

Synthesis of silver nanoparticles with antibacterial activity using the lichen *Parmotrema praesorediosum*

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Abstract: Development of a green chemistry process for the synthesis of silver nanoparticles has become a focus of interest. This would offer numerous benefits, including ecofriendliness and compatibility for biomedical applications. Here we report the synthesis of silver nanoparticles from the reduction of silver nitrate and an aqueous extract of the lichen *Parmotrema praesorediosum* as a reductant as well as a stabilizer. The physical appearance of these silver nanoparticles was characterized using ultraviolet-visible spectroscopy, electron microscopy, energy-dispersive spectroscopy, and X-ray diffraction techniques. The results show that silver nanoparticles synthesized using *P. praesorediosum* have an average particle size of 19 nm with a cubic structure. The antibacterial activity of the synthesized silver nanoparticles was tested against eight micro-organisms using the disk diffusion method. The results reveal that silver nanoparticles synthesized using *P. praesorediosum* have potential antibacterial activity against Gram-negative bacteria.

Keywords: silver nanoparticles, lichens, *Parmotrema praesorediosum*, antibacterial activity

Introduction

The “green” chemistry approach to synthesizing biocompatible silver nanoparticles (AgNPs) has gained considerable attention in recent years. Plants or natural resources has been found to be a good alternative method for nanoparticle synthesis, since this method does not use any toxic chemicals and does not require the use of high pressure, energy, and temperature. This green chemistry process has numerous benefits, including environmental friendliness, cost-effectiveness, and suitability for pharmaceutical and biomedical applications. Plants and natural resources, such as papaya,¹ *Lippia citriodora*,² *Helianthus annuus*,³ *Camellia sinensis*,⁴ and *Aloe vera*,⁵ has been used for the synthesis of metal nanoparticles.

Silver nanoparticles have become a focus of interest because they play a significant role in biological systems, living organisms, and medicine.^{6,7} In the present medical industry, silver and AgNPs have been found to be very useful in preventing infection in burns and open wounds.⁸ Silver nanoparticles have also been reported to possess antifungal,⁹ antiviral,¹⁰ and antiplatelet activity.¹¹ They are also beneficial as antimicrobials and therapeutic agents,^{12,13} and the development of AgNPs as an antibacterial agent is under way.

The aim of this study was to synthesize AgNPs using an aqueous extract of the lichen *Parmotrema praesorediosum*. Lichens are of special interest in synthesizing AgNPs because they are a composite organism consisting of a symbiotic association between a fungus and green algae.¹⁴ Lichen extracts contain a wide variety of metabolites, such

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as polysaccharide (homo-D-glucan) and phenolic (depside, depsidon, and dibenzofurane) compounds^{15,16} that can potentially act as reducing agents for silver ions. *P. praesorediosum* presents unique properties in that it has two aliphatic acids, ie, (+)-praesorediosic acid [2-(14'-carboxytetradecyl)-4-methyl-5-oxo-2,5-dihydrofuran-3-carboxylic acid] and (+)-protopraesorediosic acid [2-(14'-carboxytetradecyl)-4-methylene-5-oxo-2,5-tetrahydrofuran-3-carboxylic acid], which can play a significant role in the synthesis of AgNPs. The synthesis, characterization, and antibacterial activity of AgNPs against eight pathogenic micro-organisms were evaluated in this study.

Materials and methods

Materials

P. praesorediosum was collected from several lowland areas, including Bukit Mahkota Selangor, Malaysia. Silver nitrate (AgNO₃) was purchased from Merck (Darmstadt, Germany). Mueller-Hinton agar, Mueller-Hinton broth, and nutrient broth were purchased from Oxoid Ltd (Basingstoke, UK) for the antibacterial assays.

Synthesis of AgNPs

An aqueous extract of *P. praesorediosum* was prepared by mixing 3 g of a lichen sample with 45 mL of ultrahigh purity water (Milli-Q, Millipore, Billerica, MA, USA). The mixture was heated for several minutes at 80°C to denature the enzymes in the extract. The solution was filtered through Whatman No 1 filter paper (pore size 125 nm). The filtrate was further filtered through Whatman No 1 filter paper (pore size 25 µm) to remove plant residues. For synthesis of the AgNPs, about 10 mL of the aqueous lichen extract was mixed with 30 mL of a 1 mM AgNO₃ solution in an Erlenmeyer flask and allowed to react at room temperature for 24 hours. Use of ultrahigh purity water avoids the presence of chloride ions and prevents precipitation of silver chloride. A dried AgNP powder was obtained by freeze-drying and stored at 4°C until further analysis. The AgNPs in the freeze-drying bottle were resuspended in ultrahigh purity water for all characterization methods and antibacterial assays.

