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Biological synthesis of perga-based silver nanoparticles and their antimicrobial effects

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Abstract

Perga (bee bread) is a natural bee product that is produced by honey bees mixing with bee pollen and their secretions in honey combs. It is rich in phenolics, lipids, proteins, flavonoids, and other secondary metabolites. Due to these bioactive components, Perga has high antioxidant and antimicrobial activities. In green synthesis, these compounds are also used as electron precursors and stabilizing agents. In this study, the potential of Perga extract-derived biocomponents for the green synthesis of silver nanoparticles was evaluated. The synthesized Perga-based silver nanoparticles (SP-AgNPs) were characterized, and the antimicrobial effect of SP-AgNPs was determined according to the disk diffusion method. The obtained SP-AgNPs exhibited a maximum absorbance at 427 nm with a size range of 70-83 nm. The antimicrobial activity of SP-AgNPs was evaluated against *Escherichia coli* (*E. coli*)*, Staphylococcus aureus* (*S. aureus*)*, Bacillus subtilis* (*B. subtilis*)*,* and *Pseudomonas aeruginosa* (*P. aeruginosa*). The inhibition zones ranged from 12 to 15 mm. The results demonstrated that Perga was a good agent in green synthesis, and SP-AgNPs were a promising antimicrobial agent.

Keywords: Antibacterial, Bee bread, Bee products, Eco-friendly, Nanotechnology

INTRODUCTION

Nanotechnology is an interdisciplinary field of science concerned with the synthesis, characterization, and application of structures ranging in size from 1 to 100 nanometers across various areas, including the environment, agriculture, pharmacy, and energy, with their unique properties (Kheiriabad et al. 2024, Shahzadi et al. 2025). Nanoparticles, such as silver, gold, and zinc, which are an important part of nanomaterials, exhibit superior physical, chemical, and biological properties due to their high surface area-to-volume ratio. Silver nanoparticles (AgNPs), in particular, are used in the development of various products, such as wound dressings and disinfectants, owing to their antimicrobial activity (Deshmukh et al. 2016).

Chemical methods are commonly used in AgNP synthesis, but these methods have disadvantages in terms of energy requirements, chemical usage, environmental risks and biocompatibility (Beyene et al. 2017). For this reason, the green synthesis method, which is more environmentally friendly, cost-effective, and advantageous for biocompatibility, is becoming increasingly prominent relative to other methods. The approach that uses plants, microorganisms, fungi, algae, and various natural products for both reduction and stabilization is referred to as green synthesis (Vaseghi et al. 2018).

Plant extracts and bee products, on the other hand, reduce silver ions and help the resulting nanoparticles remain more stable thanks to the phenolic compounds, flavonoids, sugars, proteins, and other bioactive substances they contain (Bakkaloğlu 2021, Urcan et al. 2023). Silver nanoparticles obtained by green synthesis methods are widely used in biomedical applications, food safety, environmental purification, and the pharmaceutical and cosmetic fields (Dubey et al. 2023). Perga (bee bread) is one of the most widely known bee products after honey, propolis, beeswax, bee pollen, royal jelly, and bee venom (İkiz et al. 2025, Karlıdağ and Keskin 2020, Mayda et al. 2019). Bees place the pollen they collect from flowers into the honeycomb cells and mix it with honey and its secretions. As pollen undergoes lactic acid fermentation in the honeycomb cells, Perga is produced, which has higher bioavailability than pollen and possesses probiotic properties (Figure-1) (Ergün et al. 2024, Özkök et al. 2022).

Perga is rich in protein, essential amino acids, vitamins, minerals, phenolic compounds, and flavonoids (Karlıdağ and Keskin 2020, İkiz and Keskin 2024). With these components, Perga has become an important material with potential for green-synthesis of AgNPs. In this study, freshly collected Perga was extracted, and perga-based silver nanoparticles (SP-AgNPs) were obtained from the obtained extract. The antimicrobial activity of the characterized SP-AgNPs against various microorganisms was determined. The SP-AgNPs exhibited good antimicrobial activity.

