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Antioxidant and Antibacterial Activities of Biosynthesized Silver Nanoparticles using Aqueous *Terminalia catappa* Leaf Extracts as Novel Reducing Agent

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In this study, silver nanoparticles (AgNPs) were successfully synthesized from aqueous *Terminalia catappa* leaf extract that acts as a novel reducing agent. Various parameters, including pH, temperature and reaction time, were determined. The UV-visible spectra showed the main peak at 416 nm, which was the characteristic surface plasmon resonance of AgNPs. The spherical shape and particle size of 49 ± 0.01 nm were observed from SEM, TEM and laser particle size analysis (LPSA). FTIR spectra of the leaf extract exhibited the characteristic functional groups that should be responsible for Ag^+ ion reduction. The EDX spectrum proved that the obtained sample is silver. The antioxidant activity of AgNPs treated with the leaf extract as determined by the DPPH assay was higher compared to that of *Terminalia catappa* leaf extract, and the treated AgNP sample exhibited high antibacterial potential against both Gram-positive and Gram-negative bacteria.

Keywords: Antioxidant activity, Antibacterial activity, Silver nanoparticles, *Terminalia catappa*.

INTRODUCTION

Silver nanoparticles (AgNPs) have numerous applications in several industries, such as pharmaceuticals, electronics and biosensor development [1-3]. Till date, several chemical, physical and biological methods have been adopted for nanoparticle synthesis [4-7]. Biological synthesis is eco-friendly because nontoxic reducing and capping agents are used. Usually, plants containing flavonoids, alkaloids, and polyphenolic compounds are ideal for preparing nanoparticles, wherein these compounds reduce silver ions (Ag^+) to silver nanoparticles (Ag^0) and act as capping agents [8]. One such plant *Terminalia catappa* L. that has been used for AgNPs synthesis has garnered considerable attention due to its low cost and easy availability. *Terminalia catappa* L. is commonly used as a popular medicine in Southeast Asia and has been claimed to have therapeutic effects for liver-related diseases [9].

Commonly, *Terminalia catappa* L. is found in coastal areas and along the roadsides in various Southeast Asian countries [10]. Various forms of hydrolyzable tannins have been isolated from the leaves of this plant, including punicalagin, punicalin, terflavins A and B, tergallagin, tercatain, geranin, chebulagic acid, geranin, granatin B and corilagin but no caffeine has been found in it [11]. In present study, we propose the possible mechanism for the reduction process of Ag^+ by important functional groups in tannins (Fig. 1). In this mechanism, Ag^+ ions form transitional complexes with the phenolic hydroxyl groups of tannic acid that loses one hydrogen radical and one electron, and then successively undergo oxidation to quinone, followed by the reduction of Ag^+ ions to form AgNPs [12,13].

This study aimed to synthesize and characterize AgNPs synthesized using *Terminalia catappa* leaf extract as a reducing agent and determined the optimal parameters including temperature, pH and reaction time because the size and shape of silver nanoparticles are dependent on the physical and chemical factors; in present study, nanoparticle synthesis was controlled by the pH and temperature of the reaction mixture [14]. Moreover, the potential of the synthesized AgNPs as an antioxidant and antibacterial agent was investigated.

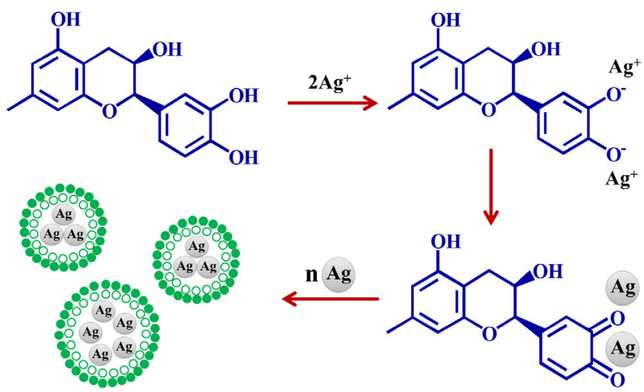

Fig. 1. Possible mechanism for the reduction process of silver ion (Ag^+)

