



Synthesis and characterization of lanthanum-doped curcumin-functionalized antimicrobial copper oxide nanoparticles

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ABSTRACT

Flavonoid-based nanomaterials have extensive potential in antimicrobial research because of their non-toxicity, large-scale producibility, and chemical stability. An efficient combination of flavonoids and rare earth metals can have excellent antimicrobial properties owing to their robust medicinal and physicochemical properties. In this study, we synthesized copper oxide (CuO) nanoparticles (NPs) using a reflux reaction and repeated doping with the rare earth element lanthanum to prepare La–CuO NPs. Next, these La–CuO NPs were functionalized with the flavonoid curcumin, and the modified NPs were termed Cu–La–CuO NPs. These NPs were chemically characterized via microscopic and spectroscopic techniques, including transmission electron microscopy (TEM), scanning electron microscopy (SEM), ultraviolet–visible (UV–Vis) adsorption, Fourier transform infrared spectroscopy (FT-IR) spectroscopy, and powder X-ray diffraction (XRD) analysis. The SEM-EDX analysis reveals the elements doped into the native CuO NPs. The TEM and XRD characterization results show, in detail, the distinct morphology and phase composition of the prepared CuO, La–CuO, and Cu–La–CuO NPs. The antimicrobial activity of the Cu–La–CuO NPs was investigated on bacteria, such as *Escherichia coli* (*E. coli*) and *Bacillus subtilis* (*B. subtilis*), and fungi, including *Aspergillus niger* (*A. niger*). The Cu–La–CuO NPs delivers excellent antimicrobial effects due to the incorporation of lanthanum and curcumin into the CuO NPs. The Cu–La–CuO NPs show excellent antimicrobial potentials with an adequate zone of inhibition values around 2.2 and 2.9 mm in *E. coli* and *A. niger*, respectively. Live and dead analysis studies suggest the antimicrobial effects of lanthanum and curcumin moieties over native CuO NPs. The obtained results are significant in developing of rare earth metal-based flavonoid-conjugated nanocomposites against typical microbes.

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1. Introduction

Microbes can have robust morphological and pathological interactions with the membranes in the human body.¹ Bacteria are the most abundant infectious microbes and are ubiquitous, with an effective reproductive capacity and the ability to resist drugs in different environments.² In this context, nanomaterials with antibiotic properties are crucial for mitigating bacterial infections.³ Suitable, efficient antibacterial precursors with multifactorial applications are urgently needed. For instance, flavonoids have potent antibacterial, antioxidant, anti-inflammatory, and anticancer

properties.^{4–7} Their non-toxicity and biocompatibility are major considerations in their utilization in antimicrobial research. Extensive studies have been conducted on the anticancer properties of flavonoids. Our lab group has focused on the exploitation of nanomaterials and metal complexes using various flavonoids, which are effective against various tumors.^{8–10} Among flavonoids, curcumin and kaempferol have been well studied for their antibacterial properties.^{11,12} However, the low bioavailability of flavonoids limits their direct use as antibiotics. Flavonoid-functionalized nanomaterials overcome this limitation because of their enhanced bioavailability and antimicrobial properties.^{13,14} Notably, flavonoids can inhibit DNA gyrase, leading to values in bacteria. For example, quercetin has been shown to have low IC₅₀ values (0.9–3.9 μmol/L against *Escherichia coli*).¹⁵ Li et al. synthesized myricetin-based Ag nanoparticles (NPs) for investigating antibacterial effects against

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human pathogenic bacteria, such as *E. coli* (*E. coli*) and *Salmonella*, which showed minimum inhibitory concentrations of approximately 10^{-4} and 10^{-5} .¹⁶ In another study, anisotropic Ag NPs were synthesized at room temperature by utilizing quercetin diphosphate (QDP) as a stabilizing and reducing agent. These Ag nanoparticles (NPs) showed substantial antimicrobial effects by expressing virulence-related genes in *E. coli* SM10, which occurs due to the release of Ag^+ ions from the QDP-Ag NPs.¹⁷

In the last two decades, CuO NPs have widely replaced Au and Ag NPs because of their cost-effectiveness, broad availability, and potent antibacterial properties.¹⁸ Rajeshkumar et al. synthesized CuO NPs by using seaweed (*Sargassum longifolium*) for antimicrobial investigations; seaweed contains many reducing agents that can be employed for the transformation of metal salts into metal NPs.¹⁹ Other researchers have synthesized CuO NPs by using brown algae such as *Sargassum polycystum*, which exhibit outstanding antibacterial and antifungal activities against *Pseudomonas aeruginosa* and *Aspergillus niger*, respectively.²⁰ Sathiyavimal et al. prepared CuO NPs by using leaf extracts of *Psidium guajava* and investigated their antibacterial applications against *E. coli* and *Sargassum pneumoniae*.²¹

Several lanthanum-based compounds can exert antimicrobial potential because of their physicochemical properties.^{22,23} For instance, Liu et al. developed lanthanum oxide (La_2O_3) hierarchical nanostructures, such as nanopolyhedra, nanospheres, and nanospindles, to investigate their antibacterial benefits.²⁴ This mainly depends on the removal of phosphate (PO_4^{3-}) groups by lanthanum, which has a strong affinity for phosphate. Antimicrobial resistance was also inhibited by lanthanum and graphene oxide (GO) nanocomposites due to lipid peroxidation, lipid dephosphorylation, and the destruction of peptidoglycan in bacteria.²⁵ These results highlight the importance of rare earth metals in antimicrobial research. Another bimetallic lanthanum and nickel-derived nanopowder, LaNiO_3 , exhibited an excellent antibacterial effect with a zone of inhibition of approximately 13 mm against *Staphylococcus aureus*.²⁶ In another study, $\text{La}^{3+}/\alpha\text{-Al}_2\text{O}_3$ NPs were synthesized using honey as a capping and reducing agent to assess their antibacterial activity against gram-positive and negative bacteria.²⁷ The main precursors of lanthanum metal include LaCl_3 , La_2O_3 , and lanthanum nitrate [$\text{La}(\text{NO}_3)_3$], which have been extensively explored for multifarious applications as antimicrobial, antioxidant, and sensing agents.²⁸ Lakshmi et al. developed lanthanum and flavonoid (rutin)-based carbon dots for sensing calcium (Ca^{2+}) ions and used LaCl_3 as a precursor to block Ca^{2+} ions in plants.²⁹ Moreover, the thermodynamic compatibility of the prepared CuO NPs, La–CuO NPs, and Cu–La–CuO NPs can be studied utilizing the Flory–Huggins interaction parameter. The following researchers investigated the above parameter using polymer nanocomposites in a detailed manner.^{30–33}

