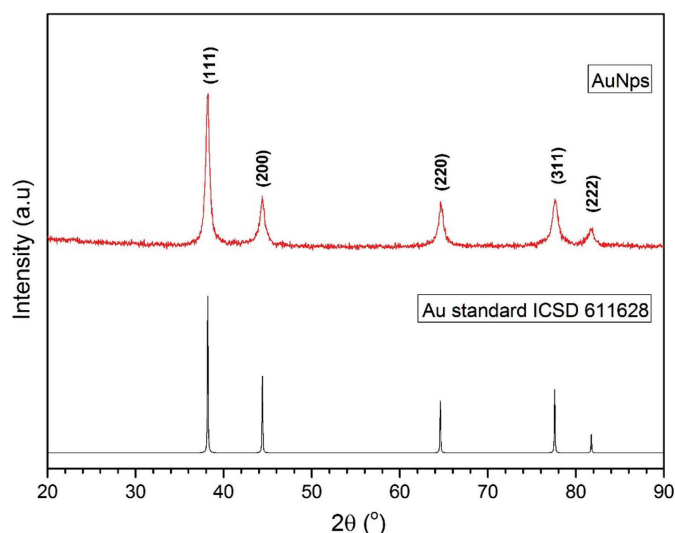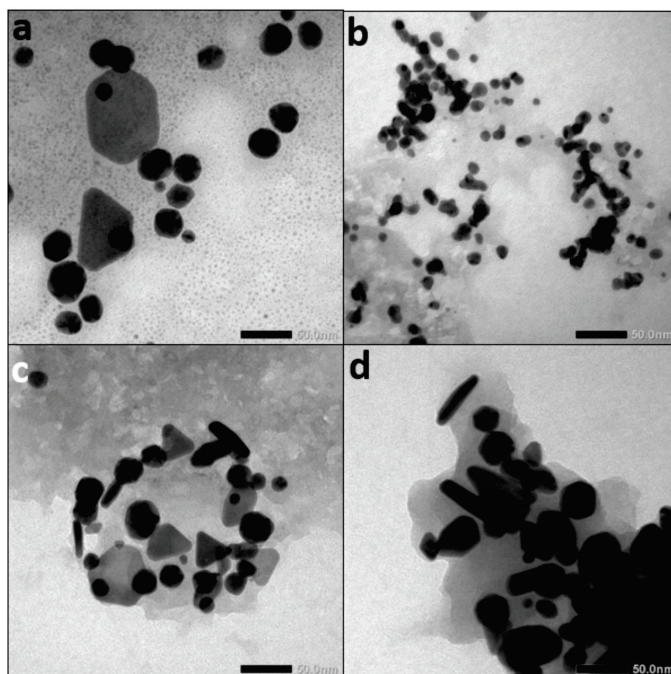

Figure 2. XRD pattern of AuNps powder.

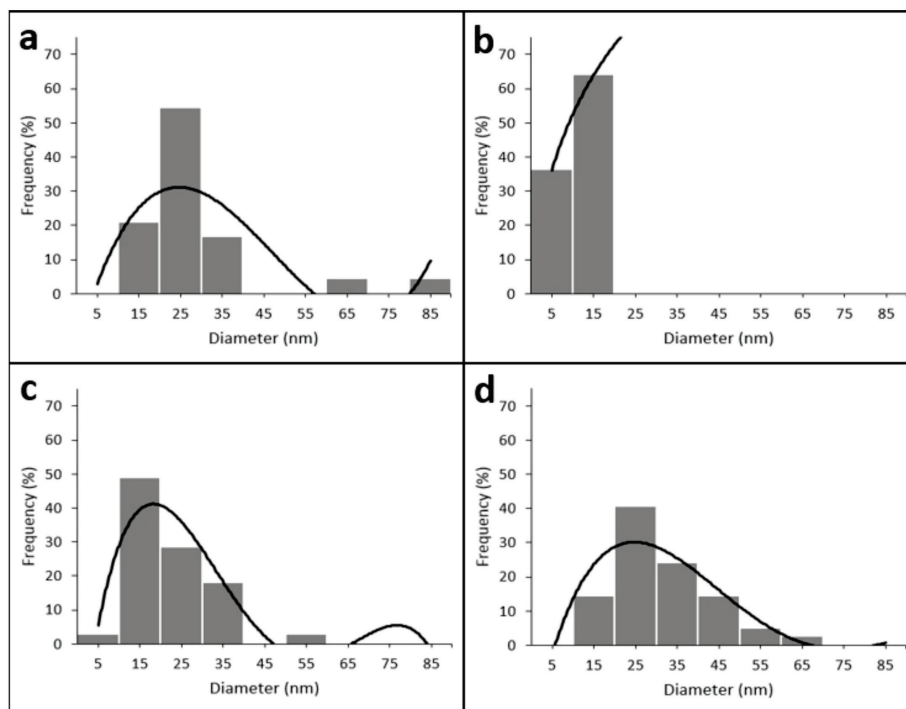**Figure 3.** TEM images of a) AuNps-1, b) AuNps-2, c) AuNps-3, and d) AuNps-4.

