



Green synthesis and optimized annealing temperature effects on zinc oxide nanoparticles using mango peel extract

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Abstract

This study presents the green synthesis of zinc oxide nanoparticles (ZnO-NPs) using mango peel extract (MgE) as a natural reducing and stabilizing agent. Phytochemical extraction was performed with deionized water, ethanol, and methanol for 1 h to 5 h, with deionized water at 4 h yielding the highest flavonoid content. Using zinc nitrate as a precursor, 25 mL of MgE successfully facilitated the synthesis of ZnO-NPs (ZnO-25MgE). It was found that annealing temperature at 400°C for 6 h produced pure ZnO. The obtained nanoparticles were characterized by X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FT-IR), and field emission scanning electron microscopy (FE-SEM). XRD confirmed the crystalline structure with an average size of 6.67 nm, while FT-IR and FE-SEM analyses revealed Zn–O vibrations, residual organics, and nanoscale morphology. Thermogravimetric analysis (TGA) indicated the appropriate annealing temperature for the removal of organics. For comparison, chemically synthesized ZnO was also characterized, and its antibacterial activity was assessed via disc diffusion against *Escherichia coli* and *Staphylococcus aureus*. The results demonstrated notable inhibition, highlighting the potential of biosynthesized ZnO-NPs for antimicrobial applications.

1. Introduction

Green synthesis refers to environmentally friendly methods for creating chemical compounds or materials that minimize or eliminate the use and production of hazardous substances, reduce energy consumption, and use non-toxic or renewable reagents and solvents. It is based on the principles of green chemistry, aiming to make chemical processes safer for both the environment and human health by reducing waste, avoiding toxic chemicals, and using sustainable resources wherever possible [1-5].

Environmental issues have become a global concern, particularly food waste, which is a result of the increasing global population. Food waste refers to food that is discarded or lost at every stage of the supply chain, including production, transportation, processing, and consumption. This waste is disposed of in various ways, such as incineration, landfilling, and composting. These methods emit CO₂ and methane, which are major greenhouse gases that directly impact climate change and the environment [6-8]. In addition, they waste valuable resources such as water, energy, and farmland [9]. Food waste can be divided into two categories: 40% edible and 60% inedible, such as fruit peels. Fruit peels are by-products of the fruit processing industry and are often discarded. However, they often contain particularly high levels of biologically active molecules, and these

bioactive compounds can be utilized to add value. They are mainly used in the food, cosmetic, and pharmaceutical industries, among others [10,11].

Mango (*Mangifera indica* L.) is a tropical fruit of significant economic importance in Thailand and other countries in Southeast Asia. It is widely cultivated for both domestic consumption and export, with Thailand being one of the world's largest mango exporters. The mango fruit consists of a seed coat (endocarp) covered with a fleshy mesocarp, which is in turn enclosed by a thin membrane (epi/exocarp). Most parts of the fruit can be eaten fresh [12]. Mango pulp can also be processed by drying, grinding, freezing, canning, or making jams and juices. In addition, mango is rich in vitamins and minerals, has high nutritional value, contains various phytochemicals, and possesses significant medicinal properties [13]. The phytochemicals found in mango peel include mangiferin, a xanthone, which exhibits antioxidant, antidiabetic, anticancer, antimicrobial, and anti-inflammatory properties [14,15]. Various techniques have been reported for mango peel extraction, such as Soxhlet extraction [16], accelerated solvent extraction [17], microwave extraction [18], batch extraction [19] and ultrasound-assisted extraction [20,21]. Another promising method is simple extraction using water or ethanol as solvents. This approach reduces the use of thermal energy, which can degrade the phytochemicals present in mango peel [11].