In this study, we synthesized CuO NPs using a simple reflux reaction at a low temperature and further doped them with lanthanum chloride (LaCl_3) to prepare bimetallic NPs. The resulting La–CuO NPs were combined with a notable flavonoid, curcumin, to develop highly effective antimicrobial agents (Cu–La–CuO NPs). The zone-of-inhibition studies were performed using these Cu–La–CuO NPs against different antimicrobial species, such as *E. coli*, *Bacillus subtilis*, and *A. niger*. The chemical characterization techniques also confirmed the formation of nanocomposites with a well-preserved crystallinity and elemental composition. Again, UV–Vis absorption and Fourier transform infrared spectroscopy (FT-IR) studies provided detailed optical properties and functional group diversities, respectively. Moreover, live/dead and LDH assay results confirmed the antimicrobial effects of synthesized Cu–La–CuO NPs. As developed nanocomposites composed of rare

earth metal (lanthanum) and flavonoid (curcumin) are potentially suitable for investigating antimicrobial effects.

2. Experimental

2.1. Materials

Copper sulfate (CuSO_4), lanthanum chloride (LaCl_3), curcumin, dimethyl sulfoxide (DMSO; 1%), ethanol, sodium hydroxide (NaOH), 2',7'-dichlorofluorescein diacetate (DCFH-DA) and agar medium were purchased from Sigma Aldrich, USA. LDH assay kit (C20300) and live/dead BacLight assay kit (L7012) were purchased from Invitrogen (USA). All chemicals and reagents used in this study were of analytical grade.

2.2. Instrumentation

Transmission electron microscopy (TEM) and high-resolution transmission electron microscopy (HRTEM) measurements of the as-prepared NPs were conducted using a JEM-3010 (JEOL Ltd., Japan). UV–visible absorption spectra were measured using a Varian Cary 100 UV–Vis spectrophotometer. The FT-IR spectra of the NPs were obtained using a JASCO FT-IR4600 spectrophotometer. X-ray diffraction (XRD) studies of the NPs were performed using the Scintag-SDS 2000 at 40 kV and 30 mA in the continuous scanning 2θ mode. Elemental composition was analyzed by a scanning electron microscope (SEM; HITACHI, S4700, Japan) with a 20 kV accelerating voltage of energy dispersive X-ray (EDX) analyzer.

2.3. Synthesis of CuO NPs

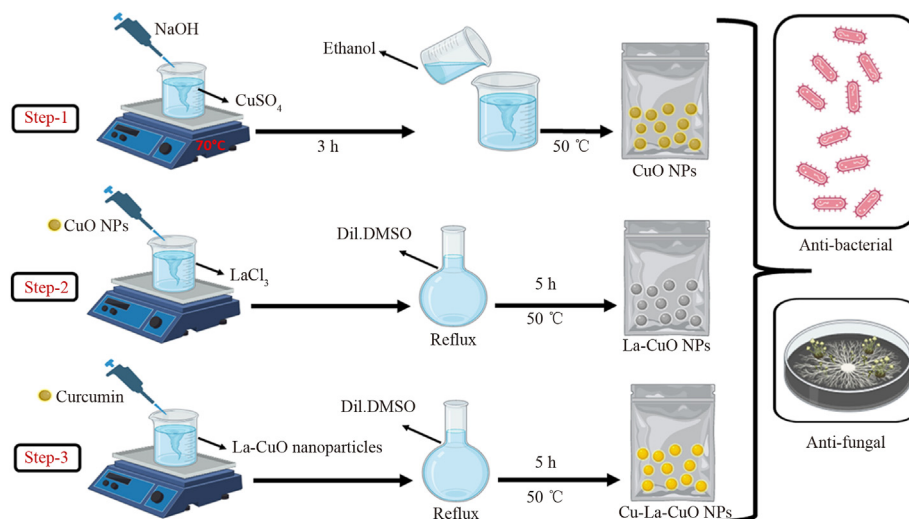
Copper sulfate (5 g) was dissolved in 100 mL deionized water (DI) water in a 500 mL beaker and stirred for approximately 5 min at 250 r/min at an ambient temperature. After 5 min, NaOH solution was added drop-wise until the solution changed to blue color and stirring was continued at 70 °C for 3 h to maintain the pH at approximately 12. Into this solution, 100 mL ethanol was added. Next, the solution was boiled at 50 °C for 5 min to obtain crystallized CuO NPs. The solution was centrifuged at 6000 r/min for 20 min to filter the impurities. The resulting CuO NPs were oven-dried and stored in a refrigerator for subsequent analysis.

2.4. Synthesis of La–CuO NPs

CuO NPs (0.75 g) and lanthanum chloride (5 g) were dissolved in 100 mL DI water and transferred into a 250 mL round bottom flask (RBF), and a diluted DMSO solution (10 mL) was added and refluxed for 5 h at 50 °C. After 5 h, the solution was collected and centrifuged at 6000 r/min for 20 min to remove the unreacted large precursors. Finally, the resulting product was dried at 60 °C for 24 h and refrigerated for further experiments.

2.5. Synthesis of Cu–La–CuO NPs

The prepared La–CuO NPs (0.75 g) and curcumin (350 mg) were dissolved in 100 mL distilled water and transferred to 250 mL RBF and a diluted DMSO solution (10 mL) was added and refluxed for 5 h at 50 °C. After 5 h, the final mixture was collected and centrifuged at 6000 r/min for 20 min, and the resulting product was dried at 50 °C for 24 h and used for further analysis. Scheme 1 illustrates the synthesis of CuO, La–CuO, and Cu–La–CuO NPs.



Scheme 1. Schematic representation of the synthesis of CuO NPs, La–CuO NPs, and Cu–La–CuO NPs and their antimicrobial applications.

2.6. Preparation of bacterial and fungal cultures

E. coli (*E. coli* C600), *B. subtilis* [*B. subtilis* (Ehrenberg) ATCC 23857™], and *A. niger* was acquired from the Korean Culture Collection of Probiotics (KCCP50037). The generic antibiotic streptomycin (control: antibacterial) was purchased from Himedia Pvt. Ltd. The cycloheximide (control: antifungal) and the agar medium were acquired from Sigma Aldrich, USA.

