Research Article

Comparative Study of the Silver Nanoparticle Synthesis Ability and Antibacterial Activity of the *Piper Betle* L. and *Piper Sarmentosum* Roxb. Extracts

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1. Introduction

*Piper betle* (*P. betle*) and *Piper sarmentosum* (*P. sarmentosum*) are the two members of the *Piper* genus, which have been reported to be rich in phytochemicals and essential oils, which showed strong antioxidant, antibacterial, and antifungal activities [1]. Besides being used as a wrapper for the chewing of areca nut, *P. betle* leaves are also used as an ingredient in stimulant, antiseptic, tonic, and other ayurvedic formulations thanks to its bioactivity components such as hydroxychavicol, allylpyrocatechol, and eugenol [2, 3]. The other *Piper* species, *P. sarmentosum*, has been reported to...
possess many potential bioactivities due to its bioactive compounds such as Vitamin E, carotenoids, xanthophylls, tannins, flavanoid, and phenolics [4–6]. The aerial parts of P. sarmentosum are consumed as a vegetable, and the whole plant are applied as a folk cure for headaches, asthma, joint aches, and toothache and to reduce fever in influenza patients [7]. In recent, these Piper species have extensively been investigated in a wide range of studies to provide scientific evidence for folklore claims or to find new therapeutic applications and thereafter to utilize in commercial products.

Silver nanoparticles (AgNPs) have attracted great attention worldwide over the last few decades due to their outstanding antimicrobial activities [8–11]. Owning to their nanoscale size and high specific surface area, AgNPs have the ability to penetrate bacterial cell walls and change the structure of cell membranes, leading to cell growth inhibition or even cell death [12]. Moreover, compared to other synthesis methods of AgNPs, biosynthesis using various sources such as microorganisms and plant extracts has been regarded as the green method and has become the inevitable trend [13–17]. The reducing agents in these green sources would transfer their electrons to reduce silver (I) ions into AgNPs without using toxic solvents and generating harmful byproducts. Additionally, these biological molecules would cover the formed AgNPs and act as capping agents to prevent the agglomeration, reduce the toxicity, and improve the antimicrobial activity of the AgNPs [18]. Therefore, P. betle and P. sarmentosum with abundant of reducing agents are expected to be effective green sources to synthesize AgNPs.

Recently, a number of research were reported about AgNPs biosynthesis using extracts of Piper species. In 2014, AgNPs were synthesized by P. betle extract and tested their antibacterial activities on Bacillus cereus, Escherichia coli, Klebsiella pneumonia, and Staphylococcus aureus. The synthesized AgNPs performed more effective antibacterial activities to the pathogens [19]. In 2019, AgNPs synthesized by the aqueous extract of P. betle were evaluated for their effect on the postharvest physiology of cut flower [20]. In 2020, the process parameters for the synthesis of AgNPs from P. betle leaf aqueous extract were optimized, and the resulted AgNPs were assessed for antiphytological activity [21]. Even though P. betle has been well studied and applied in several commercial products with antibacterial and antifungal effects, P. sarmentosum has been still little-known in this field. Moreover, there have been several researches compared the vegetative anatomy, phenolic contents, and bioactivities between P. betle and P. sarmentosum [22, 23], but their capacities in AgNP synthesis have been not compared yet.

This study was aimed to compare the silver nanoparticle synthesis ability and antibacterial activity between P. betle and P. sarmentosum extracts. P. betle and P. sarmentosum were extracted by DIW. The appropriate extract conditions for their highest total reducing capacity were determined by DPPH scavenging and Folin-Ciocalteu assays. The solutions of silver nanoparticles prepared by the extracts of P. betle and P. sarmentosum were characterized by Dynamic light scattering (DLS), Zeta potential, UV-vis, and Fourier-transform infrared (FTIR) measurements. Finally, the antibacterial activity of the synthesized silver nanoparticle solutions was tested on Escherichia coli (E. coli) using the agar diffusion well–variant method. This study would contribute useful and important information to the development of antibacterial products based on green synthesized silver nanoparticles fabricated by the extracts of the two popular Piper species in Southeast Asia, P. betel and P. sarmentosum.