Characterization of AgNPs

The reduction of silver ions to AgNPs in the solution was monitored by measuring the ultraviolet-visible spectrum of the solution using a UV 2450 double-beam spectrophotometer (Shimadzu, Tokyo, Japan) operated at a resolution of 2 nm in the range from 300–600 nm. The physical appearance of the synthesized AgNPs was analyzed by transmission

electron microscopy (TEM), energy-dispersive spectroscopy, and X-ray diffractometry. TEM analysis of the AgNPs was performed using a CM12 instrument operated at an accelerating voltage at 80 kV (Philips, Eindhoven, the Netherlands). Samples were prepared by drop-coating on formvar-coated copper TEM grids. The size distribution of the AgNPs was calculated from the TEM images by measuring the diameter of approximately 50 nanoparticles. Further, the presence of elemental silver was confirmed by energy-dispersive spectroscopy and phase identification of the AgNPs was done using X-ray diffractometry.

Evaluation of antibacterial activity of AgNPs

The antibacterial activity of the synthesized AgNPs was determined using the disk diffusion method.¹⁷ Eight types of pathogenic bacteria, including four Gram-negative bacteria (*Proteus vulgaris*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Salmonella typhi*) and four Gram-positive bacteria (*Staphylococcus epidermidis*, methicillin-resistant *Staphylococcus aureus*, *Bacillus subtilis*, and *Streptococcus faecalis*) were tested. Pure cultures of the micro-organisms were subcultured on Mueller-Hinton agar. Each strain was swabbed uniformly onto individual agar plates using sterile cotton swabs. Sterile paper disks were placed on the agar plates, and 10 µL of 100 µg/mL (w/v) samples were applied to the disks. Gentamicin (30 µg per disk) was used as the positive control and sterile distilled water as the negative control. All the plates were incubated at 37°C for 18–24 hours. The tests were repeated three times. The zone of inhibition, which appeared as a clear area around the disks, was measured.

Results

Formation of AgNPs in aqueous extract can be monitored by color change. Figure 1 shows the color changes when the aqueous extract of *P. praesorediosum* was mixed with a 1 mM AgNO₃ solution. The mixture was kept at room temperature for 24 hours. The appearance of a yellowish-brown color in the reaction vessel indicated formation of AgNPs.¹⁸ AgNPs exhibit this yellowish-brown color in aqueous solution due to excitation of surface plasmon resonance in the AgNPs.¹⁹

Ultraviolet-visible spectroscopy was used to characterize the synthesized AgNPs. As shown in Figure 2, the surface plasmon resonance of the AgNPs was centered at approximately 408 nm, indicating the presence of AgNPs in the solution.

The morphology and size distribution of the synthesized AgNPs were determined by TEM analysis. The TEM image

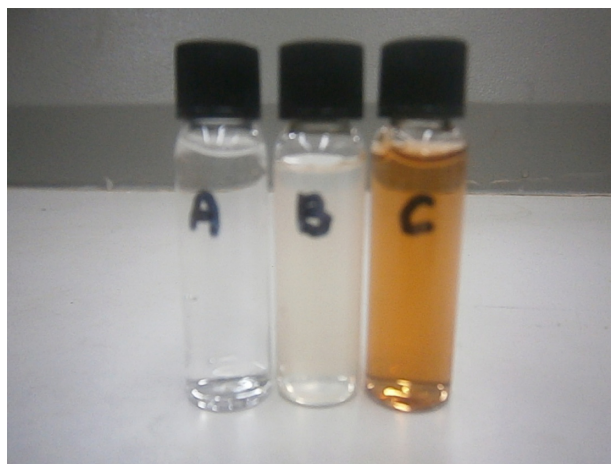


Figure 1 The 1 mM AgNO_3 solution (A), aqueous extract of *Parmotrema praesorediosum* (B), and a mixture of *P. praesorediosum* in 1 mM AgNO_3 after 24 hours of reaction (C).