2.7. Antibacterial activity

The antibacterial properties of the synthesized NPs were evaluated against various microorganisms including *B. subtilis* and *E. coli*, using the well diffusion method. In this study, streptomycin (100 µg/mL in sterile distilled water) was used as control. The nutrient agar medium was prepared systematically by dissolving 1500 mg in 150 mL distilled water and autoclaving the agar medium at 121 °C for 15 min. Subsequently, the prepared agar medium was placed into Petri plates and allowed to solidify, after which the bacterial cultures were dispersed using an L-shaped glass rod. Subsequently, 25, 50, 75, and 100 µg/mL of various concentrations of three substances were poured into prepared wells and incubated at 27 °C for 12 h. Finally, inhibitory zones (2.2 mm) were visible after incubation and were calculated.

2.8. Antifungal activity

The antifungal activity of the synthesized NPs was investigated using the well diffusion method against *A. niger*. A common fungicide (antibiotic), cycloheximide (100 µg/mL in sterile distilled water), was used as a control for antifungal activity. The potato dextrose agar medium was made using 2850 mg in 75 mL distilled water and autoclaved for 15 min at 121 °C. The medium was then placed into plates and allowed to solidify before spreading the fungal saline culture by using an L-shaped glass rod. Next, different concentrations (25, 50, 75, and 100 µg/mL) of the prepared NPs were placed into corresponding wells in three plates and incubated at 28 °C for 48 h. Finally, inhibitory zones (2.9 mm) were observed after incubation and measured.

2.9. Lactate dehydrogenase assay (LDH)

The LDH assay kit was used to evaluate membrane damage in *E. coli* cells treated with (Cu–La–CuO NPs). The *E. coli* cells were treated with different concentrations of Cu–La–CuO NPs (25, 50, 75, and 100 µg/mL) and incubated for 6 h at 37 °C. After incubation, the percentage of LDH leakage was measured by calculating the absorbance, using the following formula:

$$\text{Percentage of LDH released} = [(A-B)/(C-A)] \times 100\% \quad (1)$$

where *A* is the LDH content of the sample, *B* the LDH content of the culture medium, and *C* the LDH content of the cell lysate.

2.9.1. Live and dead cell staining assay

Cell viability of bacterial cells (*E. coli* and *B. subtilis*) treated with CuO NPs, La–CuO NPs, and Cu–La–CuO NPs was determined by using live and dead staining assay. By using the live and dead cell staining solution, cells were incubated for 60 min in a dark environment. Finally, treated cells and the untreated *E. coli* and *B. subtilis* cells alone (control) were examined under a fluorescence microscope, and images were acquired by Nikon live cell capture software.

2.9.2. Reactive oxygen species (ROS) assay

The ROS generation in the *E. coli* and *B. subtilis* cells was measured by the addition of Cu–La–CuO NPs, which were further studied using Cell Biolabs, Oxiselect™ Intracellular ROS assay kit. A fluorescent dye i.e., DCFH-DA was used for measuring the fluorescence intensity of *E. coli* and *B. subtilis* cells alone (control) and Cu–La–CuO NPs at different concentrations (25, 50, 75, and 100 µg/mL).

2.10. Statistical analysis

All data presented in this paper are the mean ± standard deviation (SD) of three individual experiments.

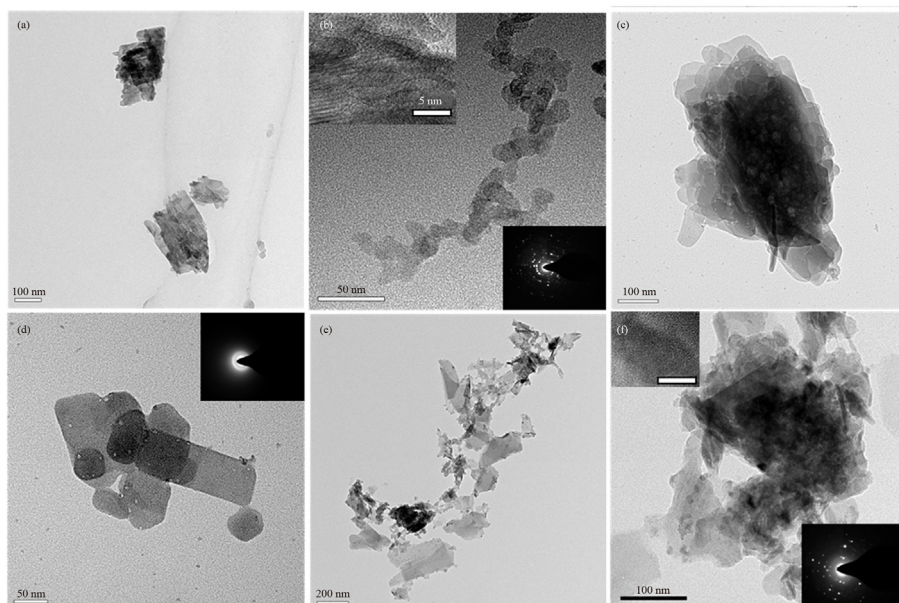


Fig. 1. TEM images of (a, b) CuO NPs, (c, d) LaCuO NPs, and (e, f) Cu–La–CuO NPs. Inset images of (b) CuO NPs (top-left: HRTEM and bottom-right: SAED pattern), (d) La–CuO NPs (top-right: SAED pattern), and (f) Cu–La–CuO NPs (top-left: HRTEM and bottom-right: SAED pattern).

3. Results and discussion

3.1. Materials characterization

The morphologies of the as-prepared CuO NPs, La–CuO NPs, and Cu–La–CuO NPs were investigated using TEM. Fig. 1(a) and (b) show the TEM images of CuO NPs, which reveal spherical and cube-like structures with an average size of approximately 20 nm. Distinct crystallinity was observed from the HRTEM images, which showed distinguishable lattice fringes (inset). The selected area electron diffraction (SAED) pattern of the CuO NPs suggests their crystalline nature (inset, Fig. 1(b)). The morphology of the CuO NPs did not change significantly, observed based on the TEM images of the La–CuO NPs (Fig. 1(c) and (d)). In contrast with the SAED pattern of the CuO NPs, the SAED pattern of the La–CuO NPs showed decreased extent of crystallinity (Fig. 1(d)). After the curcumin incorporation into the La–CuO NPs, the TEM images of the Cu–La–CuO NPs exhibited transparent sheet-like morphologies (Fig. 1(e)). Enhanced crystallinity was observed in the HRTEM image (inset) of Cu–La–CuO NPs, and it was confirmed by the SAED pattern (Fig. 1(f)).