Figure 1. The harvested Perga (bee bread) sample (Khalifa et al. 2020)

MATERIAL AND METHODS

Green Synthesis and Characterization of SP-AgNPs

Perga samples were collected from a local apiary (Bilecik (Koyunköy), 40° 8′ 12″ north latitude and 29° 45′ 24″ east longitude) in 2025. The collected samples were powdered and extracted with distilled water at a 1:5 (w/v) ratio for 24 h on a shaker. At the end of the period, the extract was filtered, and the obtained filtrate was used in the green synthesis of silver nanoparticles. Biological (green) synthesis of SP-AgNPs was performed as described by Keskin et al. (2022) with minor modifications. For this purpose, the Perga extract was mixed with a 5 mM $AgNO_3$ solution in a 1:1 (v/v) ratio, and the mixture was stirred at a constant, rapid rate at room temperature for nearly 2 hours. The color change to dark brown was observed, and the change was determined by UV-Vis spectroscopy. At the end of the period, the mixture was centrifuged at 9000 rpm, and the SP-AgNPs were dried at 75°C (Keskin 2022). The optical properties of obtained SP-AgNPs were evaluated using a UV-Vis spectrophotometer (Thermo Fisher). The functional groups of the extract responsible for the synthesis were determined using Fourier transform infrared spectroscopy (FTIR), and the size of the SP-AgNPs was determined by scanning electron microscopy (SEM).

Antimicrobial Effects SP-AgNPs

The antimicrobial effects of SP-AgNPs were determined using the disk diffusion test method (Cormican et al. 1996). *E. coli, S. aureus, B. subtilis,* and *P. aeruginosa* strains were cultured on Mueller-Hinton Agar (MHA) at 37°C for 24 hours. The overnight culture was diluted in 0.9% w/v saline to achieve a 0.5 McFarland standard. 100 µL of this suspension was spread onto agar plates. Sterile paper discs (Oxoid, CT09988, 6 mm diameter) were also placed on the agar, and 100 µL of the sample (20 mg/mL) was impregnated onto the discs. The inhibition diameter formed in the medium was measured in millimeters (mm) after incubation at 37 °C for 24 hours. All experiments were performed separately for each microorganism and repeated three times. Ampicillin was used as a positive control.

RESULTS

In this study, SP-AgNPs were synthesized and characterized using Perga, which is rich in secondary metabolites such as phenolic compounds, flavonoids, and terpenes, and the antimicrobial activities of the SP-AgNPs against various microorganisms were determined (Figure 2).

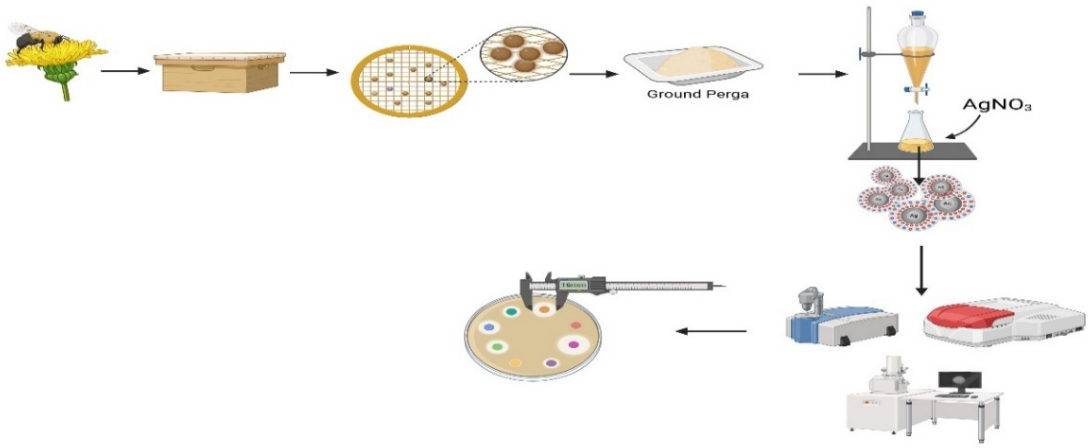


Figure 2. Synthesis scheme of Perga-based silver nanoparticles (BioRender 2026)

A UV-Vis spectrophotometer was used to determine the optical characteristics of the SP-AgNPs. The solution changed from yellow to dark brown. This indicates that the ionic silver was reduced to metallic silver. The analysis revealed that SP-AgNPs exhibited a maximum absorbance at 427 nm (Figure-3).