EXPERIMENTAL

Terminalia catappa leaves were collected from Ban Pa-yang, Tambon Tha-Ngew, Amphoe Mueang, Nakhon Si Thammarat Province, Thailand. Silver nitrate and 1,1-diphenyl-2-picryl hydrazyl (DPPH) were obtained from Sigma-Aldrich, which used as received. Other reagents of analytical grade were purchased from Merck. All the materials for bacterial studies, including nutrient agar, Mueller-Hinton broth, and agar for antibacterial activity, were bought from Hi-Media, Mumbai, India. A UV-visible spectrophotometer (Thermo scientific, Evolution 201) was used for recording the UV-Vis absorption spectra of the obtained samples. The X-ray diffraction patterns of all the samples were confirmed by an X-ray diffractometer (XRD) (Empyrean, PANalytical, Netherlands). The morphological properties of the synthesized samples were characterized by scanning electron microscopy (Merlin Compact, Zeiss) and transmission electron microscopy (TEM) (JEOL JEM-2101). Energy dispersive X-ray (EDX) was used to perform elemental analysis. The FTIR spectra of the obtained samples were verified by Fourier transform infrared spectroscopy (Bruker, Germany, VERTEX). A laser particle size analyzer (Beckman Coulter, LS 320) was used to determine the particle size of the nanomaterial.

Preparation of leaf extract: *Terminalia catappa* leaves were thoroughly washed and dried in the shade, after which they were cut into small pieces. *Terminalia catappa* leaves (10 g) were crushed with 100 mL of distilled water for 12 h at room temperature, after which the suspension was filtered through a Whatman No. 1 filter paper. The obtained filtrate in the form of an aqueous extract was stored at 4 ºC for further use as a reducing agent.

Phytochemical analysis of leaf extract: Qualitative phytochemical tests for detecting anthraquinones, flavonoids, steroids, terpenoids, saponins, alkaloids, and tannins were performed using the leaf extract according to the methods described by Ayoola *et al*. [15].

Optimization of AgNPs synthesis and their characterization: The aqueous solution of 1 mM $AgNO_3$ was prepared in an Erlenmeyer flask. Leaf extract (2 mL) was mixed with 18 mL of $AgNO_3$ solution under magnetic stirring. Silver nanoparticle formation was visually observed by the gradual change in colour of the mixture, which was incubated under conditions of different pH, time, and temperature. To achieve the maximum product yield, optimization of these factors was analyzed by UV-visible absorption. The water-suspended nanoparticles were evaporated under vacuum. After the nanoparticles were dried, SEM, TEM, EDX, laser particle size analysis (LPSA) and FTIR techniques were used to study their structure and composition.

Antioxidant activity determined using 1,1-diphenyl-2-picrylhydrazyl radical: The antioxidant activity of the leaf extract and the synthesized AgNPs were studied based on the method by Sharma and Kumar [16]. Briefly, 4 mL of 0.1 mM of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical in methanol was added to 1 mL of the samples of different concentrations. The samples were kept at room temperature in the dark, and after 0.5 h, their absorbance was measured at 517 nm against a blank of methanol. Ascorbic acid was used as the standard compound. The percentage of scavenging activity of *Terminalia catappa* leaf extract was calculated based on the following equation:

$$\text{DPPH inhibition } (\%) = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

where $\text{Abs}_{\text{control}}$ is the absorbance of DPPH taken as control and $\text{Abs}_{\text{sample}}$ is the test absorbance. All the experiments were performed in triplicate with the average values stated.

Antibacterial activity of synthesized AgNPs: The antibacterial activity of synthesized AgNPs was tested against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria by disc diffusion method. The bacterial culture was spread on a nutrient agar plate using a sterile cotton swab. Wells were prepared on agar plates, and nanoparticle solution (30 mg/L) and a standard antibiotic (chloramphenicol, 30 mg) were added to the wells. After the samples were incubated at 37 ºC for 24 h, the diameter of the inhibition zones of AgNPs was measured and compared with that around the commercial standard antibiotic chloramphenicol and aqueous leaf extract. This assay was repeated three times.