SEM analysis was performed to investigate the elemental composition of the prepared CuO, La–CuO, and Cu–La–CuO NPs. Fig. S1 shows the elemental mapping analysis for the CuO NPs, namely, the carbon (C), oxygen (O), copper (Cu), sulfur (S), and sodium (Na) elements, and the SEM image. Furthermore, the same elements and additional lanthanum (La) and chlorine (Cl) were observed in the elemental mapping analysis of the La–CuO NPs and their SEM images (Fig. S2). Finally, the elemental mapping studies for Cu–La–CuO NPs have revealed the abundance of the aforementioned elements, and their SEM images are presented in Fig. S3. The elemental mapping results correlate with the EDX spectra, which show the presence of the corresponding elements with their weight and atomic percentages (i–iii, Fig. S4).

Characteristic XRD peaks were observed for the CuO NPs at 2θ diffraction angles of 32.46° , 35.54° , 38.68° , 48.88° , 53.48° , 58.16° , 61.52° , 66.18° , 68.02° , 72.34° , and 75.12° , which were ascribed to the highly crystalline nature of CuO (curve (1), Fig. 2). However, after doping lanthanum with CuO NPs (La–CuO NPs), the peaks

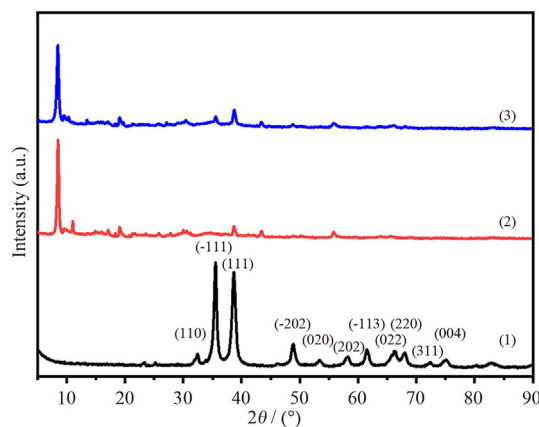


Fig. 2. XRD analysis for CuO NPs (1), La–CuO NPs (2), and Cu–La–CuO NPs (3).

were slightly reduced because of the strong interaction between lanthanum and CuO NPs (curve (2), Fig. 2). These results are consistent with the TEM results, which show the distinguished morphology of doped lanthanum particles and the SAED pattern, in which reduced crystallinity was observed in the La–CuO NPs. After the incorporation of curcumin into the La–CuO NPs (Cu–La–CuO NPs), the XRD patterns were not significantly altered compared with those of the La–CuO NPs alone; however, the crystallinity of the Cu–La–CuO NPs increased remarkably (curve (3), Fig. 2). Hence, we posit that curcumin is strongly connected to the La–CuO NPs because of the presence of multiple hydroxyl (–OH) groups.³⁴ Furthermore, the HRTEM results of the Cu–La–CuO NPs show lattice fringes, which result from their induced crystallinity and are corroborating with XRD results.

3.2. UV–Vis absorption analysis

The optical properties of the CuO NPs, La–CuO NPs, and Cu–La–CuO NPs were studied using UV–Vis absorption spectroscopy. Fig. 3 shows the absorption bands of CuO NPs (black curve) at 290 and 368 nm, which correspond to the π – π^* and

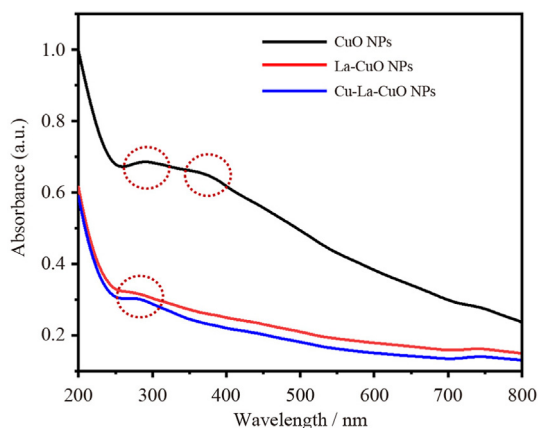


Fig. 3. UV-Vis absorption spectra for CuO NPs (black curve), La-CuO NPs (red curve), and Cu-La-CuO NPs (blue curve).

$n-\pi^*$ transitions, respectively. Furthermore, the absorption peak at 282 nm represents the $\pi-\pi^*$ transition for the La-CuO NPs (red curve) and Cu-La-CuO NPs (blue curve; Fig. 3). The blue shift in the absorption peak from 290 to 282 nm indicated the successful doping of lanthanum and curcumin in the CuO NPs. In addition, the $n-\pi^*$ transitions at 368 nm observed in the absorption spectrum of CuO NPs disappeared in both La-CuO NPs and Cu-La-CuO NPs, which may suggest the binding of (C=O) groups to the (–OH) groups of curcumin.

3.3. FT-IR analysis

Functional group analysis of the CuO, La-CuO, and Cu-La-CuO NPs was performed using FT-IR analysis. The FT-IR spectra of CuO NPs showed characteristic stretching vibrations of hydroxyl (–OH) groups at 3406 cm^{-1} .^{35–38} Stretching vibrations of the (–CH₂) and (–CH₃) groups were observed at 2850 , 2918 , and 2984 cm^{-1} , respectively.³⁹ The other stretching frequencies at 1127 and 1455 cm^{-1} were attributed to the (C–C) and (C=C) groups, respectively, and the peaks at 1508 and 1650 cm^{-1} were attributed to (C=O) (Fig. 4(a)). The FT-IR spectra of the La-CuO NPs showed peaks at 938 , 1079 , 1455 , and 1616 cm^{-1} , which were assigned to the stretching vibrations of the (C–H), (C–O–C), (C=C), and (C=O) groups, respectively. The stretching vibrations of the (–CH₂) and (–CH₃) groups were observed at 2850 , 2917 , and 2984 cm^{-1} . Stretching vibrations of the (–OH) groups were observed at 3294 cm^{-1} (Fig. 4(b)). Furthermore, the FT-IR spectra of the Cu-La-CuO NPs showed stretching vibrations of (–CH₂) and (–CH₃) at approximately 2898 and 2989 cm^{-1} . The stretching frequencies at approximately 934 , 1455 , 1509 , and 1649 cm^{-1} were ascribed to the (C–H), (C=C), and (C=O) groups, respectively

(Fig. 4(c)). Notably, the stretching vibrations of the (–OH) groups of curcumin were greatly reduced owing to the strong interactions between lanthanum and CuO in the final Cu-La-CuO NPs.