2. Materials and Methods

2.1. Materials. Silver nitrate (AgNO₃) was obtained from Guanhao High-Tech Co., Ltd. (China). Deionized water (DIW) was obtained from Milli-Q HX 7150 systems (Merck Millipore, France). pH-indicator paper was purchased from Merck (Germany). 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, and gallic acid were purchased from Sigma (Netherland). Luria Broth (LB) broth powder and agar powder were purchased from Lab M (UK).

P. betle and P. sarmentosum leaves were collected in a fresh state in Ho Chi Minh City area. After being removed the diseased, damaged, or contaminated ones, the leaves were mildly dried and ground into 2-5 mm pieces using an herbal grinder. The samples were kept in plastic zip bags and stored in fridge for further experiments.

2.2. Preparation of P. betle Extract and P. sarmentosum Extract. The dried grinding sample of each species was extracted with DIW at the ratio 1 : 15 (v/w) in an Erlenmeyer flask with magnetic stirring and heating. The extract times were 1, 2, 3, 4, and 5 h, and the extract temperatures were 30, 40, 50, 60, and 70°C. Aqueous extracts of P. betle (Pb.ext) and P. sarmentosum (Ps.ext) were obtained by centrifuging the extracted mixtures at 10,000 rpm in 10 min and consequently collecting the supernatants. These extracts were kept in the fridge for further experiments within 7 days.

2.3. Determination Total Reducing Capacity of the Extracts. It was reported that extracts with higher total reducing capacity are more potential in the green synthesis of AgNPs. The total reducing capacity of plants can be estimated by electron or hydrogen-atom transfer-based assays. DPPH and Folin-Ciocalteu assays are the two-electron transfer-based assays, in which the reducing agents present in the sample transfer electrons to oxidants such as DPPH radical or to the metal ion present in the Folin-Ciocalteu reagent. DPPH and Folin-Ciocalteu assays were used to estimate the total reducing capacity of the extracts [24–26].

DPPH assay: 100 μL of the extract (Pb.ext or Ps.ext) at concentrations of 10, 20, 30, 40, and 50% (of the initial extracts) was placed into each well of 96-well plate. Methanolic solution of DPPH (0.15 mM) was added 100 μL into every well. After being shaken vigorously, the plate was allowed for reactions in dark condition at room temperature in 30min. The control was prepared as above without the extract, and DIW was used for the baseline correction. The optical density (OD) of the samples was measured at 517 nm using a microplate reader. Ascorbic acid was used as a positive control [27]. The results were expressed as inhibition percentage of the
2.4. Preparation of Silver Nanoparticles. Silver nanoparticles were synthesized using the silver nitrate solution (1 mM) was dropped slowly into the correspondent solutions using opened syringes to form the silver nanoparticles. The silver nanoparticles synthesized by Pb.ext and Ps.ext were named as Pb.AgNPs and Ps.AgNPs, respectively. The investigated ratios of silver nitrate solution and Pb.ext or Ps.ext were prepared at the concentration range of 0-50 mg/L. The results were determined from the standard curve and were expressed as gallic acid equivalent (mg GAE/mL of extract) [28].

2.5. Characterization of the Synthesized Silver Nanoparticles. The size, size distribution, zeta potential of the synthesized silver nanoparticles were characterized using a Zetasizer Nano SZ (SZ-100, Horiba). The measurement was conducted at the detection angle of 90° and temperature of 25°C [29].

To illustrate the formation of the synthesized AgNPs, silver nanoparticle solutions with different reaction ratios and the corresponding extract were, respectively, loaded into the quartz cuvets to collect their UV-vis spectrum. The measurement was performed by Shimadzu UV-1800 machine (Shimadzu, Columbia, MD, USA) with a resolution at 1 nm and a wavelength range of 300-800 nm. DIW was used to adjust the baseline [30].

Fourier-transform infrared spectroscopy (FT-IR) was carried out by Bruker Equinox 55 FTIR spectrometer (Bruker, Ettlingen, Germany), and the KBr pellet method was used to explore the functional groups surrounding the synthesized AgNPs. Briefly, KBr was blended with each of the extracts at the ratio of 100:1 (w/w). The mixtures were then pelleted and recorded FT-IR spectroscopy at the wave-number range of 500-4000 cm\(^{-1}\) [9].