RESULTS AND DISCUSSION

Characterization of AgNPs: Phytochemical analysis of the aqueous extract of *Terminalia catappa* leaves showed the presence of different types of compounds, the main ones being steroids, flavonoids, terpenoids, saponins, alkaloids and tannins, which acted as reducing agents in the reduction reaction of AgNPs. The reduction of Ag^+ into AgNPs by the plant extract was demonstrated by the optical colour change of the solution from yellow to deep brown owing to phenomena of surface plasmon vibrations in AgNPs [17]. The surface plasmon resonance of AgNPs showed a peak centered near 424 nm in the UV-visible spectra, which corresponds to the absorbance of AgNPs [18] (Fig. 2). This observation suggested that the active compounds in the leaf extract might have reduced Ag^+ to AgNPs. However, further studies are required to identify the specific biomolecules involved in the synthesis of AgNPs.

FTIR studies: FTIR spectroscopy of the leaf extract (Fig. 3) showed peaks at 1042, 1350, 15617 and 3400 cm^{-1} attributed to C-N stretching (aliphatic amines), C-H group (aromatic),

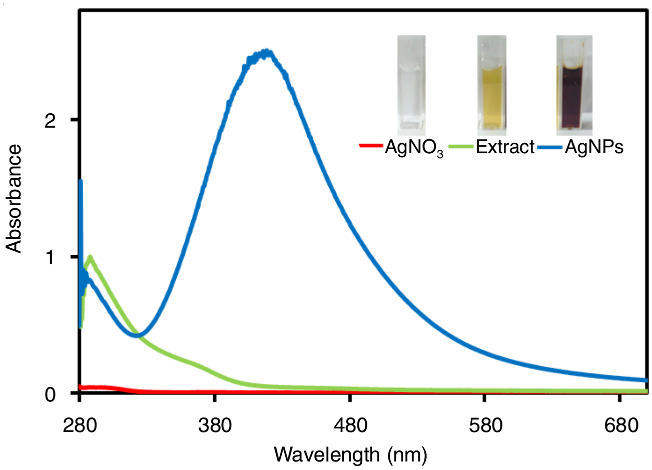

Fig. 2. UV-vis spectra of *Terminalia catappa* leaf extract, $AgNO_3$, and the AgNPs

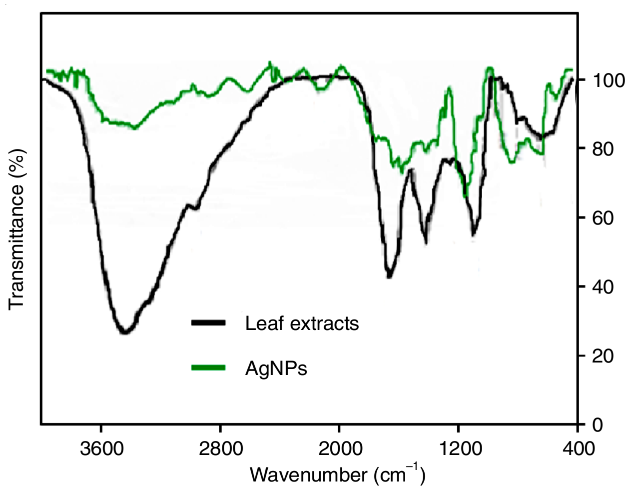

Fig. 3. FTIR spectra of AgNPs *vs.* leaf extract

C=O stretching and O-H stretching [19], respectively. The majority of the IR bands are characteristic of phenolic compounds present in the leaf extract. FTIR spectroscopy of AgNPs showed a broad peak that decreases in the intensity at around 3400 cm^{-1} corresponding to the OH stretching vibrations of phenolic compounds. The decrease in the peak shift may indicate the involvement of the OH functional group in the reduction of Ag^+ ions [20].

TEM analysis: The TEM micrograph of AgNPs is shown in Fig. 4b. The shape of AgNPs was clearly identified as spherical and LPSA analysis determined their average size to be 49 ± 0.01 nm. Furthermore, the SEM image showed a uniformly distributed spherical shape of synthesized AgNPs on the surfaces (Fig. 4a). The EDX profile exhibited a sharp peak (79%) at the energy of 3 keV for silver and also some of the weak peaks for C and O were observed, which may have initiated from the chemical composition in the extract bound to the surface of AgNPs. The emission energy at 3 keV points to the reduction of silver ions to metal silver (Ag^0) (Fig. 4c).

