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Research Article

# GREEN SYNTHESIS OF COPPER OXIDE NANOPARTICLE FROM PLANT EXTRACT AND ITS ANTIBACTERIAL ACTIVITY

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**ABSTRACT**

**Objectives:** The present study focused on the synthesis of copper oxide nanoparticles (CuO NPs) using cinchona bark extract, its characterization, and studies on antibacterial activities.

**Methods:** The CuO NPs were synthesized using 1 mM copper(II) sulfate pentahydrated with 2% (m/v) aqueous bark extract of Cinchona under optimum conditions (pH=11).

**Results:** The formation of CuO NPs has been confirmed first by the color change from colorless to light yellow and then to Brownish. Using a UV-Visible spectrophotometer, the kinetics of the reaction were studied, which showed surface plasmon resonance at 382 nm. Zeta potential and particle size were calculated to be −15.2 mV and 197 nm, respectively. The antibacterial activity of CuO NPs was tested against gram-positive and gram-negative cultures, which shows desirable activity.

**Conclusion:** The outcome of the study demonstrates that cinchona bark extract serves as a reducing and stabilizing agent, transforming $Cu^{2+}$ metallic ions into CuO NPs. The green synthesis of the CuO NPs using cinchona bark extract, their characterization, and their antibacterial activity were successfully carried out, and gram-positive *(Staphylococcus aureus) bacteria* shows more activity compared to gram-negative *(Escherichia coli)* bacteria.

**Keywords:** Copper oxide, Nanoparticles, Green synthesis, Antibacterial activity, Bark extract.

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## INTRODUCTION

Nanotechnology and nanoscience play a great role in human health improvement, food, agriculture, pharmaceuticals, and cosmetics based on their special characteristics. Nanoparticles (NPs) are particles with a size range of 1–100 nm. Extensive research has been conducted on the synthesis and utilization of NPs over the past decade. NPs have attracted a lot of interest because of their unique features and applications, which are greatly influenced by their size, shape, and structure [1]. The synthesis of NPs can be carried out using various approaches, such as physical, chemical, and biological methods. The biological method is an environmentally friendly, cost-effective, nontoxic, and safer approach for the production of NPs. Metal NPs, like silver, gold, platinum, and copper NPs, are commonly employed in different sectors to improve living standards. Pharmacologically important compounds of plants and microbial extracts are used for the reduction and stabilization of metal NPs [2,3]. Recently, different parts of plants, including stems, fruits, roots, calluses, peel, seeds, flowers, and leaves, have been used for the preparation of metal oxide NPs in several shapes and sizes using biological systems [4]. Among the NPs, gold and silver NPs were extensively studied, and reports on copper NPs were limited within the current research space. Copper oxide NPs (CuO NPs) have drawn interest due to their better electrical, conductivity, catalytic, and antimicrobial properties [2]. Several physio-chemical methods like precipitation, sonochemical, microwave radiation, electrochemical reduction, etc. were reported for CuO NPs synthesis [3,5,6]. There are several methods in practice to synthesize CuO NPs, but the use of plant extracts is a gradually evolving research area known as green synthesis of NPs. In the plant extract mediated, synthesis method, some experimental parameters, i.e., mass ratio between Copper salt and extract, nature of plant extract, temperature, and reaction time, have shown direct influence on the morphology of CuO NPs [1]. CuO NPs prepared by chemical route restrict, their applications in biological systems and also pollute the ecosystem by discharging toxic process wastes into the environment [7]. Understanding the disadvantages of physiochemical methods, researchers focused on developing an eco-friendly biological synthesis of NPs [8]. Copper is a non-toxic metal for mammals but is toxic to many microorganisms, and this offers new prospects for antimicrobial treatments [9]. Based on literature, there are several reports available on the antimicrobial properties of Copper NPs [10-13]. CuNPs have been shown to exhibit strong antibacterial activity against strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Salmonella choleraesuis* [14]. A lot of work has been done on bio reduction of metal ions to form NPs by phytochemicals and compounds found in plant extracts such as *Gum karaya* [15], *Ocimum basilicum* [16], *Momordica charantia* [17], and *Leucaena leucocephala L.* [18] In the present study, green synthesis of the CuO NPs using cinchona bark extract was aimed to explore the application of cinchona bark extract as a capping and reducing agent for the synthesized CuO NPs and evaluate the antibacterial activities of the synthesized CuO NPs against selected pathogenic organisms using the disc diffusion method [19].