The hydrodynamic size and surface charge of the synthesized silver nanoparticles were measured by dynamic light scattering (DLS) using a Zetasizer Nano SZ (SZ-100, Horiba). The measurement was determined through a Helium-neon (He-Ne) laser beam with the detection angle, and the temperatures were 90° and 25°C, respectively. Samples were dispersed in DIW prior to measurement [31].

2.6. Antibacterial Assay. Antibacterial activity was assessed against *Escherichia coli*—ATCC 25922 using the agar diffusion well–variant method. *E. coli* cultures were grown to mid-exponential phase at 37°C before being harvested and resuspended in PBS with the ratio of 100 mg wet weight cells per 1 mL PBS. Then, 1 mL of cell suspension was mixed with 10 mL of LB broth agar (containing 1.5% agar) and overlaid on the surface of solid LB agar (containing 1.5% agar) petri dishes. Four holes (8 mm in diameter, 20-30 mm apart from one to another) were then punched on the agar and 20 μL of the nanosilver suspension at the dilution of 1, 5, and 10% was dropped into the holes. DIW and each extract, which were added the same volume into every punched well, were used as blank and control, respectively. Next, the dishes were kept at about 10°C in 4–8 h for the suspension to diffuse into the agar medium. Finally, the dishes were incubated at 37°C in 24 h [32, 33]. The zones of growth inhibition were detected following the equation below:

\[
\text{Growth inhibition zone (mm)} = D - d, \quad (2)
\]

where \(D\) is the radius of sterile ring (mm) and \(d\) is the radius of the punched wells (mm).

2.7. Statistical Analysis. All the experiments were replicated 3 times, and the obtained results were represented as mean ± standard deviation. All experimental data were analysed by Student's *t*-test. \(P < 0.05\) implied that two compared results were statistically significant. \(P > 0.05\) indicated nonstatistical (NS) difference [30].

3. Results and Discussion

3.1. Appropriate Extraction Temperature. The collected Pb.ext and Ps.ext were determined their total polyphenol content and DPPH scavenging activity to choose the appropriate extract temperature. The extracts owning higher total polyphenol content and stronger DPPH activity would be regarded as having better total reducing capacity [24].

In general, the total polyphenol contents of Pb.ext were higher than that of Ps.ext in the same extract condition (Figure 1). The polyphenol contents in the extracts increased when the extract temperature increased. The phenolic amount of Ps.ext reached the highest values (~125 mg GAE/mL extract) when *P. betle* was extracted at 50 and 60°C. Meanwhile, that of Ps.ext was highest at the extract temperature of 60 and 70°C (~90 mg GAE/mL of extract). At other higher temperatures, the total polyphenol contents slightly decreased due to the degradation of phytochemicals.
exposed in water at high temperature. The phenolic compounds in aqueous extract of P. betle leaves were identified including a phenylpropanoid, five cinnamoyl and six flavonoid derivatives by F. Ferreres et al. [35]. These phenolic compounds were reported to own the redox properties which allowed them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, or metal chelators. Therefore, Pb.ext and Ps.ext with high polyphenol contents were expected to perform antioxidation and reduction. Therefore, Pb.ext and Ps.ext were expected to perform antioxidation and reduction.

The antioxidant capacity of Pb.ext and Ps.ext was tested by DPPH free-radical scavenging assay and the results were presented in Table 1. The Pb.ext at 50°C and Ps.ext at 60°C performed the strongest DPPH scavenging activity with IC50 values of 1.45% and 2.00% (percentage concentration of the initial extract), respectively. The results suggested that P. betle has stronger antioxidant activity than P. sarmentosum. This was consistent with the published literature about the phytochemistry and pharmacology of the two Piper species [36, 37]. The IC50 value of ascorbic acid as the positive control was 5.21 μg/mL, which was relevant with the previous study [38, 39]. These results suggested that P. betle should be extracted at 50°C and P. sarmentosum should be extracted at 60°C to get a better total reducing capacity.

3.2. Appropriate Extraction Time. To determine the appropriate extract time, P. betle and P. sarmentosum were extracted at the chosen temperatures for each sample at different times from 1 to 5 h. Subsequently, the total polyphenol content and DPPH scavenging activity of the obtained extracts were determined.