Effect of time: Time was one of the most important factors in the identification of nanoparticles during the green synthesis process. Fig. 5a shows that an increase in the reaction time resulted in a gradual increase in the absorbance at 420 nm and it was founded that the colour intensity increased with the duration of incubation with the maximum absorbance reached at 3 h. When incubation time was 5 h, the absorbance values decreased and there was a shift in the colour of peak to red, indicating instability in the formation of AgNPs possibly due to increased particle aggregation and precipitation when the reduction time increased [21].

Effect of temperature: The UV-visible spectra of AgNPs at three different temperatures *viz.* room temperature, 40 ºC and 60 ºC is shown in Fig. 5b. It is found that the rate of AgNPs

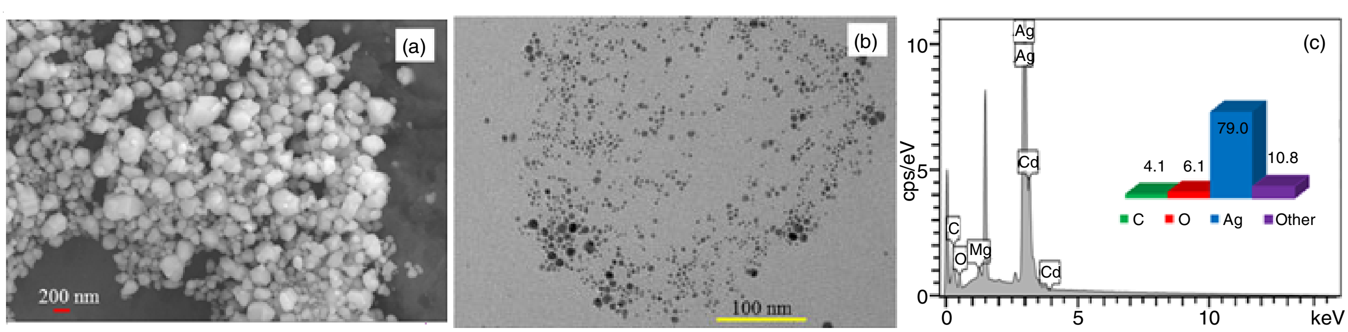

Fig. 4. (a) SEM image (b) TEM image (c) EDX spectrum of AgNPs

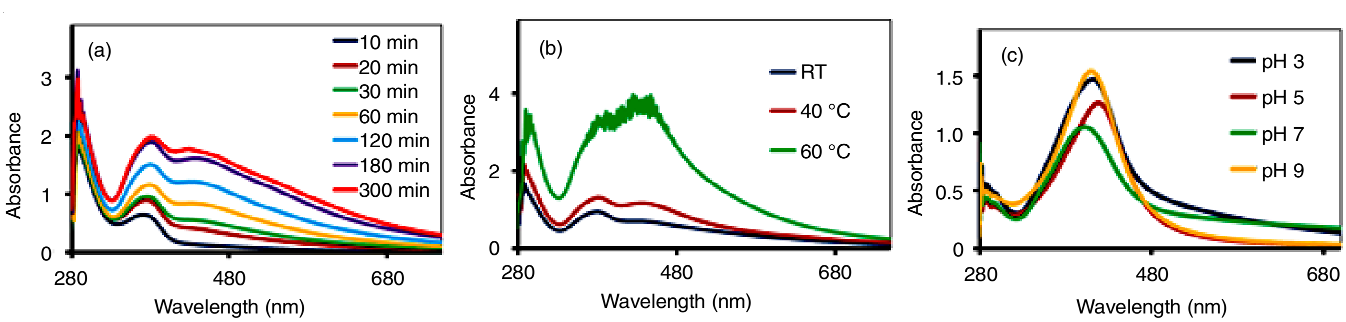

Fig. 5. UV spectra showing (a) effect of time (b) effect of temperature and (c) effect of pH on the nanoparticles synthesis

formation increased with higher temperatures. The maximum synthesis of AgNPs occurred at 60 ºC. However, when the reaction was performed at > 60 ºC, the leaf extract degraded.