Abbreviation: AgNO_3 , silver nitrate.

in Figure 3 shows that the particles were polydispersed and mostly spherical. The particle size histograms for the AgNPs (shown on the right side of Figure 3) suggest that the particles ranged in size from 5 nm to 40 nm with an average diameter of 19 nm.

The synthesized AgNPs were further characterized using X-ray diffractometry (Figure 4). The X-ray diffraction pattern showed four intense peaks (38.2° , 44.3° , 64.5° , 77.5°) in the whole spectrum of 2θ values ranging from 20° to 80° , which correspond to the (111), (200), (220), and (311) crystallographic planes of face-centered cubic silver, respectively.

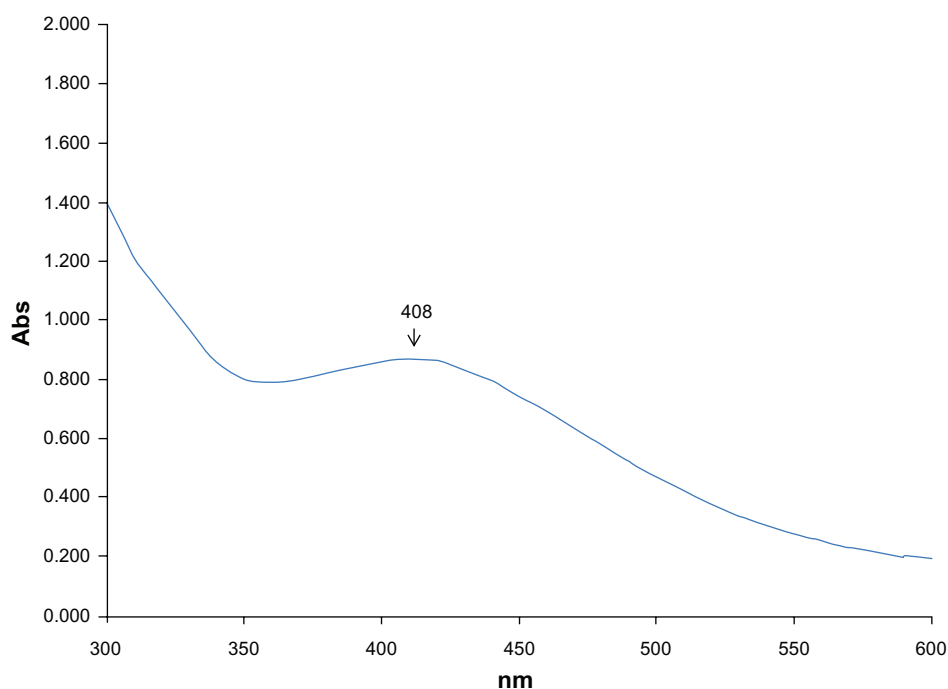


Figure 2 Ultraviolet-visible absorption spectrum of silver nanoparticles synthesized using aqueous extract of *Parmotrema praesorediosum*.

The crystallite domain size was calculated using the Debye-Scherrer formula: $D = 0.94 \lambda / \beta \cos \theta$, where D is the average crystallite domain size perpendicular to the reflecting planes, λ is the wavelength of $\text{Cu}_{\text{K}\alpha}$, β is the full width at half maximum, and θ is the Bragg diffraction angle. The average crystallite size of the synthesized AgNPs was 15.4 nm, derived from the full width at half maximum of peak corresponding to the (111) plane, which is in a good agreement with the particle size determined by TEM analysis.

Reduction of silver ions to elemental silver by the aqueous extract of *P. praesorediosum* was confirmed by energy-dispersive spectroscopy (Figure 5). The silver nanocrystallites displayed an optical absorption band peak at approximately 3 KeV, which is typical of the absorption of metallic silver nanocrystallites due to surface plasmon resonance.²⁰

The antibacterial effects of silver salts have been known since antiquity,²¹ but the use of AgNPs as an antibacterial agent is relatively new. Therefore, the antibacterial activity of the synthesized AgNPs against Gram-negative and Gram-positive bacteria was investigated. At a concentration of 100 $\mu\text{g/mL}$, the AgNPs efficiently inhibited the growth of Gram-negative bacteria. Figure 6 shows the antibacterial activity of AgNPs against *S. typhi*. The diameters of the inhibition zones for the all tested pathogens are listed in Table 1. Thus, our results show that AgNPs synthesized using an aqueous extract of *P. praesorediosum* have potential antibacterial activity against Gram-negative bacteria.