Zinc oxide nanoparticles (ZnO NPs) represent one of the most extensively studied and commercially applied nanomaterials, particularly in electrochemical systems, biomedical devices, cosmetic formulations, and textile manufacturing. Their widespread use is primarily attributed to advantageous properties such as high surface area, excellent biocompatibility, effective ultraviolet (UV) absorption and scattering, and pronounced antibacterial activity. ZnO nanoparticles can be fabricated through various synthesis routes, including physical, chemical, and biological approaches, which are commonly categorized based on their underlying physicochemical mechanisms. Annealing is a crucial post-synthesis process for improving the properties of zinc oxide (ZnO) nanoparticles, especially those prepared via green synthesis routes. The application of heat enhances crystallinity by allowing atoms to rearrange into a more ordered crystal structure. Annealing also helps reduce structural defects and removes residual organic compounds originating from plant extracts used during synthesis. In addition, the annealing temperature influences particle size, morphology, and surface characteristics of ZnO nanoparticles. These structural modifications significantly affect optical, electrical, and functional properties such as photocatalytic and antimicrobial performance. Therefore, careful control of the annealing process is essential to optimize the quality and performance of ZnO nanoparticles for various applications [22–26].

This research focuses on adding value to ripe mango peel by utilizing it as a reducing agent in a green chemical process for the synthesis of zinc oxide nanomaterials with antibacterial properties. Flavonoids were extracted from mango peel extract (MgE) using three different solvents; deionized water, ethanol, and methanol for 1 h, 2 h, 3 h, 4 h, and 5 h, respectively. The total polyphenol content was quantified using a UV–Vis spectrophotometer to determine the optimal extraction time. Subsequently, zinc oxide nanoparticles (ZnO NPs) with antibacterial activity were synthesized via a green synthesis process employing MgE. The synthesized ZnO NPs were characterized using X-ray diffraction (XRD) for crystal structure analysis, Raman and Fourier transform infrared (FT-IR) spectroscopy for molecular bond analysis, and field emission scanning electron microscopy (FE-SEM) for morphological analysis. Furthermore, the effect of annealing temperature on the removal of organic impurities was investigated, resulting in the formation of pure ZnO NPs. Finally, the antibacterial activity of the pure ZnO NPs was evaluated using the disc diffusion method to determine the sample exhibiting the highest antibacterial efficacy.

2. Materials and methods

2.1 Materials

Zinc Nitrate 6-Hydrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) was purchased from KemAus, Australia, and laboratory grade was used as the zinc precursor. Mango peels waste (*Mangifera indica*) from the local market in Bangkok, Thailand. *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) from Department of Biology, School of Science, KMITL.

2.2 Methods

2.2.1 Preparation of mango peel extract (MgE)

Mango peels obtained from local markets were washed three times with tap water and once with deionized (DI) water. The cleaned peels were cut into small pieces, dried in an oven at 60°C until completely dry, and then pulverized into powder using a blender. Two grams of mango peel powder were mixed with 100 mL of different solvents (methanol, ethanol, and deionized water) and extracted for varying durations (1 h, 2 h, 3 h, 4 h, and 5 h). The resulting extracts were filtered through Whatman No. 1 filter paper. Finally, the total flavonoid content of each extract was quantified using a Thermo Scientific Orion AquaMate 7000 Vis spectrophotometer.

2.2.2 Preparation of ZnO NPs by green synthesis method

In this step, two grams of zinc nitrate were mixed with MgE in different ratios (5 mL, 10 mL, 15 mL, 20 mL, 25 mL, and 30 mL) in a beaker. The mixture was stirred at 300 rpm for 1 h and then placed in a water bath at 60°C for an additional hour. The resulting solution was dried in an oven at 60°C until completely dry. The obtained zinc oxide nanoparticles are hereafter referred to as ZnO–MgE.

2.2.3 Annealing

The synthesized ZnO–MgE was studied for its effect on the removal of organic impurities by 1 g of ZnO–MgE being oven-dried for 6 h at different temperatures of 350°C, 375°C and 400°C, respectively.

2.2.4 Analysis of ZnO NPs

The crystallinity of the synthesized ZnO nanoparticles was analyzed using XRD with a Smart Lab diffractometer (Rigaku) to confirm their identity. The Raman spectra were recorded using a DXR Raman spectrometer (Thermo Scientific), and FTIR spectroscopy was employed to identify the functional groups on the nanoparticles. The morphology of the nanoparticles was examined using a FE-SEM (Apreo S-Thermo Fisher Scientific).