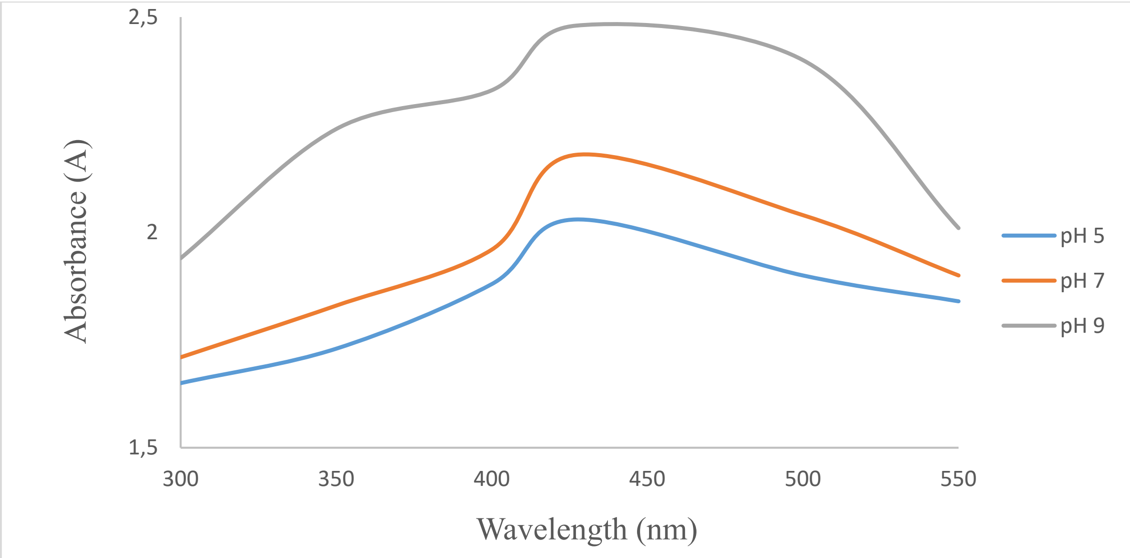


Figure 3. UV-Vis spectrum of SP-AgNPs and the effects of pH

FTIR was used to identify potential reducing and oxidizing groups involved in the synthesis of silver nanoparticles. For this purpose, FTIR analysis was performed on the Perga extract and the supernatant remaining after synthesis (Figure 4 and Figure 5). Upon examination of Figures-4 and 5, the peaks around 3200 cm⁻¹ are associated with O-H vibrations, while the peaks around 2110 cm⁻¹, 1637 cm $^{-1}$, and 1300 to 1070 cm $^{-1}$ are related to C-C, N-H, C=C, and C≡C vibrations, respectively (Mallikarjuna et al. 2011, Sharifi-Rad et al. 2024, Soto et al. 2024).