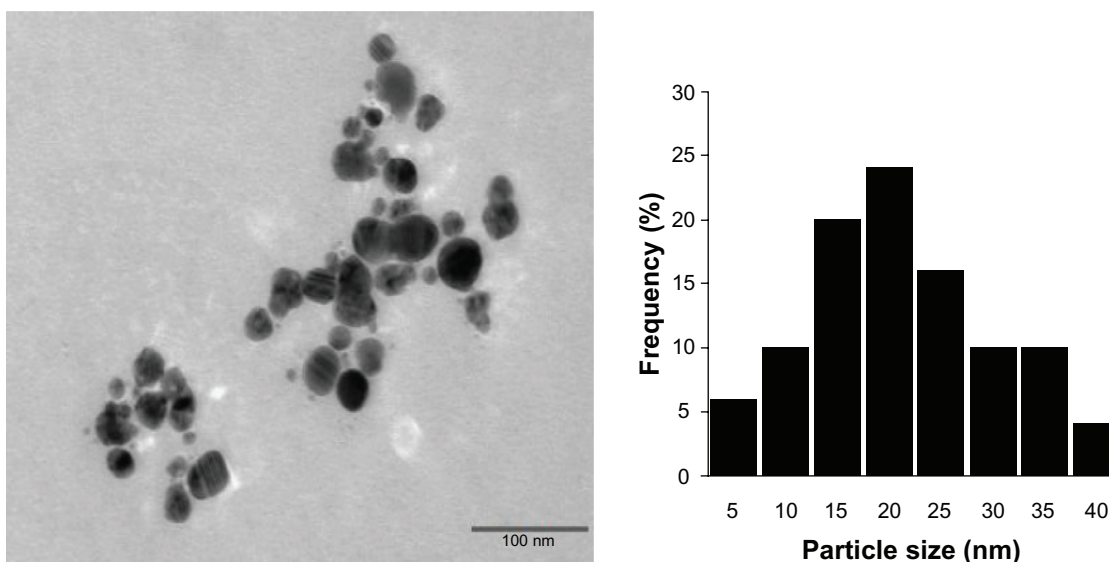


Figure 3 Transmission electron microscopic image of silver nanoparticles obtained using *Parmotrema praesorediosum* extract after 24 hours of reaction (left) and particle size distribution (right).

Discussion

The development of easy, reliable, and ecofriendly methods helps to increase interest in the synthesis and application of nanoparticles that are beneficial for mankind.^{22,23} Recently, plant extracts and natural resources have been found to be useful in the reduction of silver ions to AgNPs. Silver nanoparticles show a dark reddish-brown color in aqueous solution due to the surface plasmon resonance phenomenon.²⁴ The surface plasmon resonance band of AgNPs can be monitored through the ultraviolet-visible spectrum ranging from 400 nm to 450 nm, indicating formation of AgNPs.²⁵ Also, it is well

recognized that the absorbance of AgNPs depends mainly upon size and shape.²⁶ The technique outlined above has proven to be very useful for analysis of nanoparticles.^{27–29} According to Mock et al, the morphology of nanoparticles is highly variable, and includes spherical, rod-like, decahedral, triangular, and platelet shapes. This could be due to correlation of the absorption spectrum for individual AgNPs.³⁰

The chemical composition and crystal structure of a material are often determined by X-ray diffraction analysis. The presence of AgNPs in plant extracts can be identified using X-ray diffractometry to examine the diffraction peaks