The phenolic contents of the Pb.ext and Ps.ext, which were showed in Figure 2. It was confirmed that P. betle contained a higher amount of phenolic compounds than P. sarmentosum. The polyphenol contents in the Pb.ext gradually increased and reached the highest value (~125 mg GAE/mL extract) when the extraction time increased from 1 to 3 h and, then slightly decreased in the longer extraction time. For the Ps.ext, the total polyphenol reached the highest values and remained stable when the samples were extracted from 1 to 3 h (~90 mg GAE/mL extract). However, there was no statistically difference between 2 and 3 h extract samples of Pb.ext and among 1, 2, and 3 h extract samples of Ps.ext.

Additionally, the results of DPPH free-radical scavenging activity (Table 2) were consistent with the total polyphenol contents in the extracts. The Pb.ext in 2 and 3 h showed their best DPPH scavenging activity with IC50 of about 1.4%; meanwhile, Ps.ext in 1, 2, and 3 h got their lowest IC50 of about 2.4% (concentration of the initial extract). Consequently, the appropriate extract time was chosen as 2 h for P. betle and 1 h for P. sarmentosum. The correlation between polyphenol contents and DPPH scavenging activities of Pb.ext and Ps.ext suggested that the phenolic compounds of both extracts were responsible for their antioxidant activities [23]. These results also revealed that P. betle has stronger antioxidant activity as well as reducing power than P. sarmentosum. This was in line with the published literature about the two Piper species’ phytochemistry and pharmacology [36, 37].

![Figure 1: Total polyphenol content (mg GAE/mL) of Pb.ext (solid) and Ps.ext (pattern) extracted in 3 h at different temperatures. Bars show means ± SD. Bars with the same letters are not statistically different based on the least significant difference at P < 0.05.](image1)

![Figure 2: Total polyphenol content (mg GAE/mL) of Pb.ext (solid) and Ps.ext (pattern) extracted at 50°C and 60°C, respectively, in different time. Bars show means ± SD. Bars with the same letters are not statistically different based on the least significant difference at P < 0.05.](image2)
3.3. Characterization of the Synthesized Silver Nanoparticles. *P. betle* and *P. sarmentosum* were extracted by DIW in the chosen conditions of 50°C in 2 h, and 60°C in 1 h, respectively. Then, the obtain of each extract was used for green synthesis of silver nanoparticles with the silver nitrate solution and the leaf extract ratios of 1:2, 1:4, 1:6; 1:8, and 1:10 (v/v). After 8 h reacting in dark condition with magnetic stirring at room temperature, the result silver

![Graphs showing UV-vis spectra and FTIR spectra of Pb.ext and Pb.AgNP, Ps.ext and Ps.AgNP at different reacting ratios.](image)

**Figure 3**: The UV-vis spectra of (a) Pb.ext and Pb.AgNP at different reacting ratio, and (b) Ps.ext and Ps.AgNP (b) at different reacting ratio.

**Figure 4**: The FTIR spectra of (a) Pb.ext (i) and Pb.AgNP (ii) and (b) Ps.ext (i) and Ps.AgNP (ii).

| Silver nanoparticles | Pb.AgNP | Ps.AgNP |
|----------------------|---------|---------|
| Z-average (nm)       | 16.28 ± 3.84 | 14.51 ± 2.90 |
| Zeta potential (mV)  | -23.06 ± 1.52 | -19.83 ± 2.57 |

**Table 3**: Hydrodynamic size and Zeta potential of Pb.AgNP and Ps.AgNP.

3.3. *Characterization of the Synthesized Silver Nanoparticles*. *P. betle* and *P. sarmentosum* were extracted by DIW in the chosen conditions of 50°C in 2 h, and 60°C in 1 h, respectively. Then, the obtain of each extract was used for green synthesis of silver nanoparticles with the silver nitrate solution and the leaf extract ratios of 1:2, 1:4, 1:6; 1:8, and 1:10 (v/v). After 8 h reacting in dark condition with magnetic stirring at room temperature, the result silver

![Graphs showing UV-vis spectra and FTIR spectra of Pb.ext and Pb.AgNP, Ps.ext and Ps.AgNP at different reacting ratios.](image)

**Table 4**: Growth inhibition diameter of Pb.AgNP and Ps.AgNP against *E. coli* obtained by the agar diffusion well-variant method.

