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Antibacterial Activity of *Borassus flabellifer* Vinegar-Graphene Quantum Dots Against Gram-Positive and Gram-Negative Bacteria

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Borassus flabellifer vinegar–graphene quantum dots (BFV-GQDs) were successfully synthesized using a pyrolysis method with *Borassus flabellifer* vinegar (BFV) as the precursor. All the samples were characterized using ultraviolet-visible spectrophotometry (UV-Vis), scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX). The antibacterial activities of BFV-GQDs against strains of Gram-negative bacteria (*Escherichia coli*) and Gram-positive bacteria (*Staphylococcus aureus*) were determined using the agar well diffusion method for preliminary screening, while minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using the broth macro-dilution method. The zones of inhibition were compared with those of citric acid–graphene quantum dots (CA-GQDs). It was observed that the synthesized BFV-GQDs demonstrated excellent antibacterial activity against *Staphylococcus aureus* (82.3%) and good antibacterial activity against *Escherichia coli* (73.3%). The MIC of BFV-GQDs against *E. coli* was 6.25 mg/mL and *S. aureus* was 12.5 mg/mL, whereas the MBC of BFV-GQDs against *E. coli* was 12.5 mg/mL and *S. aureus* was 25.0 mg/mL.

Keywords: *Borassus flabellifer* vinegar, Graphene quantum dots, Antibacterial activity.

INTRODUCTION

Presently, the increase of resistance among bacteria *via* evolutionary processes toward conventional antibiotics, including penicillin and amoxicillin, is a main global health concern [1]. The abuse of these antibiotics has resulted in the occurrence of bacterial resistance, as well as adverse side effects [2]. Therefore, effective treatment of bacterial infections utilizing these antibiotics is critical [3]. Currently, carbon nanomaterials such as graphene oxide (GO), graphene quantum dots (GQDs) and carbon quantum dots (CQDs) have attracted increasing attention because of their unique properties, including high mechanical flexibility, high conductivity, low resistivity, excellent thermal and chemical stability and good antimicrobial activity [4-6]. The unique characteristics of these nanomaterials make them attractive to a variety of applications, such as simulated systems [7], electrochemical applications [8], thin-film microelectrodes [9], dye-sensitized solar cells [10], adsorbents for wastewater treatment [11-13], catalysts [14,15] and sensors [16,17].

Particularly, GQDs can destroy the structure of bacterial cells [18]. Several researchers have tried to apply GQDs and their composites in the production of antibacterials. For instance, Sheik Mydeen *et al.* [19] applied GQDs/ZnO nanocomposites against *Pseudomonas aeruginosa*, Teymourinia *et al.* [20] applied cotton-silver-graphene quantum dots (cotton/Ag/GQDs) nanocomposites as novel antibacterial nano pads against *Staphylococcus aureus* and *Escherichia coli*, Sen & Nyokong

[21] applied nitrogen and sulfur co-doped graphene quantum dots (N and S-GQDs) against *Staphylococcus aureus*, Habiba *et al.* [22] applied PEGylated silver-graphene quantum dots nanocomposites against both Gram-negative and Gram-positive bacteria, including *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Wang *et al.* [23] applied GQDs@hMSN(EM)), which was prepared through the loading of both GQDs and erythromycin into the hollow mesoporous silica nanoparticle against *Escherichia coli* and *Staphylococcus aureus*.

Generally, carbon, glucose, citric acid and various fruit juices are excellent precursors for the synthesis of GQDs due to their low carbonization temperature [24]. The sour taste of fruit juice is due to the presence of acids, among which the major acid is citric acid [25]. To this end, we have reported for the first time the synthesis of graphene quantum dots (BFV-GQDs) using *Borassus flabellifer* vinegar that was prepared from fermented palmyra palm juice, which is an abundant carbon source, as the precursor, using an eco-friendly green pyrolysis synthesis method. The synthesized BFV-GQDs were characterized using SEM, EDX and UV-Vis. The antibacterial activity of BFV-GQDs was determined by the well diffusion method against strains of Gram-negative bacteria (*Escherichia coli*) and Gram-positive bacteria (*Staphylococcus aureus*).