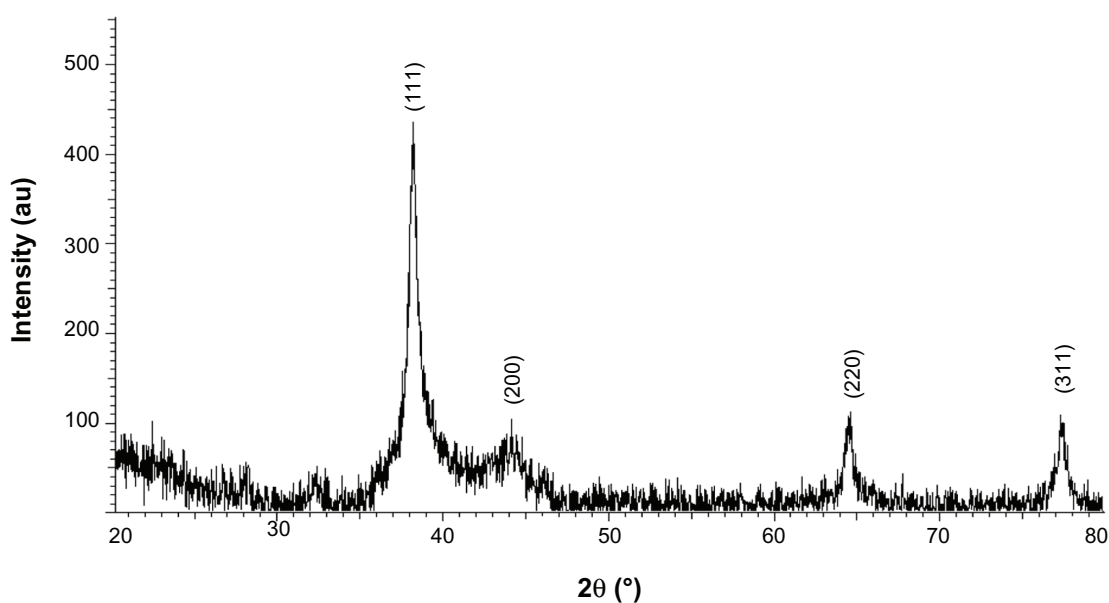


Figure 4 X-ray diffraction pattern of the prepared silver nanoparticles.

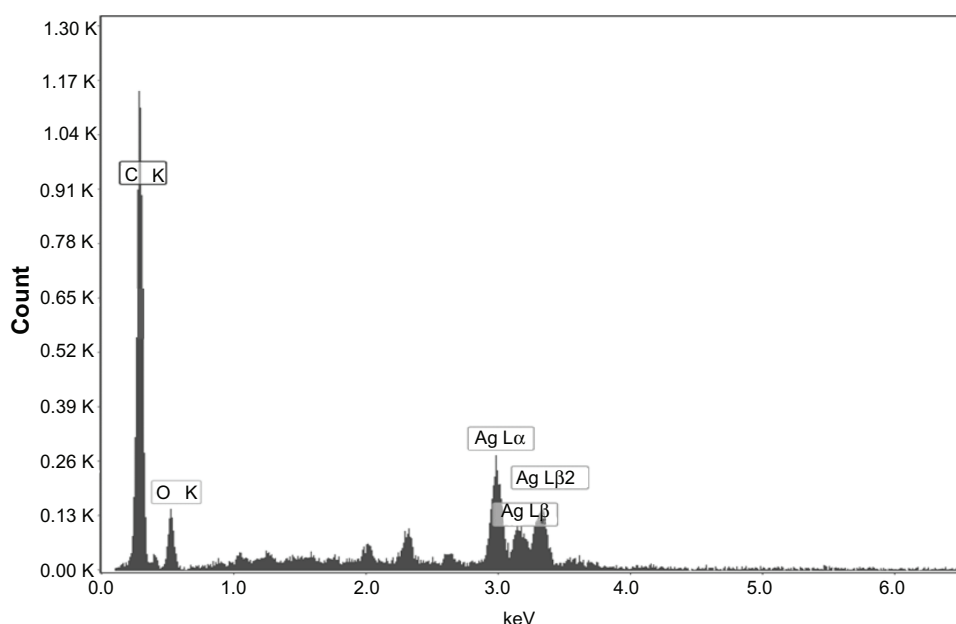


Figure 5 Energy-dispersive spectrum of silver nanoparticles synthesized by treating 1 mM AgNO₃ with an aqueous extract of *Parmotrema praesorediosum*.
Abbreviations: CK, carbon peak; OK, oxygen peak.

of the plant.³¹ In this work, we discovered that the synthesized AgNPs match the face-centered cubic crystal structure of metallic silver. This result is consistent with the results reported by Jain et al,¹ Cruz et al,² and Gilaki.³