## METHODS

All chemicals were of analytical grade. Copper (II) sulfate pentahydrated ($CuSO_4.5H_2O$), ferric chloride ($FeCl_3$), Sodium hydroxide (NaOH), deionized water, hydrochloric acid (HCl), sulfuric acid ($H_2SO_4$), and ethanol ($C_2H_5OH$) were from Loba Chemie Pvt. Ltd. India; Benedict's solution; and iodine solution.

### Bacterial strain

Gram-positive and Gram-negative bacteria strains were used for this experiment.

**Collection of bark**
The bark of Cinchona was collected from local areas. The plant was identified and authenticated by the Department of Botany, Dr. R.G. Bhoyar New Arts Commerce and Science College, Wardha with reference number of 20/rgbacsbotany/2022-23. Then the washed and air-dried bark of Cinchona was reduced to a fine powder, and around 100 g of powder was obtained.

**Preparation of cinchona bark extract**
The extraction was carried out by the decoction method. Afterward, it was allowed to cool down to room temperature. The mixture was first filtered using Whatman filter paper, after which the filtrate was collected and kept at 4°C for further synthesis of CuO NPs.

**Phytochemical screening**

*Test for alkaloids (Wagner's test: Iodine-potassium iodide solution)*
1.2 g of iodine and 2 mL of $H_2SO_4$ were mixed and diluted to a 100 mL solution. 10 mL of the alcoholic extract was acidified by adding 1.5% (v/v) of HCl, and then a few drops of Wagner's reagent were added. The formation of yellow or brown precipitates was assessed to confirm the presence of alkaloids.

*Test for glycosides*
Aqueous NaOH solution was diluted in 1 mL of distilled water, and it was added to the alcoholic extract after a little portion of the extract had been mixed with the distilled water. The formation of a reddish brown color was taken as an indicator for the presence of glycosides.

*Test for tannins ($FeCl_3$ test)*
1 mL of extract was stirred with 1 mL of $FeCl_3$ solution; the occurrence of a greenish-black precipitate indicated the presence of tannins.

*Test for flavonoids*
2 mL of extract was added to 2 mL of 10% (m/v) $FeCl_3$ solution, and the mixture was shaken. A woody, brownish precipitate indicated the presence of flavonoids.

*Test for phenols*
The extract was treated with 3–4 drops of $FeCl_3$ solution. It was then left to form a bluish-black color that indicates the presence of phenols.

*Test for carbohydrates (benedict test and iodine test)*
A few drops of Benedict solution were added to the plant extract, and it was checked for its formation of brick red, color which is used to confirm the presence of glucose. A few drops of iodine were added to the other extract, where a dark blue color formation confirmed the presence of starch.

**Green synthesis of CuONPs**
1 mM $CuSO_4 \cdot 5H_2O$ solution was treated with 2 mL of 2% (m/v) bark extract of cinchona and stirred magnetically at room temperature until the light blue color was changed to light yellow. Then the mixture was heated at 80°C for 30 min. Following that, it was allowed to cool down. Afterward, the mixture was treated with a 1 M NaOH solution drop by drop for a specific pH=11. The mixture was centrifuged at 10,000 rpm for 15 min and then left to dry at 100°C for 24 h. The synthesized CuO NPs were characterized using visual observation and UV-Visible spectroscopy [20].

**Characterization**

*Visual observation*
The appearance of a brown color indicates the formation of copper NPs during the bio-reduction of the copper sulfate aqueous solution using extract.