Effect of pH: The effect of pH was determined under acidic, neutral and basic conditions. The UV-visible spectra of AgNPs were measured at pH 3.0, 5.0, 7.0 and 9.0. The particle size of AgNPs was measured under the best conditions. The results showed that the synthesis of AgNPs occurred under a neutral pH condition and yielded more products under the basic condition. Fig. 5c shows the effect of pH on AgNP synthesis. At pH 3, the colloid solution was colourless and changed to brown at pH 9. As the pH increased, the colour of the solution became more intense, indicated that the formation of AgNPs was more effective at higher pH levels.

Antioxidant activity: The free radical-scavenging activity was studied in terms of electron donating or radical scavenging ability using the stable radical DPPH. Previous studies showed that the plant-mediated nanoparticle synthesis includes reduction followed by capping with plant constituents [22]. These results confirmed the antioxidant activity of AgNPs capped with the essential functional groups possessing free radical-scavenging activity. As shown in present results, capped AgNPs of *Terminalia catappa* leaf extracts were found to be a strong free radical scavenger when compared to the standard ascorbic acid. This antioxidant activity may be caused by the capping constituents present in the plant extract and present on the surface of the nanomaterials.

The IC_{50} value of *Terminalia catappa* leaf extracts and AgNPs was 89.12 and 10.41 mg/mL, respectively, indicating that AgNPs exhibited a better antioxidant activity in comparison to the leaf extract. Moreover, the DPPH free radical-scavenging assay of AgNPs when compared with that of ascorbic acid showed promising results. The bioconjugated AgNPs exhibited a comparable free radical scavenging activity to that of ascorbic acid, which has an IC_{50} value of 13.40 mg/mL.

As well the antioxidant activities of *Terminalia catappa* leaf extracts and AgNPs compared with standard ascorbic acid in Fig. 6 showed %DPPH inhibition of *Terminalia* leaf extracts and AgNPs compared with standard ascorbic acid. Again AgNPs exhibited the highest antioxidant capacity and showed greater than antioxidant capacity of *T. catappa* leaf extracts.

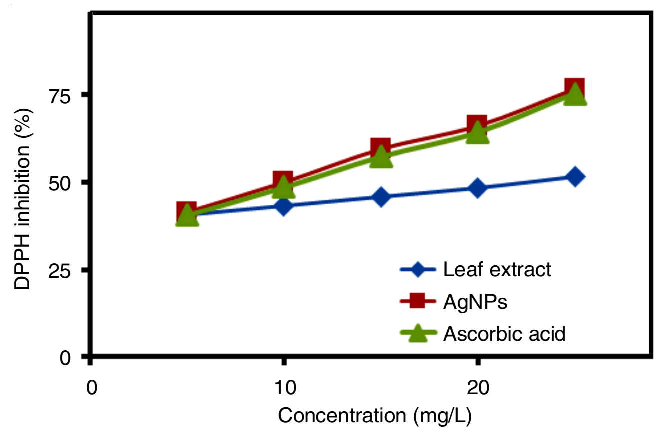

Fig. 6. Antioxidant activities of *Terminalia* leaf extracts and AgNPs compared with standard ascorbic acid

The antibacterial properties of the synthesized AgNPs were tested against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria by well diffusion method. The antibacterial activities of *Terminalia catappa* leaf extracts and their corresponding AgNPs colloidal solutions are shown in Fig. 7. The inhibition zone around the well (Table-1) showed that synthesized AgNPs exhibited strong antibacterial