3.4. Antimicrobial studies

The antibacterial properties were analyzed from the well diffusion method using CuO, La-CuO, and Cu-La-CuO NPs against gram-positive and gram-negative bacteria such as *B. subtilis* and *E. coli* (Fig. 5). The zone of inhibition was observed using the above-mentioned NPs against *B. subtilis*. However, the La-CuO NPs have shown the highest zone inhibition (2 mm) when compared to that (1.8 mm) of *E. coli* at $100\text{ }\mu\text{g/mL}$ concentration. Whereas the largest zone of inhibition was observed around 2.2 mm in *E. coli* after the treatment with Cu-La-CuO NPs ($100\text{ }\mu\text{g/mL}$) in contrast to the other concentrations (25, 50, and $75\text{ }\mu\text{g/mL}$). However, significant zone of inhibition results was not observed in *E. coli*, when treated with the CuO and La-CuO NPs. The achieved antibacterial activity for Cu-La-CuO NPs might have been accredited to the intracellular generation of ROS in bacterial cells. The zones of inhibition results for *B. subtilis* and *E. coli* are summarized in Table 1. As the concentration of the nanomaterial increases, the antibacterial activity also increased. But in all the cases the antibacterial activity is less than the positive control, streptomycin. However, in case of Cu-La-CuO NPs the maximum inhibitory activity is observed with $2.2 \pm 0.16\text{ mm}$ against *E. coli*; but the streptomycin shows maximum inhibitory activity with $2.8 \pm 0.14\text{ mm}$ against *E. coli*. Maximum zone of inhibition was observed with $2.0 \pm 0.21\text{ mm}$ when treated with La-CuO NPs against *B. subtilis*, however it was significantly less than the positive control, streptomycin, which has inhibited growth with $3.3 \pm 0.16\text{ mm}$. Generally, gram-positive bacteria have a rigid plasma membrane that enables the establishment of plasma membrane damage, whereas gram-negative bacteria have a flexible membrane which eventually makes it persist the external damage. However, the synthesized NPs have a similar effect under the same conditions in both species. More importantly, the zone of inhibition was proportional to the concentration of NPs. The Cu-La-CuO NPs ($100\text{ }\mu\text{g/mL}$) have demonstrated the maximum zone of inhibition (2.9 mm) in *A. niger* when compared to other concentrations (25, 50, and $75\text{ }\mu\text{g/mL}$) as shown in Fig. 6. We believe that the obtained results are attributed to the high sensitivity and susceptibility of the synthesized Cu-La-CuO NPs. Table 2 shows the zone of inhibition results for *A. niger* using CuO, La-CuO, and Cu-La-CuO NPs. To our surprise, even at $100\text{ }\mu\text{g/mL}$, all of the nanomaterials employed in this investigation demonstrated high antifungal efficacy than the study's positive control, cycloheximide.

3.5. LDH assay

To evaluate the antibacterial activity in *E. coli* using Cu-La-CuO NPs, an LDH assay was performed. Fig. 7 demonstrates the LDH leakage percentage in the *E. coli* cells treated with the Cu-La-CuO

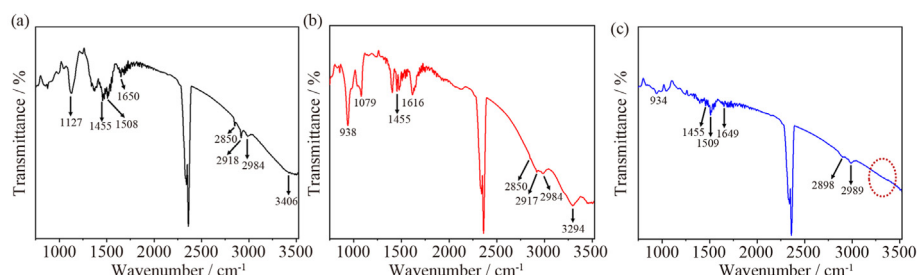


Fig. 4. FT-IR spectra for the CuO NPs (a), La-CuO NPs (b), and Cu-La-CuO NPs (c).

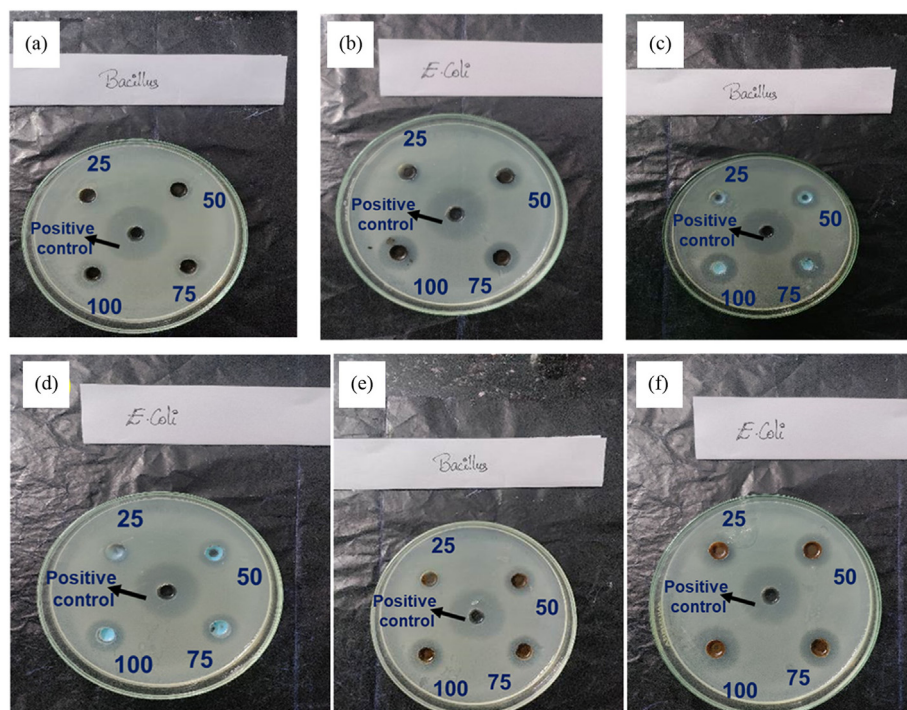


Fig. 5. Antibacterial activity of CuO NPs (a, b), La–CuO NPs (c, d), and Cu–La–CuO NPs (e, f) at different concentrations (25, 50, 75, and 100 µg/mL) treated with *B. subtilis* and *E. coli*. The streptomycin was used as a positive control.