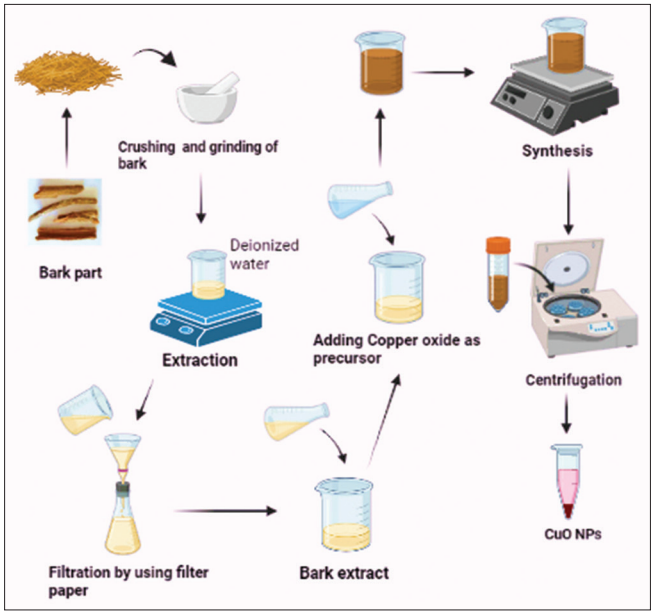


**Fig. 1: Schematic illustration of Synthesis of copper oxide nanoparticles created with Biorender.com**

*UV-Vis spectroscopy*
The reduction of copper sulfate to copper was monitored by recording UV-Vis spectra between wavelengths 200 and 700 nm. The measurements were recorded on a Perkin Elmer spectrometer lambda 25.

*pH analysis*
With the addition of NaOH, the pH of the extract, precursor, and final combination were all measured using a microprocessor pH meter (ESICO model 1010).

*Particle size and zeta potential measurement*
The particle size and zeta potential were determined by the dynamic light scattering (DLS) method using the Malvern Zetasizer instrument.

**Antibacterial activity of CuO NPs using the disc diffusion method**
The discs were soaked with double-distilled water, 2% (m/v) bark extract, 1 mM $CuSO_4 \cdot 5H_2O$ solution, and solutions containing CuO NPs of each type separately. Tetracyclin was placed at the center of the plates as a positive control. Then, the discs were air-dried in sterile conditions. The plates containing nutrient agar media were prepared by swabbing them with the microbial cultures (*S. aureus* and *E. coli*). Previously prepared discs were placed on each part of the plate. The discs were placed in the following order: Disc soaked with double-distilled water as a negative control; disc soaked with solutions of 1:2 and 1:3 containing extract-mediated CuO NPs; disc soaked with bark extract of cinchona. The plates were incubated at 37°C for 24 h. Finally, the zone of inhibition was observed and measured against each type of test microorganism [21].

**RESULTS AND DISCUSSION**

**Phytochemical test**
The results of the phytochemical analysis of the extract are shown in Fig. 2, which revealed the presence of secondary metabolites.

**Visual observation**
- The color changes arise due to the excitation of surface plasmon resonance in the metal NPs, indicating the formation of CuO NPs.
- The colorless 1 mM $CuSO_4 \cdot 5H_2O$ solution started changing its color to light yellow as soon as the bark extract of cinchona was added

to it. At the time when the NaOH solution came in contact with the solution, it had a brownish color (Fig. 3), indicating the formation of CuO NPs.

**UV-Vis analysis**

The UV-Vis spectra result revealed a strong absorbance at 382 nm, suggesting the formation of CuO NPs, while the pure bark extract of Cinchona has shown a strong absorbance at 294 nm. This result definitely agrees with the range of λmax values of the CuO NPs, 200–400 nm, at different previous works using plant extracts other than cinchona. The measurements were recorded on a PerkinElmer spectrometer lambda 25.

*Optimization of precursor concentration*

To optimize the concentration of the precursor, two steps were taken. The first stage was at a time when pH was not adjusted. Here, the peaks for the CuO NPs were all closer to the peak of the pure extract at 294 nm. According to different previously done studies on the synthesis of CuO NPs, there was a need to change the media to a basic condition by adding NaOH solution so as to adjust the pH. Hence, adding a NaOH solution with the recommended pH=11 (before optimization) was taken as the next step. As soon as it was added, a remarkable change in the position of the peaks was observed [20].