| Tested disk | Tested object | Growth inhibition diameter (nm) |
|-------------|---------------|---------------------------------|
| Blank       | DIW           | —                               |
| Control     | Extract       | —                               |
| Sample      | AgNP 1%       | 2.85 ± 0.91                     | 7.55 ± 0.12 |
|             | AgNP 5%       | 5.19 ± 0.45                     | 12.82 ± 0.18 |
|             | AgNP 10%      | 8.93 ± 0.28                     | 15.64 ± 0.14 |

**Table 4**: Growth inhibition diameter of Pb.AgNP and Ps.AgNP against *E. coli* obtained by the agar diffusion well-variant method.
nanoparticle solutions (named as Pb.AgNP 1 : x and Ps.AgNP 1 : x; where x were 2, 4, 6, 8, and 10) were separately loaded into the quartz curvettes to collect their UV-vis spectrum at the wavelength range of 350-650 nm (Figure 3).

Figure 3(a) presented the UV-vis spectrum of Pb.ext and Pb.AgNP at different reacting ratios; meanwhile, Figure 3(b) presented those of Ps.ext and Ps.AgNP. The UV-vis spectra of both extracts of Pb.ext and Ps.ext showed no peak in the wavelength range of 350–650 nm. However, the UV-vis spectra of reacted mixtures of both Pb.AgNP and Ps.AgNP showed the peaks in the wavelength of 400–450 nm. This demonstrated that silver nanoparticles with a surface plasmon resonance occurred in the reacted mixtures and silver nanoparticles were synthesized [40–42]. The pointed shape of these peaks helped predict that the particle size distribution of AgNPs synthesized by Pb.ext as well as Ps.ext was relatively narrow. There was a similarity in the correlation of the reacting ratio and the absorbance between Pb.AgNP and Ps.AgNP. When the reacting ratio of silver nitrate and the extract increased, the absorbance increased and peaked at the ratio of 1 : 8, then decreased slightly at 1 : 10. These results suggested that the green synthesis process of silver nanoparticles by Pb.ext and Ps.ext was efficient at the reacting ratio of 1 : 8. Therefore, both Pb.AgNP and Ps.AgNP with a ratio of 1 : 8 were used for further experiments.

The phytochemicals not only play the role of reducing silver (I) ions into AgNPs but also chelate with AgNPs which helps stabilize the biosynthesized AgNPs. Therefore, FTIR spectroscopy was performed to identify the possible biomolecules responsible for the reduction of silver (I) ions and the functional groups surrounding the synthesized silver nanoparticle. Figure 4 presented the FTIR spectra of Pb.ext versus Pb.AgNP 1 : 8 (Figure 4(a)) and the FTIR spectra of Ps.ext versus Ps.AgNP 1 : 8 (Figure 4(b)). In general, both spectra showed strong and broad peaks at the wavenumber region of 3470-3309 cm⁻¹ which was attributed to O-H stretching (arising from alcohols and phenolic compounds) [43, 44]. However, the OH peaks were more broadening in the FTIR spectra of the two extracts than those of the corresponding silver nanoparticle solutions. This was due to the stronger hydrogen bonding of the phytochemical in the extracts as well as demonstrated that silver nanoparticles were capped by phytoconstituents [45, 46]. The peak at 2385 cm⁻¹ was due to the O=C=O stretching vibrations indicated as CO₂. Moreover, peaks appeared at the wavenumber region of 1629-1638 cm⁻¹, which attributed to the C=C groups from alkenes [44, 47, 48], and reduced their intensity after silver reduction (Figure 4(a) and Figure 4(b)). This phenomenon revealed that the bioactive compounds in the two extracts including antioxidants, phenols, and flavonoids with abundant aromatic C=C groups took part in the reduction of silver (I) ions into AgNPs [43]. In Figure 4(a), peaks appeared at the wavenumber region of 1380-1385 cm⁻¹ attributed to C-H bending vibrations of C-H (alkanes); meanwhile, the absorption band at 1256 cm⁻¹ indicated the presence of C-N stretching vibrations in Pb.ext. In Figure 4(b), some peaks in Pb.ext’s IR spectra were absent in Pb.ext’s IR spectra. This is probably due to the abundance of bioactive compounds in P. betle leaf versus P. sarmentosum leaves, which also explains why extracted leaves of P. betle are more commonly used in commercial products than P. sarmentosum [36, 37].