EXPERIMENTAL

Preparation of *Borassus flabellifer* vinegar-graphene quantum dots (BFV-GQDs): All samples of palmyra palm juice and *Borassus flabellifer* vinegar were collected from the Songkhla and Nakhon Si Thammarat provinces of Thailand. For the preparation of BFV-GQDs, approximately 200 mL of palmyra palm juice sample was added into a beaker. Then, the beaker was heated to 200 ºC for about 30 min using a paraffin oil bath. The sample was slowly liquified to a yellow colour. The liquid was then transferred into a beaker containing 10 mL of 0.25 mol/L NaOH, with continuous stirring for 30 min. The obtained sample was dried at 90 ºC and kept in a desiccator before use. To confirm the formation of BFV-GQDs, the prepared samples were characterized using scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX) and ultraviolet-visible spectroscopy (UV-Vis) techniques.

Antibacterial activity of BFV-GQDs: The antibacterial activity of BFV-GQDs against strains of Gram-negative bacteria (*Escherichia coli*) and Gram-positive bacteria (*Staphylococcus aureus*) was determined using the agar well diffusion method for preliminary screening, while minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using the broth macro dilution method. Two bacterial strains obtained from the Science Center, Nakhon Si Thammarat Rajabhat University, Thailand, were used. The agar well diffusion method was applied to test the antimicrobial activity of the BFV-GQDs. The inoculum was prepared using fresh cultures of bacterial strains cultured on sterile Muller Hinton Agar (MHA). A loopful of bacterial culture was inoculated into a Muller Hinton Broth medium and incubated at 37 ºC for 24 h. The culture size was adjusted to 0.5 McFarland standard turbidity, which corresponded to approximately 10^8 colony-forming units (CFU/mL). Cell suspensions (100 mL of the target strain) were introduced into MHA plates and spread thinly on the plates using a sterile wire loop. Once the medium had solidified, disks of a diameter of 8 mm each were cut out of the agar and 100 µL of BFV-GQDs or citric acid-graphene quantum dots (CA-GQDs) as control were placed into each well. The plates were then incubated at 37 ºC for 24 h. The diameters of the zones of inhibition (in mm) around the well were measured after 24 h. The tests were performed in triplicate. The MIC and MBC were determined using a modification of the dilution tube method. Briefly, 1 mL of Muller Hinton Broth (MHB) was added to eight sterile test tubes. Then, 100 mg/mL of dissolved BEV-GQDs was added to the first tube and shaked well. Now, 1 mL was transferred from the first test tube to the second test tube and then to the third test tube. This was carried on till the eighth test tube, after which, 1 mL of this content was withdrawn and thrown to waste. Each test tube was inoculated with 0.5 McFarland standard 1 mL of *E. coli* culture. The entire procedure was similarly repeated for *S. aureus* too, mixed well and incubated at 37 ºC for 18-24 h after confirming that the negative control tube showed no growth. Then, a loopful of broth from each test tube not showing growth was inoculated into an MHA plate and incubated further for 24 h at 37 ºC. The tubes and agar plates were examined for the growth of the bacteria. The experiment was repeated thrice.

RESULTS AND DISCUSSION

Characterization: To analyze the morphological changes in the prepared samples, scanning electron microscopy (SEM, Aztec, U.K.) was used. Fig. 1 shows the SEM images of BFV (Fig. 1a) and BFV-GQDs (Fig. 1b). The morphology of BFV was a sheet form, while BFV-GQDs were spherical with particle sizes in nanometers. The elemental analysis of the obtained samples was done using an SEM employing the energy dispersive X-ray spectroscopy technique (EDX, Oxford, Aztec, United Kingdom). The EDX spectrum of BFV showed various elements, including C, O, Na, Mg, P, Cl and K, which accounted for 46.3, 39.6, 2.4, 0.7, 1.1, 5.0 and 5.0%, respectively of the BFV (Fig. 2a). On the other hand, the BFV-GQDs only showed strong peaks of C (41.2%) and O (58.8%) in the prepared sample (Fig. 2(b)), indicating the successful synthesis of BFV-GQDs [17]. The UV-Vis spectrum (Fig. 3b) also confirmed the formation of BFV-GQDs with absorption peaks at 278 nm and 350 nm, assigned to $\pi \rightarrow \pi^*$ transition of C = C and $n \rightarrow \pi^*$ transition of C=O, as previously reported for similar GQD-based materials [26].