*Ratio of volume of extract: $CuSO_4$*

The synthesis of CuO NPs for 1:3 ratios of extract and copper sulfate was carried out at different pHs, namely 4, 5, 6, 7, 8, 9, 10, and 11. The time taken for color change as well as the UV-visible spectrum of the reaction mixture was monitored. A blue shift in the wavelength from 382 to 328 nm was observed with the increase in the amount of precursor salt. This shift can be explained on the basis of an increased nucleation rate due to a greater amount of $cu2^+$ ions and the generation of smaller nanoparticles in the solution. However, with a further increase in the precursor ion from 1:2 to 1:3, a red shift was observed in SPR from 328 to 294 nm. This may be due to collisions between smaller NPs, which lead to particle growth [22]. The brown color was noted for the optimum amount of precursor and extract, producing a greatest number of copper nanoparticles in aqueous medium. (1:3). This ratio was found to be ideal, as the biosynthesized NPs showed maximum absorption at 382 nm.

*Effect of temperature on biosynthesis of CuONPs*

The effect of temperature on the rate of formation of CuO NPs was studied for the 1:3 composition of the extract and $CuSO_4$ solution. The CuO NPs were formed within 30 min at 80°C. However, at room temperature and 60°C, the formation of CuO NPs occured after 1 day and 2 h, respectively, and above 80°C under boiling conditions, the solution becomes charred and no particle formation is seen. Hence, the reaction at 80°C favors the biosynthesis of CuO NPs using extract.

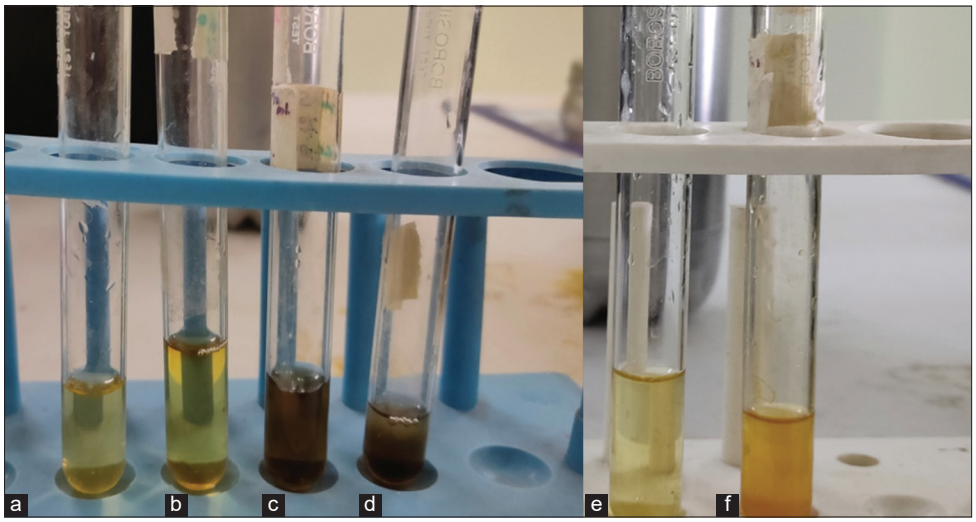


**Fig. 2: The color change observed when the bark extract of cinchona was tested the presence of (a) alkaloids, (b) glycosides, (c) Tannins, (d) phenols, (e) flavonoids, (f) carbohydrates**

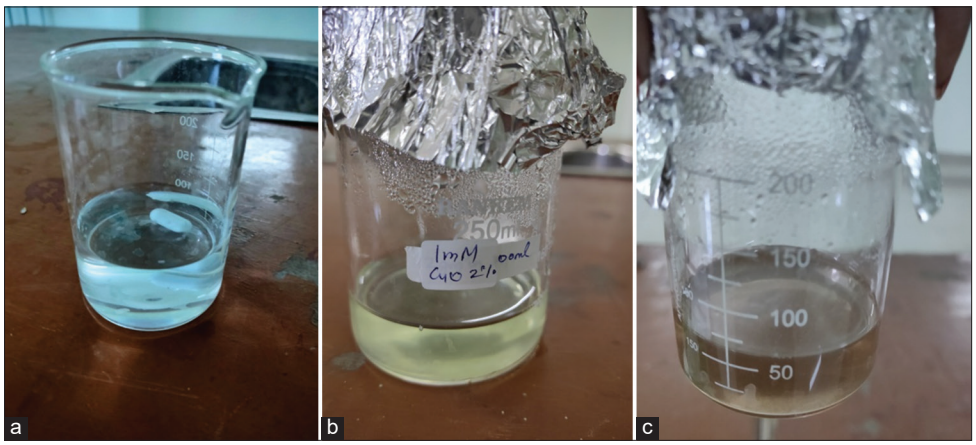


**Fig. 3: Color changes observed before and after the formation of copper oxide nanoparticles. (a) Copper(II) Sulphate pentahydrated precursor, (b) bark extract of cinchona and (c) copper oxide nanoparticles**