Table 1

Antibacterial activity (mm) for the CuO NPs, La–CuO NPs, and Cu–La–CuO NPs at different concentrations.

Nanomaterials	Bacteria	25 (µg/mL)	50 (µg/mL)	75 (µg/mL)	100 (µg/mL)	Positive control (Streptomycin)
CuO NPs	<i>E. coli</i>	0.9 ± 0.08	1.2 ± 0.08	1.5 ± 0.02	1.7 ± 0.05	2.9 ± 0.14
	<i>B. subtilis</i>	0.2 ± 0.04	0.6 ± 0.03	0.7 ± 0.04	1.2 ± 0.12	3.2 ± 0.16
La–CuO NPs	<i>E. coli</i>	0.7 ± 0.04	0.9 ± 0.08	1.6 ± 0.12	1.8 ± 0.21	3.1 ± 0.14
	<i>B. subtilis</i>	1.0 ± 0.08	1.3 ± 0.12	1.7 ± 0.05	2.0 ± 0.21	3.3 ± 0.16
Cu–La–CuO NPs	<i>E. coli</i>	1.2 ± 0.12	1.4 ± 0.13	1.7 ± 0.05	2.2 ± 0.16	2.8 ± 0.14
	<i>B. subtilis</i>	1.1 ± 0.09	1.3 ± 0.12	1.7 ± 0.05	1.8 ± 0.21	3.2 ± 0.16

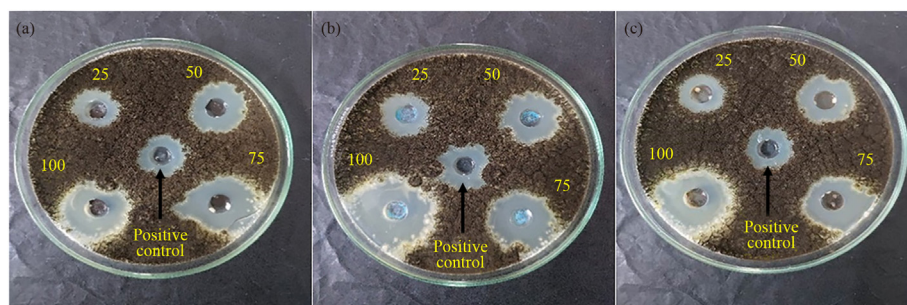


Fig. 6. Antifungal activity of CuO NPs (a), La–CuO NPs (b), and Cu–La–CuO NPs (c) at different concentrations (25, 50, 75, and 100 µg/mL) treated with *A. niger*. The cycloheximide was used as a positive control.

Table 2

Antifungal activity (mm) for the CuO NPs, La–CuO NPs, and Cu–La–CuO NPs at different concentrations.

Nanomaterials	Fungi	25 µg/mL	50 µg/mL	75 µg/mL	100 µg/mL	Positive control (Cycloheximide)
CuO NPs	<i>A. niger</i>	0.9 ± 0.08	1.3 ± 0.12	2.0 ± 0.12	2.5 ± 0.16	1.2 ± 0.18
La–CuO NPs	<i>A. niger</i>	0.7 ± 0.05	1.2 ± 0.08	1.9 ± 0.12	2.8 ± 0.21	1.4 ± 0.18
Cu–La–CuO NPs	<i>A. niger</i>	1.0 ± 0.08	1.2 ± 0.16	1.9 ± 0.20	2.9 ± 0.15	1.2 ± 0.18

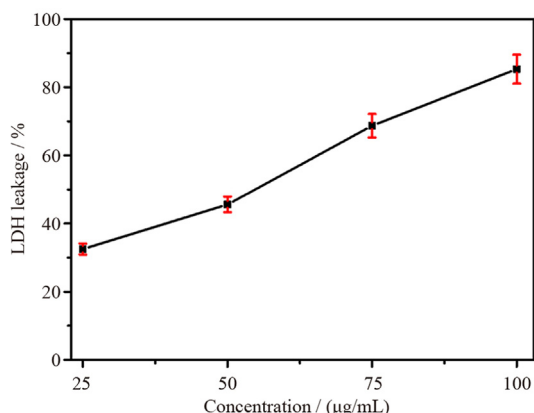


Fig. 7. LDH assay after the treatment of Cu–La–CuO NPs at different concentrations in *E. coli*.

NPs. An increase in the leakage percentage was observed with an increase in the concentration of the Cu–La–CuO NPs. The percentage leakage of LDH was minimal in untreated *E. coli* as compared to the Cu–La–CuO NPs-treated *E. coli*. In this manner, LDH assay results are encouraged to investigate the cell death or cytotoxicity in the bacterial cells. More importantly, the cell wall of bacterial cells i.e., *E. coli* was permitted to be damaged by the antimicrobial activity of Cu–La–CuO NPs in a concentration-dependent manner. Also, these results are signifying bacterial cell damage via effective usage of nanocomposites.