2.2.5 Antimicrobial activity assay

The antibacterial activity of the synthesized zinc oxide nanoparticles was evaluated using the disc diffusion method. The nanoparticles were dispersed in water at a 0.3 mg·mL⁻¹ concentration. Aliquots of 30 µL of the nanoparticle suspension were dropped onto sterile filter paper discs (6 mm diameter) placed on the surface of agar plates previously inoculated with the test bacteria (*Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*)). The plates were incubated at 37°C for 24 h. The inhibition zones around the discs were measured and recorded. The larger the inhibition zone, the greater the antibacterial activity of the nanoparticles.

3. Results and discussion

3.1 Preparation of mango peel extract (MgE)

Figure 1 shows the total flavonoids extracted from mango peel using different solvents and time. The results showed that deionized

water solvent at 4 h gave the highest flavonoid value because deionized water is a polar solvent and the flavonoids present in mango peel are due to the fact that flavonoids are phytochemicals with polar structures, especially in the form containing hydroxyl groups ($-OH$) and sugar groups (glycosides) [27]. Therefore, water can extract the most flavonoids from mango peel.

3.2 Characterization of synthesized zinc oxide nanoparticles

ZnO-MgE refers to ZnO synthesized using mango peel extract (MgE) as a reducing agent. The characterization of the synthesized zinc oxide is shown in Figure 2(a), which presents the analysis using the XRD technique. It can be observed that under the condition of using 5 mL of extract (orange line, ZnO-MgE5), ZnO could not be synthesized. Only the phase of $Zn(OH)(NO_3)(H_2O)$ was detected, which matched the data from JCPDF 01-084-1907, indicating that the amount of extract used was insufficient. When the amount of extract was increased to 10 mL (pink line, ZnO-MgE10), 15 mL (blue line, ZnO-MgE15), and 20 mL (green line, ZnO-MgE20), the $Zn(OH)$ compound phase increased, while the $Zn(OH)(NO_3)(H_2O)$ phase decreased. When the extract volume was further increased from 25 mL to 30 mL (red and black lines, ZnO-MgE25 and ZnO-MgE30), the ZnO phase appeared at the characteristic 2θ positions corresponding to specific crystal planes, with no other phases detected. Additionally, the presence of other molecules in the structure of ZnO-MgE under all conditions was examined using the FTIR technique, as shown in Figure 2(b). The FTIR spectra of ZnO-MgE samples show a broad peak

at 3250 cm^{-1} and a band at 1659 cm^{-1} corresponding to $O-H$ stretching and bending vibrations, indicating adsorbed water and surface hydroxyl groups. The band at 1316 cm^{-1} and peaks at 1017 cm^{-1} and 812 cm^{-1} are attributed to $C-O$ and $C-H$ vibrations from residual organic compounds in the mango peel extract, suggesting their role as capping or stabilizing agents. Characteristic $Zn-O$ stretching bands at 513 cm^{-1} and 423 cm^{-1} confirm the successful formation of the ZnO structure. These results indicate the coexistence of inorganic and organic functional groups on the ZnO surface [28].

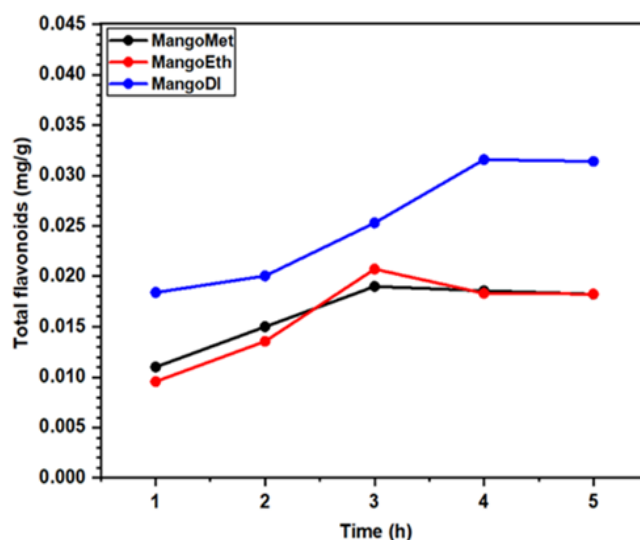


Figure 1. Effect of extraction time on the flavonoid content of mango fruit extracts.