Antibacterial activity: The zones of inhibition produced by sample BFV-GQDs against *E. coli* and *S. aureus* bacterial strains are presented in Fig. 4. These zones of inhibition were compared with citric acid-graphene quantum dots (CA-GQDs), pure NaOH and pure BFV. The results indicated that the synthesized BFV-GQDs demonstrated excellent antibacterial activity against *E. coli*. at 73.3%, whereas *S. aureus* showed an inhibition zone at 82.3% (Table-1). Moreover, the MIC values were 6.25 mg/L and 12.5 mg/L against *E. coli* and *S. aureus*, respectively, while the MBC of BFV-GQDs against *E. coli* was 12.5 mg/mL

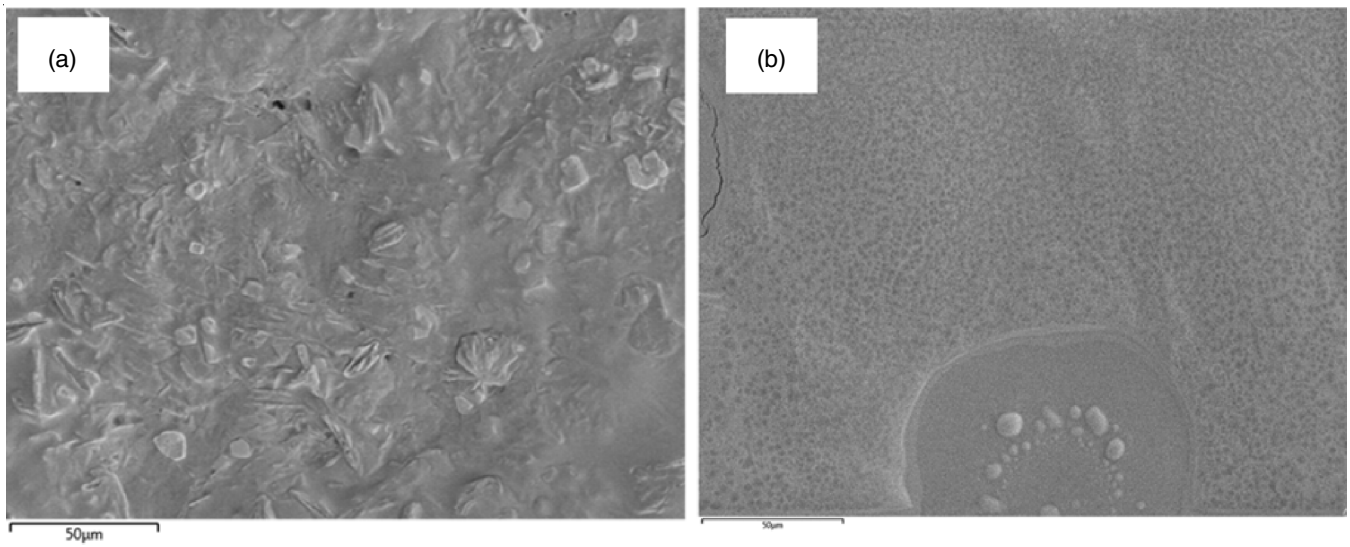

Fig. 1. SEM images of (a) BFV and (b) BFV-GQDs

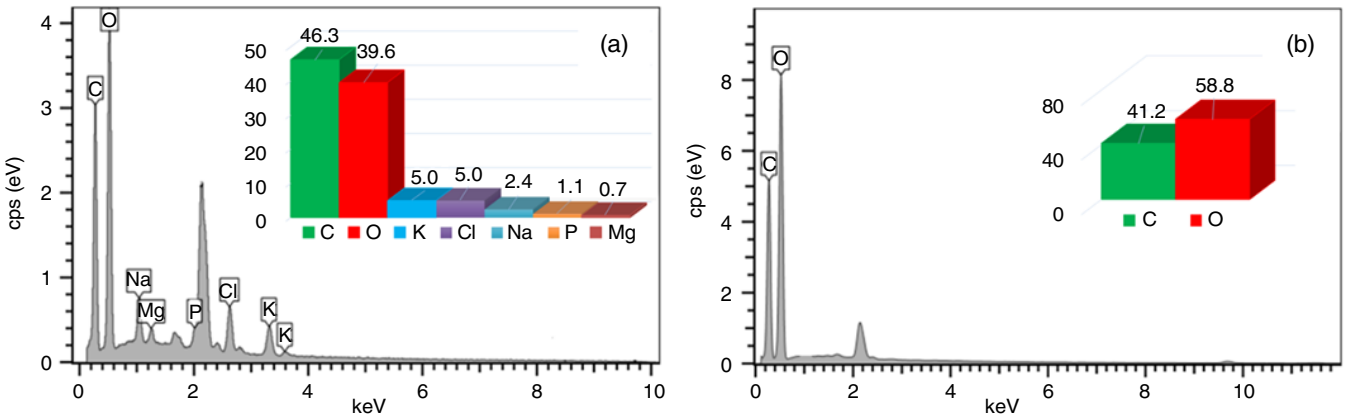

Fig. 2. EDX spectrum of (a) BFV and (b) BFV-GQDs

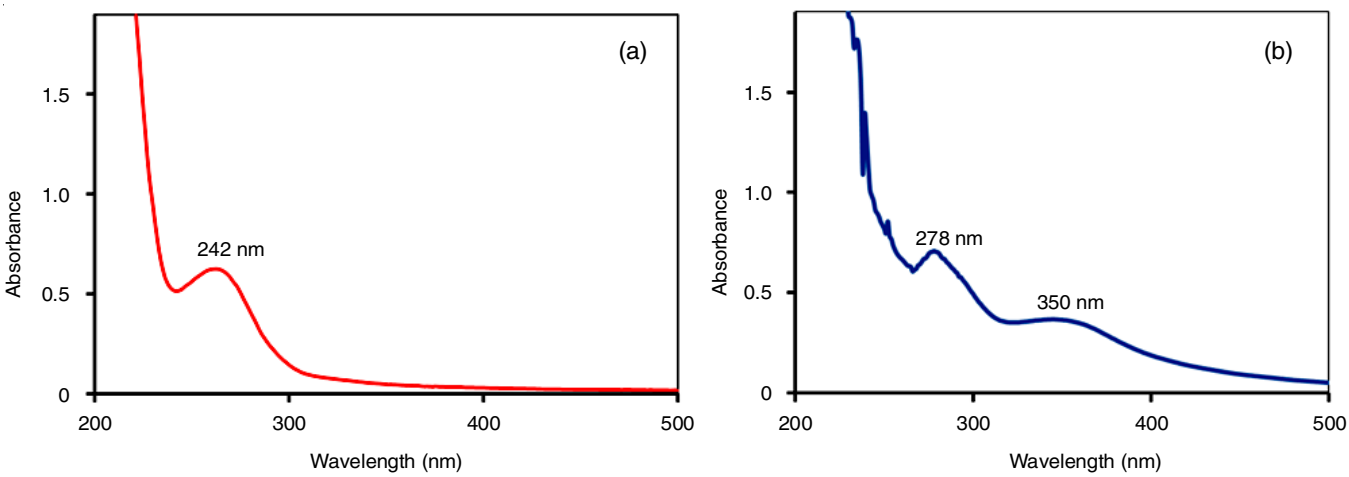

Fig. 3. UV-vis spectrum of (a) BFV and (b) BFV-GQDs

and against *S. aureus* was 25.0 mg/mL (Table-2). The MIC and MBC values of the prepared BFV-GQDs against the tested bacteria are summarized in Table-3.