3.6. Live and dead assay

The ability of CuO NPs, La–CuO NPs, and Cu–La–CuO NPs-treated *E. coli* and *B. subtilis* cells was investigated using the live and dead assay. These studies were allowed to observe the damaging membranes in both *E. coli* and *B. subtilis*. For this, the

bacterial cells were stained with SYTO 9 and propidium iodide for color differentiation of green and red, respectively. The cells showing intact cell membranes are representing green fluorescence and those with damaged cell membranes are denoting red fluorescence. Fig. 8(a) shows the *E. coli* cells alone (control), which exhibit a distinctive intact morphology. The CuO NPs-treated *E. coli* cells were loosening their intact morphology (green fluorescence) and few cells showed red fluorescence as shown in Fig. 8(b). In the case of La–CuO NPs-treated cells the still less intact (green fluorescence) and more dead cells (red fluorescence) were observed (Fig. 8(c)). Finally, the Cu–La–CuO NPs-treated *E. coli* cells have shown maximum dead cells (red fluorescence) as compared to that of La–CuO NPs-treated cells as shown in Fig. 8(d). These results are signifying that the synthesized Cu–La–CuO NPs have magnificent bactericidal properties against *E. coli* cells. Similar results were found in the *B. subtilis* cells also, which proved the maximum antimicrobial efficiency of the developed Cu–La–CuO NPs (Fig. 9). The obtained results are indicating that curcumin-conjugated La–CuO NPs are highly efficient for antimicrobial studies.

3.7. Morphological studies in bacterial cells

The SEM images clearly show the morphology of *B. subtilis* and *E. coli* before and after the treatment of CuO NPs, La–CuO NPs, and Cu–La–CuO NPs. Initially, untreated *B. subtilis* cells (control) were exhibiting definite bacterial morphology without any damage as shown in Fig. 10(a). Further, the SEM images of CuO NPs-treated *B. subtilis* cells exerted very limited damaging potential (Fig. 10(b)). This explains the negligent antimicrobial potential of the native CuO NPs. However, La–CuO NPs-treated cells of *B. subtilis* have shown moderate changes in their morphology as shown in Fig. 10(c). These changes are attributed to the dual effect of two metals namely CuO and lanthanum. Finally, distinctive bacterial damage was observed in the SEM images of Cu–La–CuO NPs-treated *B. subtilis* cells (Fig. 10(d)). Also, similar results were observed in the *E. coli* cells, which confirms the excellent antimicrobial potential of the Cu–La–CuO NPs (Fig. S5). These results

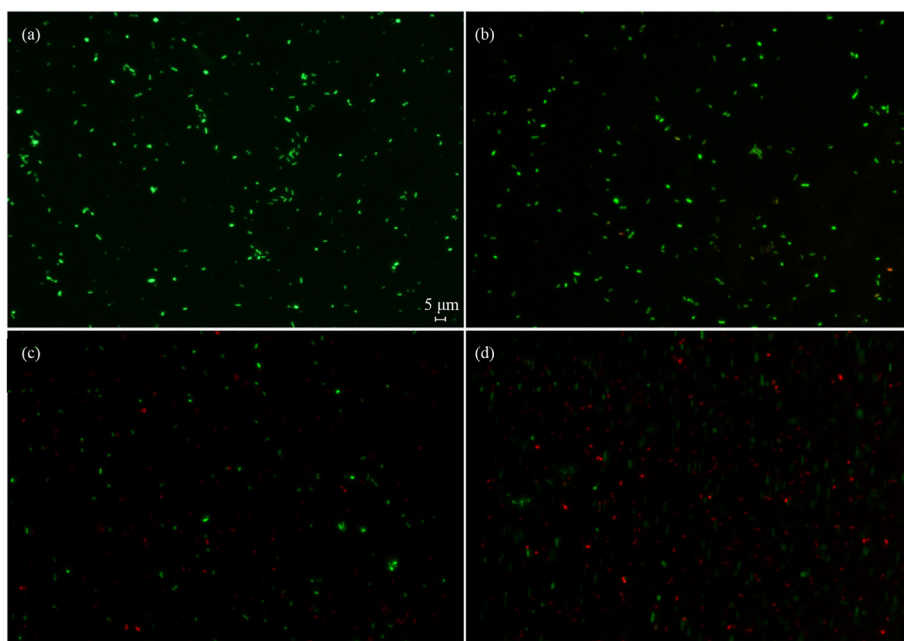


Fig. 8. Live and dead cell staining assay images of *E. coli* cells untreated (control) (a), and after treating with CuO NPs (b), La–CuO NPs (c), and Cu–La–CuO (d) NPs. Green and red color fluorescence represents live and dead cells, respectively. *E. coli* cells were incubated for 60 min.

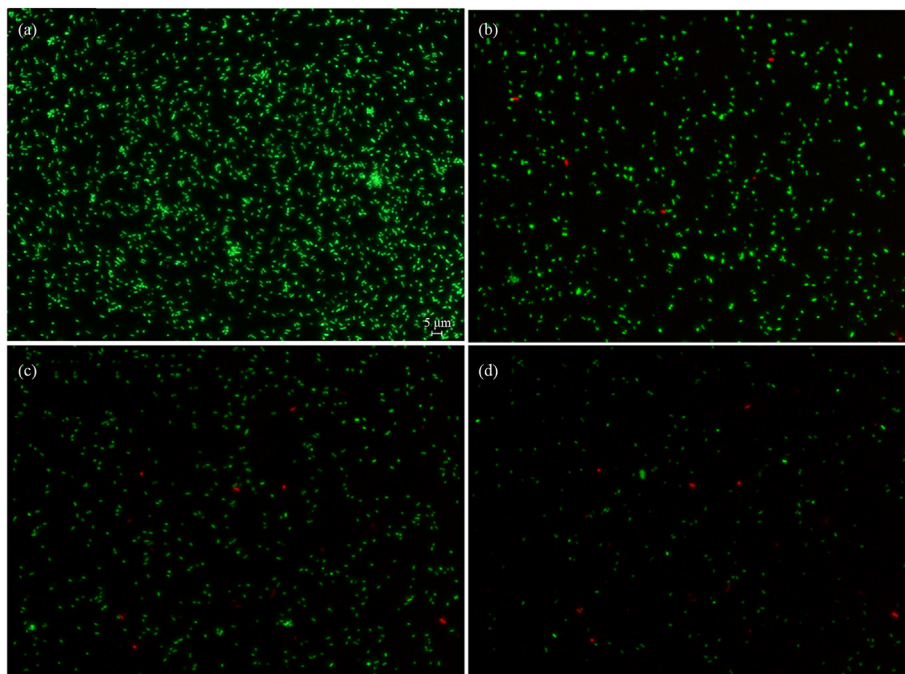


Fig. 9. Live and dead cell staining assay images of *B. subtilis* cells untreated (control) (a), and after treating with CuO NPs (b), La–CuO NPs (c), and Cu–La–CuO NPs (d). Green and red color fluorescence represent live and dead cells, respectively. *B. subtilis* cells were incubated for 60 min.