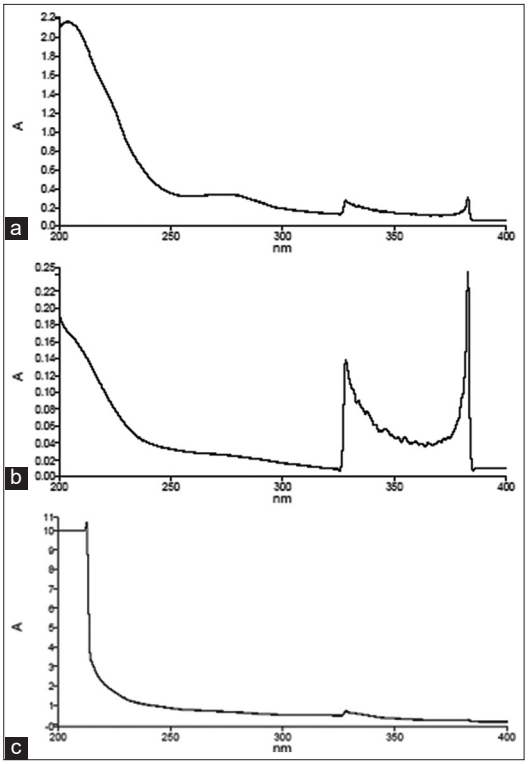


**Fig. 4: UV-visible absorption spectra of copper oxide nanoparticles synthesized by using Cinchona bark extract as shown in (a) UV-Visible spectrum of bark, (b) UV-visible spectrum of $CuSO_4$, (c) UV-visible spectrum for the formation of CuONPs under optimum conditions**