Possible mechanisms for the antibacterial activity of BFV-GQDs: To evaluate the possible mechanisms for the antibacterial activity of BFV-GQDs, the changes in the cell walls/cell membrane of the prepared samples were evaluated by SEM. Fig. 5. shows SEM images of *S. aureus* (a) before treatment with BFV-GQDs and (b) after treatment with BFV-GQDs. The cell walls of S. *aureus* show obvious damage (Fig. 5b) after treatment with BFV-GQDs. Moreover, Fig. 6 shows the SEM images of *E. coli* (a) before treatment with BFV-GQDs and

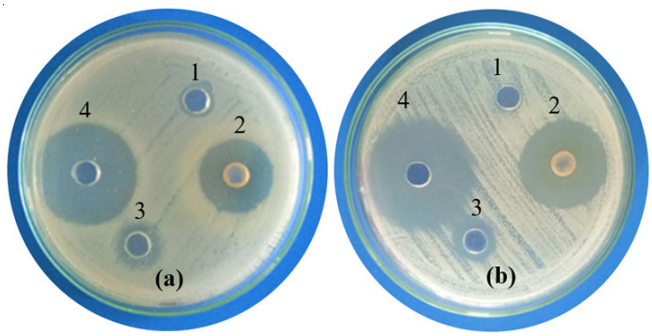

Fig. 4. Zone of inhibition produced by GQDs against (a) *E. coli* and (b) *S. aureus* (1 = NaOH, 2 = BFV-GQDs, 3 = BFV and 4 = CA-GQDs)

TABLE-1
ZONE OF INHIBITION OF BFV-GQDs COMPARED TO THAT OF CA-GQDs AS STANDARD

Type of active material	Inhibition zone (mm)	
	E. coli	*S. aureus*
Borassus flabellifer vinegar-graphene quantum dots (BFV-GQDs)	24.55	28.98
Citric acid-graphene quantum dots (CA-GQDs)	33.40	35.23
Zone difference (%)	73.30	82.30

TABLE-2
BACTERIAL GROWTH IN DIFFERENT CONCENTRATIONS OF BFV-GQDs IN THE BROTH AFTER 24 h

Type of bacterial	Concentration of BFV-GQDs (mg/mL)			
	50.00	25.00	12.50	6.25
E. coli	–	–	–	+
S. aureus	–	–	+	+

Positive (+) = Indicating growth; Negative (-) = Indicating absence of growth

TABLE-3
MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL CONCENTRATION (MBC) OF BFV-GQDs AGAINST GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

Type of active material	Gram-negative (*E. coli*)		Gram-positive (*S. aureus*)	
	MIC	MBC	MIC	MBC
BFV-GQDs concentration (mg/mL)	6.25	12.50	12.50	25.00

(b) after treatment with BFV-GQDs. The cell walls of *E. coli* also showed damage (Fig. 6b) after treatment with BFV-GQDs. These results indicate that the antibacterial activity of BFV-