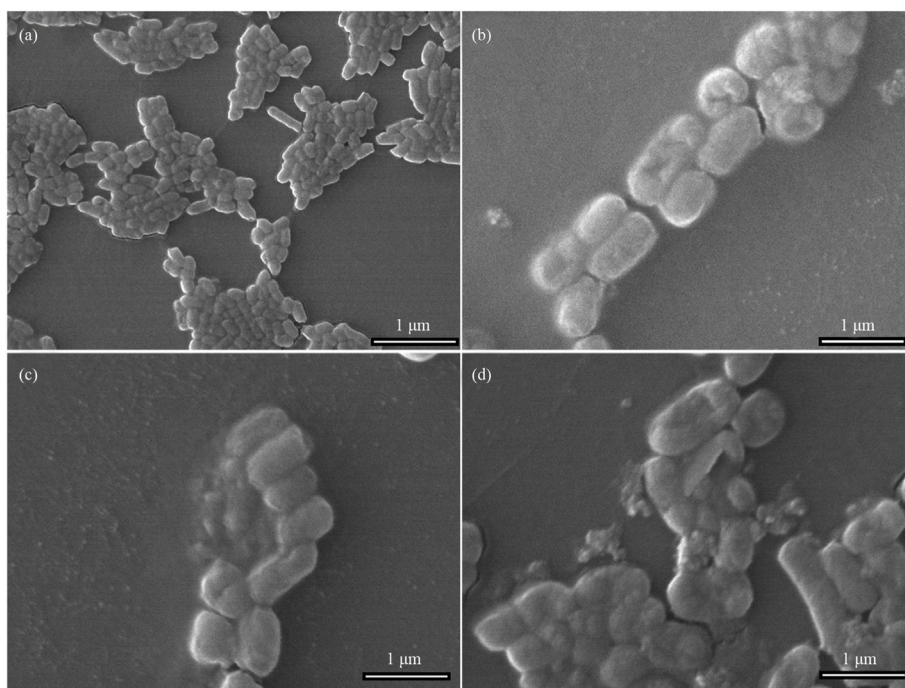
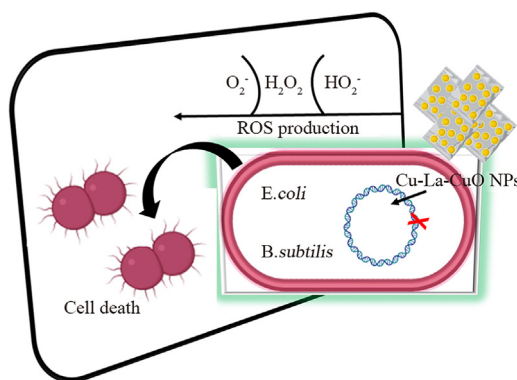


Fig. 10. SEM images of *B. subtilis* cells untreated (control) (a), and after treating with CuO NPs (b), La–CuO NPs (c), and Cu–La–CuO NPs (d). *B. subtilis* cells were incubated for 60 min.

were achieved due to the potential antibacterial properties of curcumin along with the other metals like CuO and La. It suggests the benefits of flavonoids like curcumin when synthesized in the form of nanocomposites. From these results, we believe that nanomaterials synthesized from the efficient conjugation of rare earth metals and flavonoids are having excellent antibacterial effects compared to the CuO NPs alone.

3.8. ROS assay

ROS assay was investigated in *E. coli* and *B. subtilis* using various concentrations of Cu–La–CuO NPs (25, 50, 75, and 100 $\mu\text{g/mL}$) by employing DCFH-DA. *E. coli* and *B. subtilis* cultures (each 1 mL) were incubated in a six-well plate with the above-mentioned concentrations of Cu–La–CuO NPs at 37 $^{\circ}\text{C}$ for 5 h. Immediately, these bacterial solutions were treated with DCFH-DA (100 $\mu\text{mol/L}$) and



Scheme 2. Schematic representation for the bactericidal property of Cu–La–CuO NPs through the ROS generation.

then incubated for 30 min in dark conditions. The fluorescence intensity was measured and evaluated as shown in Figs. S6 and S7.

3.9. Mechanism for bactericidal effects

The possible mechanism for the bactericidal property was explained in using ROS assay and is presented in Scheme 2. The destruction of cellular components such as DNA, proteins, and lipids may have occurred in the bacterial cells due to ROS production. Generally, the internalization and the direct contact of initial H_2O_2 may have damaging effects on the cells owing to the major loss of cellular integrity. However, this process may occur by utilizing their metabolic products including O_2^- and HO_2^- . In general, they cannot penetrate the membrane without the nanocarriers like nanoparticles, and thereby no damage can be observed. Noteworthy, their direct contact through the nanoparticles can lead to the damaging of bacterial cells. In this context, the prepared Cu–La–CuO NPs may have facilitated the direct exposure of the radicals like O_2^- and HO_2^- towards the bacterial cells and can lead to the destruction of major cellular components. From Figs. S6 and S7, we assume that these effects are substantially based on the concentrations of the Cu–La–CuO NPs.

4. Conclusions

In this study, we synthesized CuO NPs using reflux reaction and the synthesized CuO NPs show spherical and cube-like morphology, which have an average size of around 20 nm. Again, the synthesized CuO NPs were doped with a rare-earth compound (i.e., LaCl_3) to develop highly antimicrobial bimetallic NPs (La–CuO NPs). The resulting La–CuO NPs react with curcumin to produce effective antimicrobial agents (Cu–La–CuO NPs). These NPs (CuO NPs, La–CuO NPs, and Cu–La–CuO NPs) were well-characterized by employing TEM, SEM, XRD, UV–Vis, and FT-IR techniques. The zone of inhibition was performed using these Cu–La–CuO NPs against microbes, such as *E. coli*, *B. subtilis*, and *A. niger*. The Cu–La–CuO NPs deliver a significant zone of inhibition in *E. coli* and *A. niger* with 2.2 and 2.9 mm, respectively. The UV–Vis absorption and FT-IR studies were investigated to understand the detailed optical properties and functional group analysis. The LDH and live/dead assay results prove the antimicrobial effects of final Cu–La–CuO NPs. The prepared flavonoid-based NPs are highly effective as antimicrobial compounds. In the future, these

nanomaterials can be thoroughly investigated for *in vivo* and molecular level applications.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jre.2022.08.020>.

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