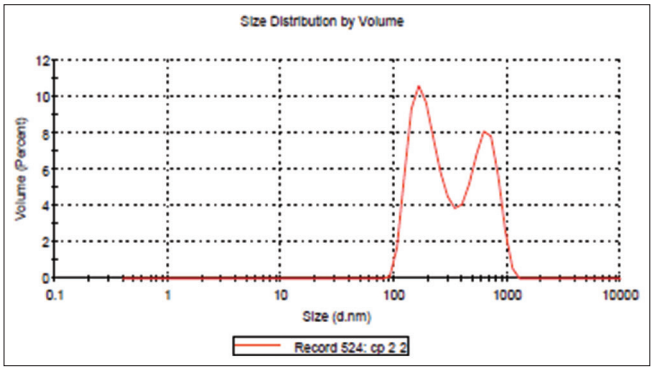


**Fig. 5: Particle size distribution of Copper(II) Sulphate pentahydrated measured by Dynamic light scattering**

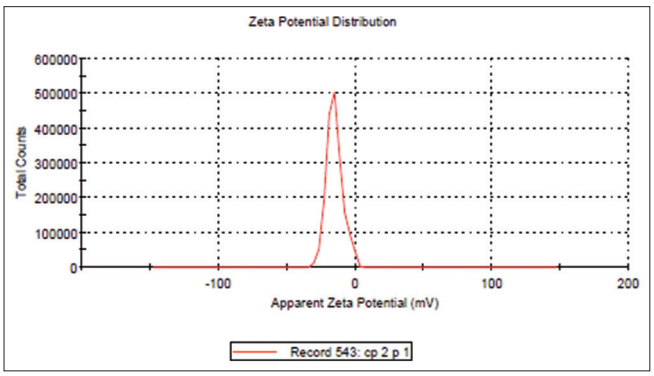


**Fig. 6: Zeta potential of precursor**

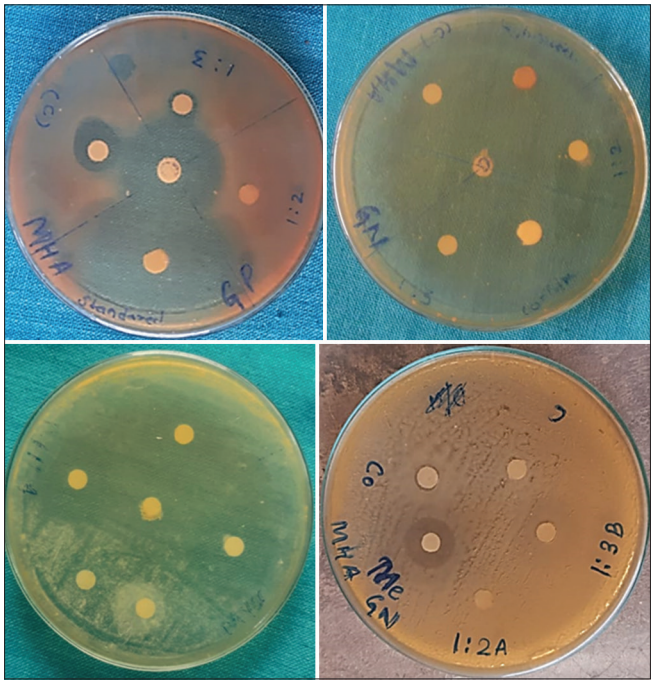

**Fig. 7: Zone of inhibition produced by copper oxide nanoparticles**

**Table 1: The result of phytochemical screening test**

| Test | Result |
|---|---|
| Alkaloids | + |
| Glycoside | – |
| Tannins | + |
| Phenols | + |
| Flavonoids | + |
| Carbohydrate | – |

*Optimization of the pH of the mixture of extract and the precursor*

The pH values of the precursor, the extract, and the NPs before pH was adjusted were 4, 5, and 5.4, respectively. The CuO NPs synthesized at different pH values (6, 7, 8, 9.5, 10, 11, and 12) were optimized based on the intensity of their peak and the red shift of their respective λmax values. The best result for the synthesis of CuO NPs was obtained at pH=11. This shows that a more basic media is very suitable for the synthesis of CuO NPs. The added NaOH solution is expected to act as a catalyst for the formation of the nanoparticle by making particles collide and connect to each other so as to form homodispersed NPs [23]. But increasing the pH above 12 might hinder the formation of the CuO NPs. The stability of the synthesized CuO NPs was assessed by comparing the UV-Vis spectrum of CuO NPs at different ages [24].

*Particle size and zeta potential measurement*

The particle size and zeta potential were determined by the DLS method. Malvern Zetasizer instrument was 197 nm and −15.2 mV, respectively.

**Antibacterial activity of CuO NPs**

The antibacterial effect of copper NPs was analyzed on the basis of the zone of inhibition. CuO NPs exhibited antibacterial activity against both gram-positive and gram-negative bacteria such as *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa* by the disc diffusion method. The synthesized CuO NPs show effects against gram positive and gram negative bacteria, respectively, with a zone of inhibition of 12 mm and 10 mm in Table 2. These results were also compared with those of standard antibiotics.

**Table 2: Antibacterial activity of CuO NPs using some human pathogenic bacteria by disc diffusion method**

| Test organism | Zone of inhibition (mm) |
|---|---|
| *Staphylococcus aureus* | 12 |
| *Escherichia coli* | 10 |

CuO NPs: Copper oxide nanoparticles

Obtained experimental results have shown that synthesized CuO NPs have antibacterial activity on both gram-positive and gram-negative bacteria.

## CONCLUSION

CuO NPs have now been developed using an easy, simple, cost-effective, and environmentally friendly process. Cinchona bark extract serves as a reducing and stabilizing agent, transforming $Cu^{2+}$ metallic ions into CuO NPs. The green synthesis of the CuO NPs using cinchona bark extract, their characterization, and their antibacterial activity were successfully carried out. The antibacterial activities of the synthesized CuO NPs against gram-negative (*E. coli*) and gram-positive (*S. aureus*) bacterial strains were evaluated by measuring the zone of inhibition, and gram-positive (*S. aureus*) bacteria showed more activity compared to gram-negative (*E. coli*) bacteria.

## AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

## CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

## AUTHORS FUNDING

No funding has been received for this research work.

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