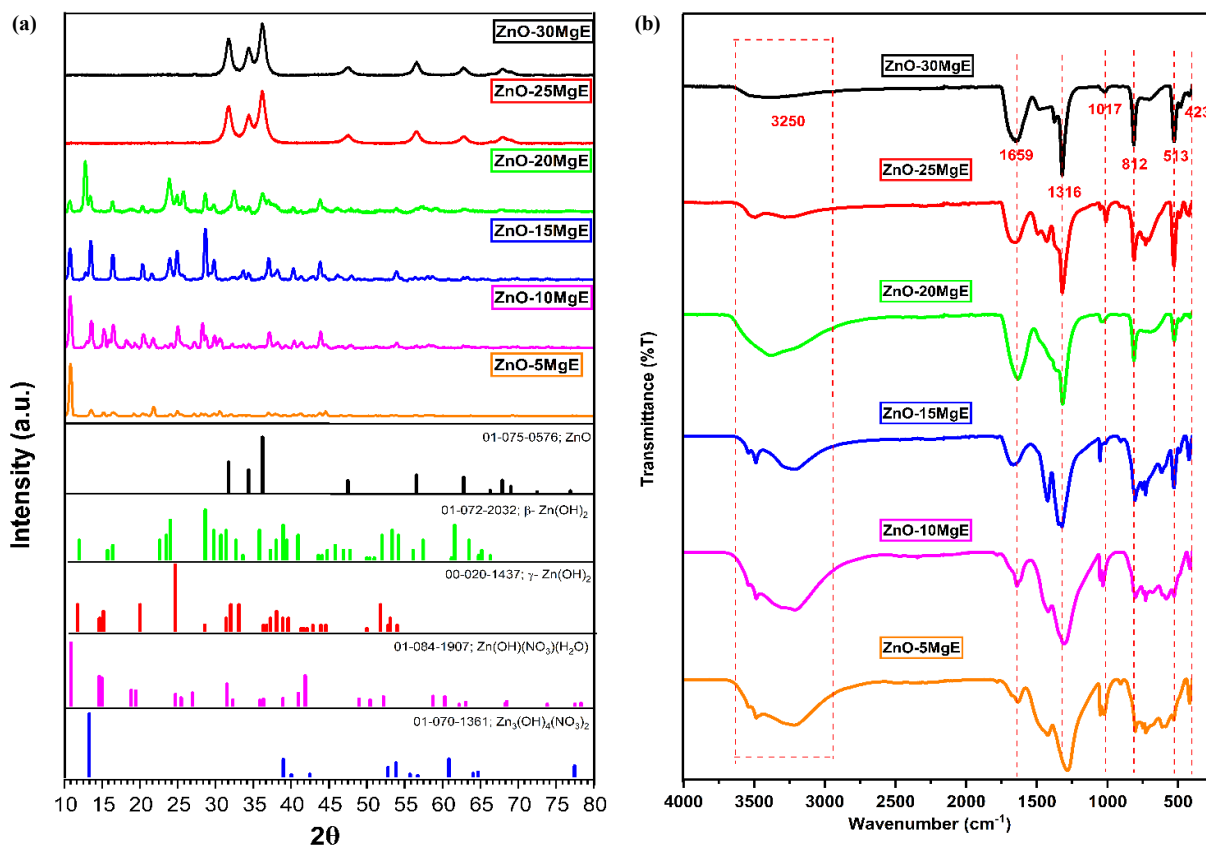


Figure 2. Structure Characterization of ZnO synthesized : (a) XRD, and (b) FTIR.

3.3 Influence of annealing temperature for removing organic impurity

Figure 3(a) presents the TGA curve, which illustrates the effect of temperature on the removal of organic groups from the synthesized ZnO. A significant weight loss was observed between 350°C to 400°C, indicating the decomposition and removal of organic residues, leading to the formation of pure ZnO. Based on these results, three annealing temperatures: 350°C, 375°C, and 400°C were selected for further investigation over a 6 h duration. The FTIR spectra in Figure 3(b) confirm that impurity peaks remained at 350°C and 375, suggesting that some organic compounds were strongly bonded and not fully eliminated at these temperatures. In contrast, at 400, the absence of these peaks indicates the complete removal of organic impurities, consistent with the TGA results and confirming the formation of pure ZnO.