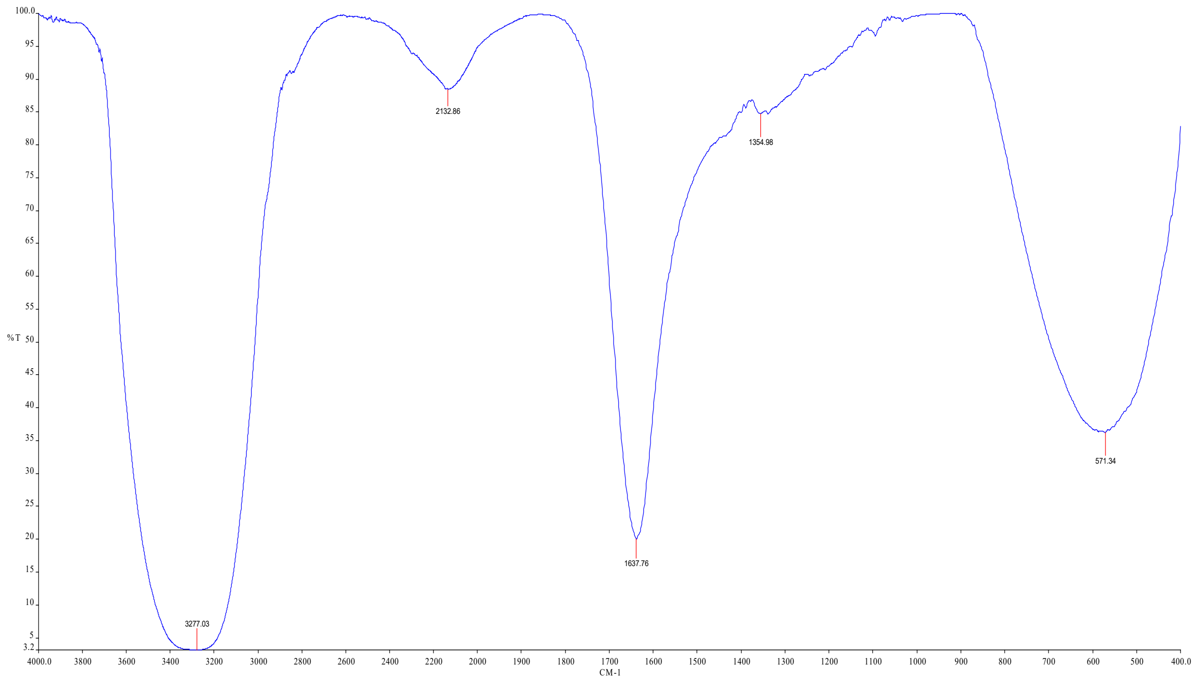


Figure 4. FTIR spectrum of Perga extract

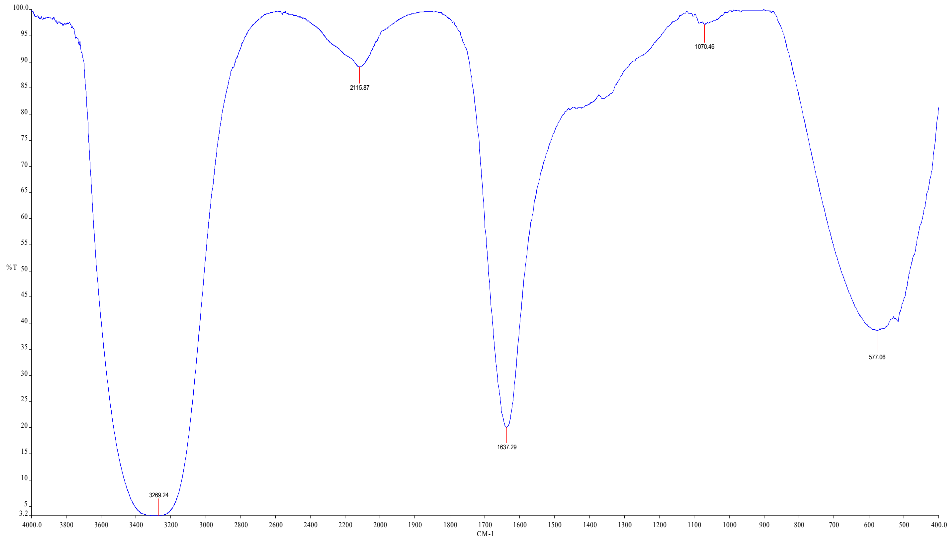


Figure 5. FTIR spectrum of AgNPs supernatant

The sizes of the SP-AgNPs ranged between 70 and 83 nm (Figure 6).