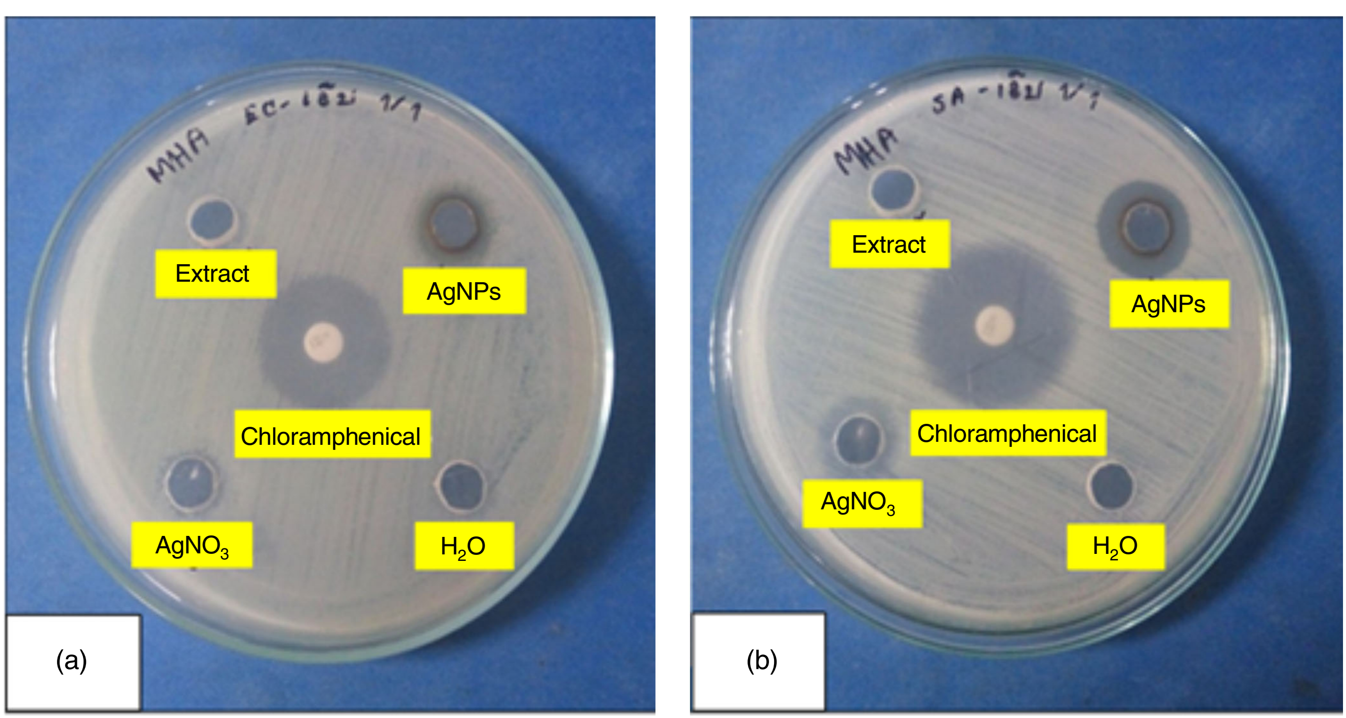

Fig. 7. Antioxidant activities of *Terminalia* leaf extracts and their corresponding AgNPs colloidal solutions

TABLE-1
INHIBITORY ACTION OF AgNPs AGAINST
S. aureus AND *E. coli* BACTERIA

Sample	Solvent	Inhibition zone (mm)	
		S. aureus	*E. coli*
$AgNO_3$	H_2O	9.5 ± 0.70	11 ± 1.41
Leaf extract	H_2O	No inhibition	No inhibition
AgNPs	H_2O	13.5 ± 0.82	16 ± 1.54

activity than that of leaf extract and $AgNO_3$. *E. coli* (Gram-negative bacterium) produced the maximum zone of inhibition possibly due to its cell wall composition constituting a thick peptidoglycan layer that forms a rigid structure obstructing the penetration of AgNPs; the Gram-negative bacteria, on the other hand, have a relatively thinner peptidoglycan layer [23].

Conclusion

A simple and efficient green synthesis method of AgNPs using aqueous leaf extract of *Terminalia catappa* is developed. The synthesized AgNPs were characterized by UV-visible spectroscopy, FTIR, LPSA, SEM and TEM analyses. The UV-visible spectra displayed a maximum absorption peak at 416 nm, which is a characteristic of nanosilver particles. TEM and SEM analyses confirmed the spherical shape of the particles. FTIR analysis was performed to reveal the potential functional groups involved in the synthesis of AgNPs. The mean particle size was 49 ± 0.01 nm, which was calculated using LPSA. It is found that one-step green method proposed in present study to be effective and economical, and it could serve as an alternative for the rapid production of AgNPs. In addition, obtained AgNPs exhibited better antioxidant activity against DPPH radicals than the standard ascorbic acid. The antibacterial activity of the synthesized AgNPs was also investigated by the well diffusion assay using both Gram-positive and Gram-negative bacteria. It is believed that the present findings can be appropriately used in various fields ranging from biomedicine to addressing environmental issues.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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