Energy-dispersive spectroscopy is used to gain further insight into the features of AgNPs. Throughout the scanning range of binding energies, the peak around 3.40 keV corresponds to the binding energies of AgL.³² Some impurity peaks were detected below 1 keV, corresponds to a carbon peak (CK) and oxygen peak (OK), which was probably related to the presence of crystalline biomolecules in the extract. The *P. praesorediosum* extract contains several metabolites,

including antranorin, (+)-praesorediosic acid, and (+)-proto-praesorediosic acid,³³ that may play a significant role as reducing agents for silver ions. Special consideration is needed to investigate the synergistic effects of AgNPs with biomolecules.

Silver has been used for many years for antimicrobial applications in the medical field.^{34,35} Recently, in the field of nanotechnology, applications of AgNPs as antimicrobial agents have been expanding. The exact mechanism by which silver ions and AgNPs exert their antibacterial effect remains to be identified. A literature survey shows that smaller AgNPs having a large surface area available for interaction would have a stronger antibacterial effect than larger AgNPs.³⁶ It is also possible that AgNPs not only interact with the membrane surface, but may also penetrate inside bacteria.³⁷

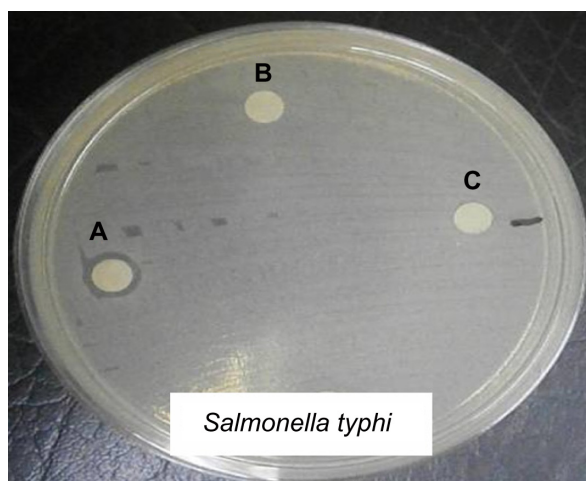


Figure 6 Antibacterial activity assay against *Salmonella typhi* by the disk diffusion method. (A) Synthesized silver nanoparticles, (B) distilled water, and (C) *Parmotrema praesorediosum* aqueous extract.

Table 1 Mean zone of inhibition (mm) of silver nanoparticles synthesized by *Parmotrema praesorediosum* against eight pathogenic micro-organisms

Pathogens	Zone of inhibition (mm)	
	100 µg/mL aqueous extract	100 µg/mL silver nanoparticles
<i>Proteus vulgaris</i>	6	8±0.0
<i>Pseudomonas aeruginosa</i>	6	8±0.8
<i>Serratia marcescens</i>	6	8±0.3
<i>Salmonella typhi</i>	6	7.5±0.0
<i>Staphylococcus epidermidis</i>	6	6
Methicillin-resistant	6	6
<i>Staphylococcus aureus</i>		
<i>Bacillus subtilis</i>	6	6
<i>Streptococcus faecalis</i>	6	6

Note: The disk diameter is 6 mm.

According to Kim et al,¹³ the antibacterial effects of AgNPs may be associated with the characteristics of certain bacterial species. In this investigation, we found that AgNPs synthesized using *P. praesorediosum* have potential antibacterial activity against Gram-negative bacteria, in a manner similar to that reported by Shrivastava and Dash.³⁸

Conclusion

We have demonstrated a good method for developing a simple, safe, cost-effective, and ecofriendly preparation of AgNPs in an aqueous extract of *P. praesorediosum*. To the authors' knowledge, this is the first report concerning use of a lichen species to reduce silver ions to AgNPs. The synthesized AgNPs had an average particle size of 19 nm and a cubic structure, and remained stable without agglomeration for more than one month. They showed potential antibacterial activity against Gram-negative bacteria. Thus, application of these synthesized AgNPs based on our findings may lead to valuable discoveries in various fields, including medical devices and in the pharmaceutical and biomedical industries. Toxicity studies of these AgNPs using human pathogens may open the door to a new range of antibacterial agents.

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Disclosure

The authors report no conflicts of interest in this work.

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