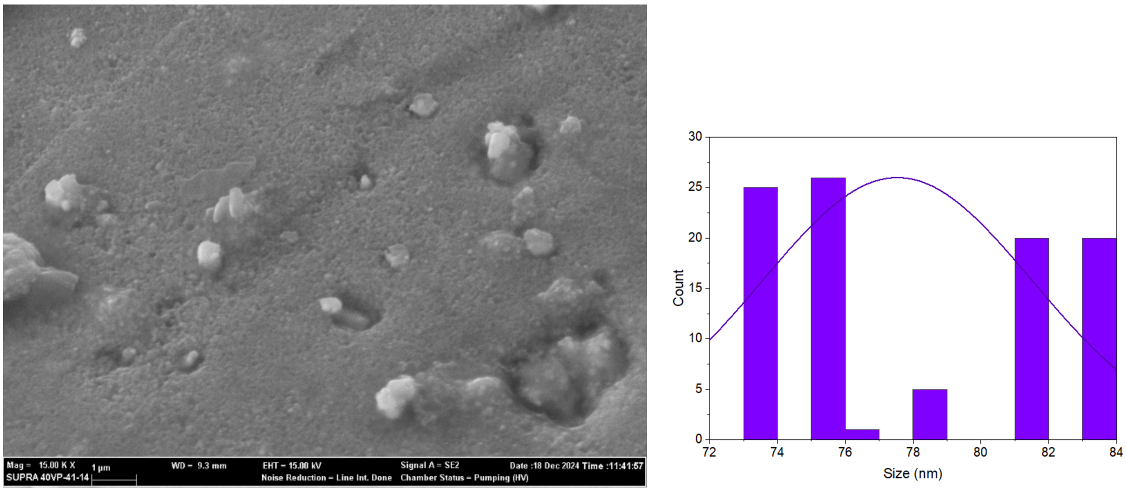
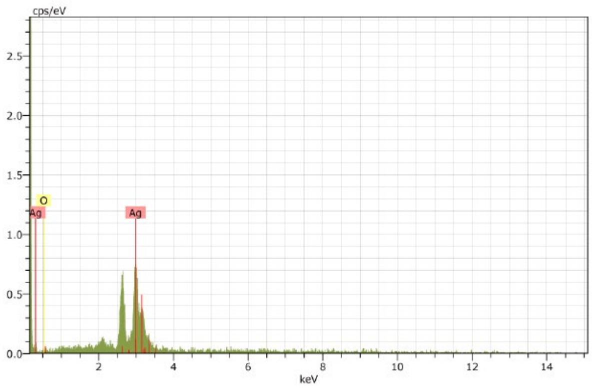


Figure 6. SEM image and histogram of SP-AgNPs

The EDX spectrum of the obtained nanoparticles showed a peak at 3 keV (Figure 7).



Element	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (1 Sigma) [wt.%]
Silver	L-series	52.87	98.21	89.03	2.10
Oxygen	K-series	0.97	1.79	10.97	1.17
	Total:	53.84	100.00	100.00	

Figure 7. EDX spectrum and elemental composition of SP-AgNPs

Silver nanoparticles exhibit broad-spectrum antimicrobial activity due to their surface properties (De Mel et al. 2025). The SP-AgNPs synthesized were found to be against *P. aeruginosa* (12±0.5 mm), *S. aureus* (13±0.5 mm), *B. subtilis* (14±0.8 mm), *E. coli* (15±1.0 mm) and Ampicillin (11±0.5 mm) (Figure 8). This indicates that the synthesized nanoparticles exhibit a moderate antimicrobial activity.

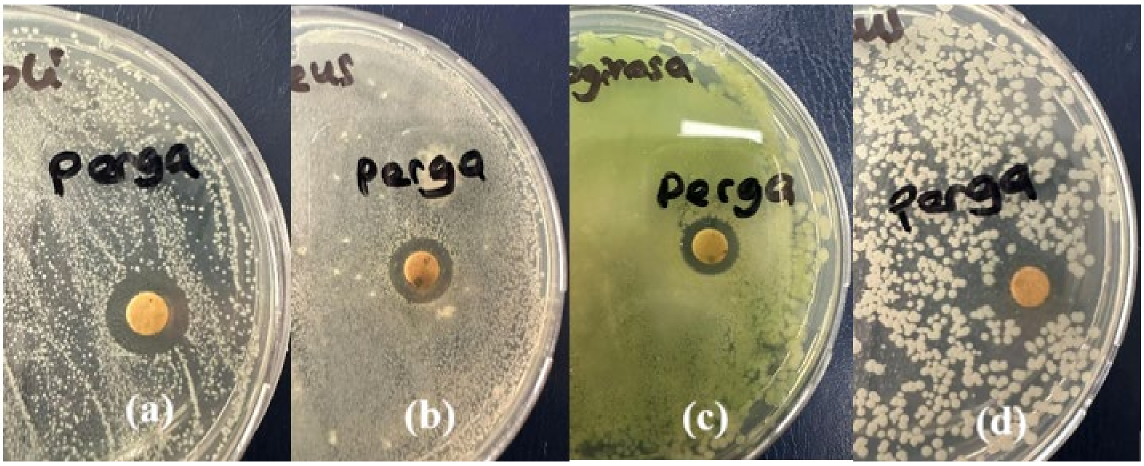


Figure 8. (a) *E. coli,* (b) *S. aureus,* (c) *P. aeruginosa*, and (d) *B. subtilis* inhibition zones