3.4 The morphology of synthesized ZnO and pure ZnO nanoparticles

Figure 4 shows the morphology of ZnO nanoparticles synthesized using ripe mango peel extract. It was found that ZnO-25MgE before annealing (Figure 4(a-b)), had larger particles and still contained impurity clusters on the surface. When ZnO-25MgE was annealed at various temperatures, the agglomerated particles disintegrated and became smaller, becoming spherical in shape. As shown in Figure 4(c-f), and when the temperature was increased to 400°C (Figure 4(g-h)), it was found that the impurities disappeared, the particles obtained had a smoother surface and were homogeneous. This transformation indicates that the annealing process plays a crucial role in improving the purity and uniformity of ZnO nanoparticles. Higher temperatures promote the removal of organic residues and enhance crystallinity, leading to more defined particle boundaries. Consequently, the morphology becomes more stable, which can positively influence the material's optical and catalytic properties [29].

3.5 The antibacterial efficacy

For the antibacterial efficacy test of ZnO-25 MgE at 400°C against Gram-negative bacteria (*E. coli*) and Gram-positive bacteria (*S. aureus*), as shown in Figure 5 (a-c), it can be seen that as the concentration of ZnO solution increases, the antibacterial efficacy also increases. The annealing temperature of 400°C was selected because it produced the highest purity ZnO, as confirmed by the TGA analysis. The concentration of ZnO at 0.7 mg·mL⁻¹ showed the best antibacterial performance, with the clear zone values of 9.54 mm and 8.32 mm (*E. coli* and *S. aureus*, respectively). The primary antibacterial mechanism of zinc oxide nanoparticles involves the generation of reactive oxygen species (ROS), such as hydroxyl radicals ([•]OH) and superoxide (O₂^{•-}), upon ZnO disintegration. These ROS damage the bacterial cell membrane and DNA, ultimately leading to cell death. It can be seen that *E. coli* exhibits better antibacterial resistance than *S. aureus* due to *E. coli* has an outer membrane acts as an additional protective barrier, making it harder for many antibiotics to enter the cell [30,31].

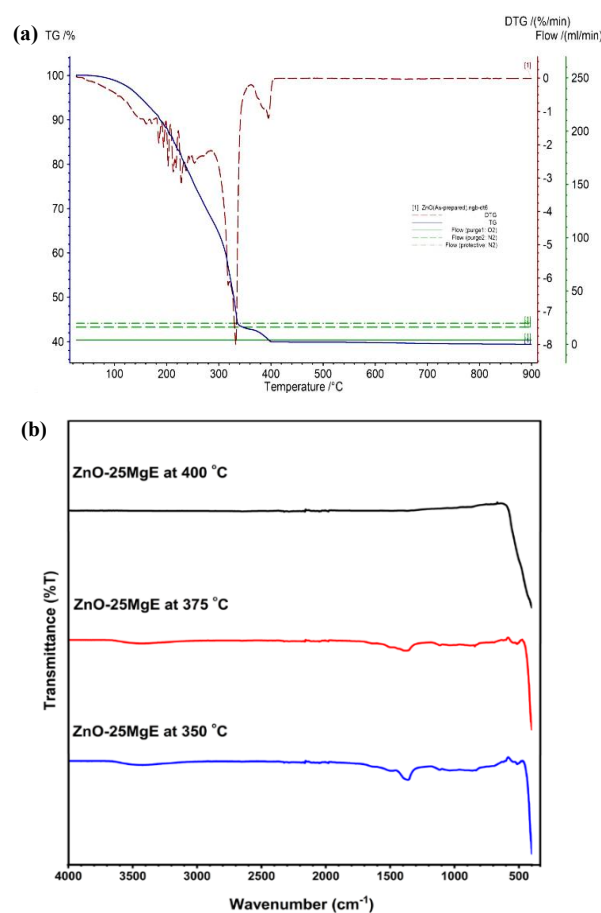


Figure 3. (a) the TGA curve, and (b) FTIR to examine the influence of the annealing temperatures used for the removal of organic groups in the synthesized ZnO.