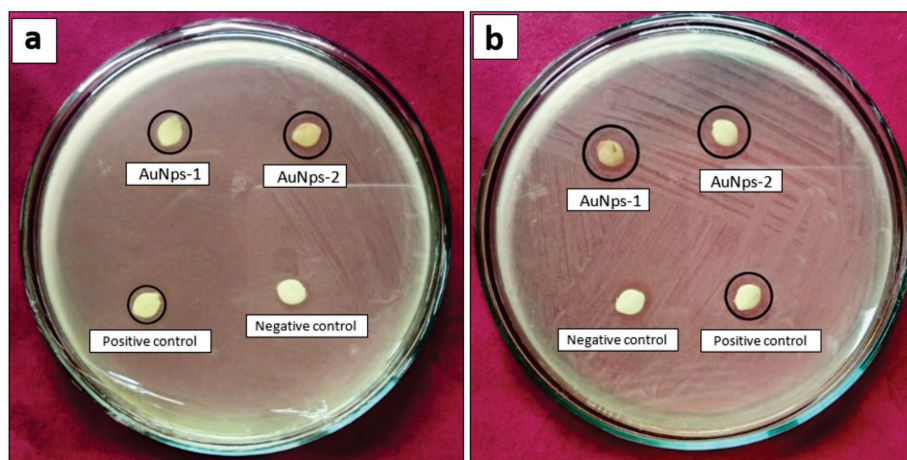**Figure 4.** Particles size distribution of a) AuNps-1, b) AuNps-2, c) AuNps-3, and d) AuNps-4.

Figure 5. Antibacterial activity of gold nanoparticles against a) *S. aureus* and b) *E. Coli*.

Table 2. Inhibition zone of gold nanoparticles.

Bacteria	Inhibition zone (mm)					
	(-) controll	(+) controll	AuNPs-1	AuNPs-2	AuNPs-3	AuNPs-4
S. aureus	-	4	6	7	6	6
E. coli	-	5	8	9	7	6

The results showed that different $HAuCl_4$ concentrations in synthesizing gold nanoparticles did not show any significant effect on the inhibition zone. In addition, a higher inhibition zone was performed by AuNps-2 both against *S. aureus* and *E. coli*. This might be due to the size of AuNps which allowed it to easily enter into the cytoplasm of the cell. AuNps stimulated the formation of biofilm and interacted with the element of the cell wall, leading to the change of the structure, degradation, and hence the death of the cell. While interacting, AuNps was trapped in the biofilm and released some compounds, which caused a distortion in the cell wall. Some previous studies on green synthesized gold nanoparticles and their antibacterial activity with the comparison to our samples are shown in Table 3. These results showed that *U. gambir* Roxb. mediated gold nanoparticles with TEA since capping agents have the potential to be developed as antibacterial agents in biomedical applications.

Table 3. Comparison of particle size and inhibition zone of green synthesized gold nanoparticles.

Extract	Average particles size (nm)	Bacteria strains	Inhibition zone (mm)	References
Gloriosa superb leaf extract	20	*B. subtilis*	-	(Gopinath *et al.*, 2016)
		E. coli	-	
Deinococcus radiodurans bacteria	43.75	*S. aureus*	8.95 ± 0.17	(Li *et al.*, 2016)
		E. coli	9.21 ± 0.20	
Nephentes khasiana leaf extract	50	*Bacillus sp.*	10.0	(Bhau *et al.*, 2015)
		E. coli	8.0	
U. gambir Roxb leaf extract	11	*S. aureus*	7	this work
		E. coli	9	

CONCLUSION

Gold nanoparticles of different shapes and sizes were successfully synthesized using *U. gambir* leaf extract by the addition of TEA and varying the concentration. The UV-Vis spectrophotometry analysis confirmed the formation of AuNps by SPR absorption at a wavelength of 515–570 nm. The XRD analysis results showed the formation of high-crystalline FCC-structured AuNps. The TEM analysis result showed that the formed AuNps were spherical, hexagonal, and triangular with 4–82 nm in size. The utilization of TEA as a capping agent can maintain the particle size, even in 5× precursor concentration. The prepared AuNps showed excellent antibacterial activity against *E. coli* and *S. aureus*.

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CONFLICT ON INTEREST

Authors declared that they do not have any conflicts of interest.

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