DISCUSSION

Silver nanoparticles obtained via green synthesis generally exhibit maximum absorbance at 400–500 nm (Geoprincy et al. 2013). The wavelength at which maximum absorbance is observed varies with the synthesis source and conditions. In a study by Kavin et al. (2025), silver nanoparticles derived from *Coffea arabica* husk exhibited maximum absorbance at 400 nm. In another study, silver nanoparticles derived from *Astragalus fasciculifolius* exhibited a maximum absorbance at 443 nm. The maximum absorbance was also reported at 430 nm (Di Fraia et al. 2025), between 200 and 300 nm (Mirbagheri et al. 2025), and at 440 nm (Sadeghi-Kiakhani et al. 2025). pH is one of the most important factors affecting nanoparticle synthesis. Secondary metabolites, such as phenolic compounds, flavonoids, and terpenes, present in plants or bee products used as electron sources, exhibit different ionization properties across pH values. At high pH values, the π-electrons in the carbonyl/carboxyl groups of phenolic compounds and flavonoid rings, in particular, facilitate the conversion of Ag^+ ions into Ag NPs through redox (oxidation-reduction) reactions (Lopes and Courrol 2018).

In this study, the maximum absorbance was observed at pH 9 (Figure 3). A review of the literature indicates that optimal synthesis was achieved at different pH values, including 10 (Magdy et al. 2025), 8 (Di Fraia et al. 2025), and 9 (Keskin et al. 2025). The sizes and shapes of AgNPs synthesized using the green synthesis technique vary in different ranges. A study by Madivoli et al. (2020) reported that the nanoparticles ranged in size from 35 to 65 nm. In a study by Singh et al. (2025), the synthesized nanoparticles ranged in size from 4 nm to 14.81 nm. Another study reported an average particle size of 40 nm (De Mel et al. 2025).

In a study by Varadavenkatesan et al. (2025), the synthesized nanoparticles were reported to be less than 100 nm. Nanoparticle sizes vary widely, as they are influenced by the source used and the synthesis conditions (Shahzadi et al. 2025). The 3 keV peak is a characteristic feature of silver nanoparticles. It was observed that the nanoparticles obtained in this study exhibited a peak at 3 keV, consistent with the literature (Pektaş et al. 2024).

A study performed by Singh et al. (2025) reported that the synthesized nanoparticles exhibited good antimicrobial activity against *C. albicans, Lactobacillus, S. aureus*, and *S. mutans*. A study by Das et al. (2025) indicated that the synthesized nanoparticles exhibited antimicrobial activity against S. aureus, with increasing inhibition zones at higher concentrations. A study by Chabuck et al. (2025) reported good antimicrobial activity against *Klebsiella, E. coli,* and *S. mutans*. It was stated that one of the main reasons for silver nanoparticles' good antimicrobial activity was their ability to bind to cells through surface properties and disrupt cell membrane integrity. A review of the literature indicates that silver nanoparticles exhibit good antimicrobial activity against various microorganisms generally (Aliero et al. 2025, Hinis et al. 2025, Moges and Misskire 2025).

Conclusion

The production of silver nanoparticles used in nanotechnology applications via green synthesis techniques is highly significant both environmentally and for human health. In this study, the potential of Perga, a highly bioactive compound, for the synthesis of silver nanoparticles was evaluated, and the nanoparticles' antimicrobial activities were assessed. It was found that the synthesized nanoparticles were below 100 nm in size and exhibited moderate activity against the infection-causing microorganisms *E. coli, S. aureus, P. aeruginosa, and B. subtilis*. Future studies will focus on determining Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC), and on developing new products for wound dressings, disinfectants, or solutions containing SP-AgNPs.

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AI declaration: AI was not used in the article.

Author contributions: Merve Keskin: Writing–review & editing, Methodology, Data curation, Conceptualization, Formal analysis. Adem Kaya: Writing–original draft, Formal analysis. Both authors read and approved the final manuscript.

Conflict of interest: The authors declare no conflict of interest.

Data availability status: All data used in this study are available within the article. Requests for additional data can be directed to the corresponding author via email.

Ethical statement: Not applicable

Financial source: No financial source

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