Figure 5: Antibacterial activity on E. coli of Pb.AgNP and Ps.AgNP at different concentrations.

The synthesized silver nanoparticles in Pb.AgNP and Ps.AgNP solutions were washed and collected by repetitively centrifuging the reacted mixtures with DIW at 10000 rpm in 10 min for each time. Subsequently, these AgNPs were dispersed in DIW for DLS and Zeta potential measurements (Table 3). The DLS measurement showed that the hydrodynamic size of Pb.AgNP and Ps.AgNP was 16.28 ± 3.84 and 14.51 ± 2.90 nm, respectively. The silver nanoparticles synthesized by Pb.ext were larger than those synthesized by Ps.ext. This was due to the higher total reducing capacity of P. betle compared to P. sarmentosum which was proved in the previous experiments. On the other hand, the negative zeta potential values of both Pb.AgNP and Ps.AgNP in the range of -30 to 0 mV suggested that the obtained silver nanoparticles were stable and did not agglomerate. These results were agreed with other green synthesized AgNPs fabricated by phytochemicals [49].
3.4. Antibacterial Activity of the Synthesized Silver Nanoparticles. In the antibacterial activity test, the blank disks were treated by DIW, and the control disks were treated by Pb.ext and Ps.ext (added DIW into the initial extracts with the ratio 1:8 (v/v) and then diluted 10 times). The sample disks were treated by the green synthesized nanosilver solutions, Pb.AgNP 1:8 and Ps.AgNP 1:8, at the concentrations of 1, 5, and 10%. The tested objects were tested for antibacterial ability against *E. coli* by the agar diffusion well–variant method and the results were showed in Table 4 and Figure 5.

Firstly, there was no growth inhibition area on the DIW disks, confirming that no antibacterial activity was made by DIW and the experiment was conducted under aseptic condition. Secondly, the antibacterial activity of Pb.AgNP and Ps.AgNP was concentration–dependent manner. Thirdly, at the same treated concentration of 10%, the growth inhibition areas caused by the extracts was obviously smaller than those caused by the respective nanosilver solutions. These results suggested that the green synthesized silver nanoparticles by the extracts enhanced the antibacterial activity of the initial extracts. Interestingly, no growth inhibition activity of 10% Pb.ext was observed, which demonstrated that the aqueous extract of *P. betle* at the tested concentration performed no inhibition activity on *E. coli*. This finding was consistent with the previous publication [50]. From the results of antibacterial test, it could be concluded that the antibacterial activity of Pb.AgNP on *E. coli* was totally caused by the synthesized silver nanoparticles.

Taken together, even though Pb.ext performed stronger reducing power than Ps.ext, Ps.AgNP showed markedly better antibacterial activity against *E. coli*, a common bacterium, than Pb.AgNP. This finding would be a great reference for manufacturers of antibacterial products to expand their raw materials as well as to enrich their product categories. The research also offers good recommendations for combining the two extracts to achieve antibacterial products with wide-effect on bacteria.

4. Conclusions

The appropriate aqueous extract conditions of the two *Piper* species—*P. betle* and *P. sarmentosum*, were determined for their highest total reducing capacity as 50°C in 2 h and 60°C in 1 h, respectively. Pb.ext showed better total reducing capacity with higher total polyphenol content (~125 mg GAE/mL extract) and stronger DPPH activity (IC50 was 1.45%). The ratio of 1 mM silver nitrate solution and the extracts for better nanosilver synthesis was determined as 1:8 (v/v). At the same dilution, both Pb.AgNP and Ps.AgNP performed significantly stronger antibacterial activity against *E. coli* compared to their initial extracts. The growth inhibition diameter caused by 10% Ps.AgNP (15.64 ± 0.14 mm) was nearly 2 times higher than that caused by 10% Pb.AgNP (8.93 ± 0.28 mm). This study would contribute useful and important information to the development of antibacterial products based on green synthesized silver nanoparticles fabricated by the extracts of the two popular plants in Southeast Asia, *P. betle* and *P. sarmentosum*. It also offered good recommendations to combine the extracts of *P. betle* and *P. sarmentosum* for wide-effect antibacterial products.

Data Availability

The experimental data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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