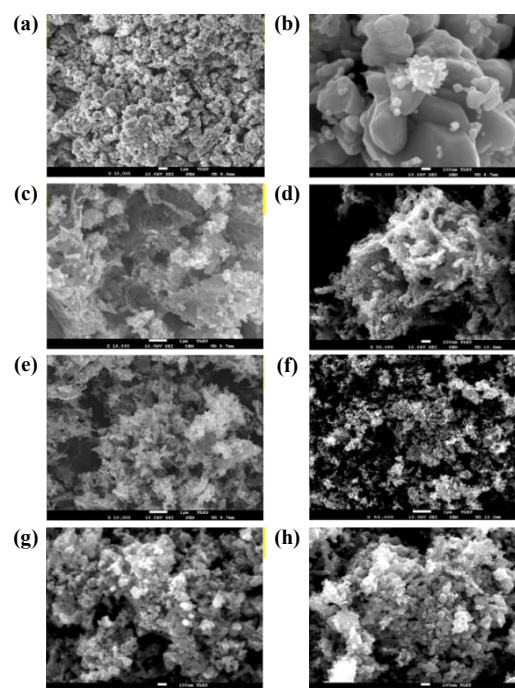


Figure 4. FE-SEM images of ZnO at 10,000 (left) and 50,000 (right) magnifications: (a,b) ZnO-25MgE, (c,d) annealed at 350°C, (e,f) annealed at 375°C, and (g,h) annealed at 400°C.

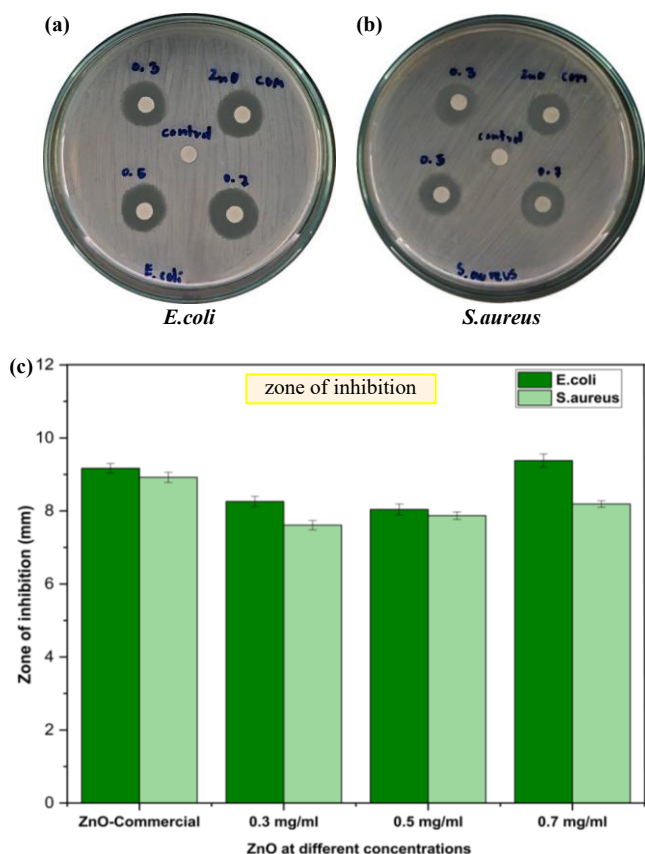


Figure 5. Antibacterial activities (a) *E. coli*, (b) *S. aureus*, and (c) graph of inhibition zone.

4. Conclusions

This research focused on the effect of annealing temperature on the green synthesis of ZnO nanoparticles using mango peel extracts prepared with different solvents, emphasizing an environmentally friendly approach. Among the various extracts, the one obtained using deionized water exhibited the highest flavonoid content, suggesting its strong potential as both a reducing and stabilizing agent in the synthesis process. ZnO-25 MgE effectively facilitated the synthesis of ZnO nanoparticles, and annealing at 400°C for 6 h resulted in the formation of high-purity ZnO. The purity of the ZnO nanoparticles was characterized using various techniques, including X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, and TGA analysis. Scanning electron microscopy (SEM) revealed that the annealing process plays a vital role in enhancing the structural purity and morphological uniformity of ZnO nanoparticles. Regarding antibacterial performance, the ZnO nanoparticles displayed stronger inhibitory effects against *Escherichia coli* (*E. coli*) than *Staphylococcus aureus* (*S. aureus*), underscoring their potential as effective antibacterial agents.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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