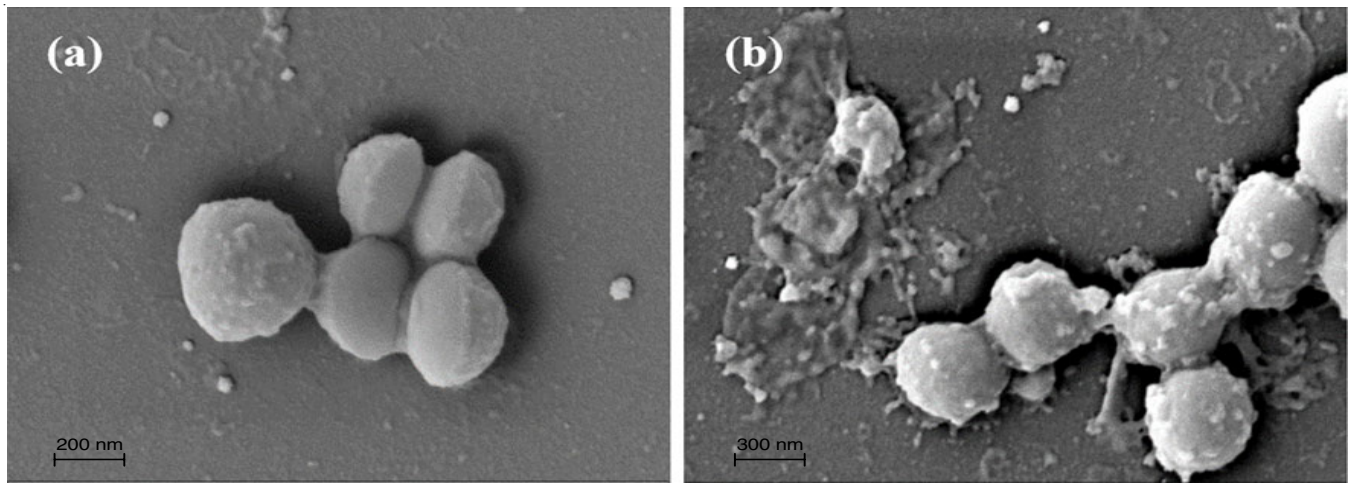

Fig. 5. SEM images of *S. aureus* (a) before treatment with BFV-GQDs and (b) after treatment with BFV-GQDs

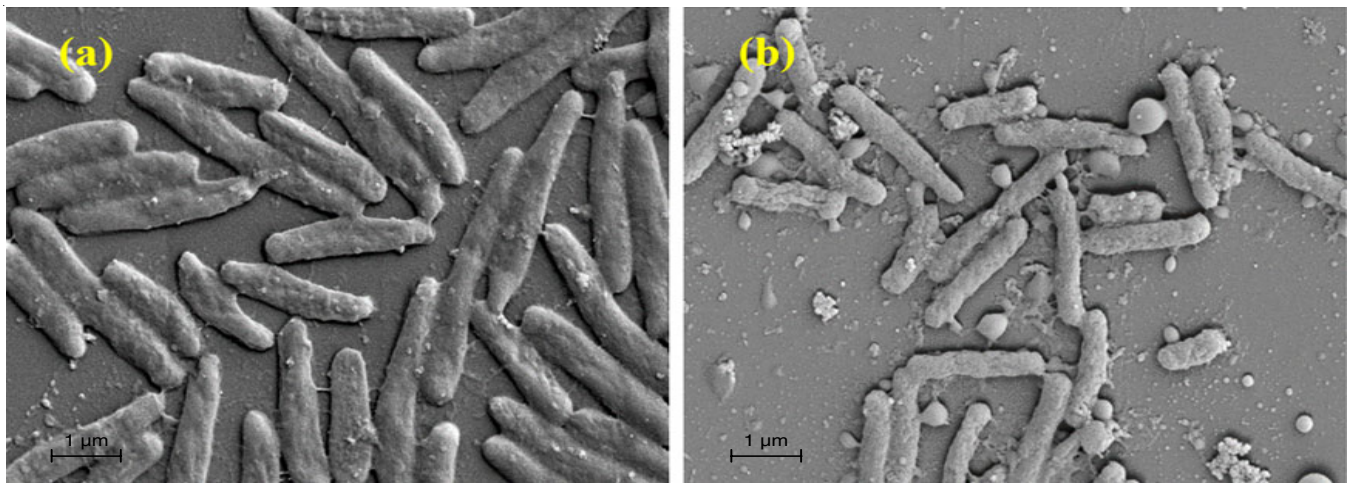

Fig. 6. SEM images of *E. coli* (a) before treatment with BFV-GQDs and (b) after treatment with BFV-GQDs

GQDs occurs *via* mechanical damage to the cell membrane. However, literature reviews have proposed several other mechanisms for the antibacterial activity of nanomaterials, including the production of reactive oxygen species (ROS), mechanical damage to the cell membrane, entrapment and release of metal ions [27].

Conclusion

In this work, the preparation and antimicrobial activity of graphene quantum dots (GQDs) derived from *Borassus flabellifer* vinegar was reported for the first time. Different characterization techniques confirmed the successful synthesis of GQDs. The prepared GQDs exhibited antibacterial activity against both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria. The synthesized BFV-GQDs demonstrated excellent antibacterial activity against *E. coli.* bacteria at 73.3%, while *S. aureus* was inhibited at 83.3%. The MIC of BFV-GQDs was 6.25 mg/mL and 12.5 mg/L for *E. coli* and *S. aureus*, respectively, whereas the MBC of BFV-GQDs against *E. coli* was 12.5 mg/mL and against *S. aureus* was 25 mg/mL.

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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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