JPPIPA 11(4) (2025)

Jurnal Penelitian Pendidikan IPA

http://jppipa.unram.ac.id/index.php/jppipa/index

Antibacterial Activity of Indonesian Medicinal Plant Extracts *Tinospora crispa, Averrhoa bilimbi* and *Syzygium polyanthum* against *Shigella sonnei*

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Received: October 09, 2024
Revised: January 11, 2025
Accepted: April 25, 2025
Published: April 30, 2025

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DOI: 10.29303/jppipa.v11i4.9378

Abstract: Diarrhea and dysentery are major health problems in Indonesia, with *Shigella sonnei* being one of the primary causative agents. Excessive use of antibiotics has led to bacterial resistance, necessitating safe and effective alternative treatments. This study evaluates the antibacterial potential of extracts from brotowali (*Tinospora crispa*), wuluh starfruit (*Averrhoa bilimbi*), and bay leaf (*Syzygium polyanthum*) against *Shigella sonnei*. Plant materials were sourced from Kakap Village, West Kalimantan, and extracted using maceration with 96% ethanol. Phytochemical tests were conducted to identify active compounds in the extracts, while antibacterial activity was assessed using the disk diffusion method at extract concentrations of 20%, 40%, 60%, and 80%. All three plant extracts contained active compounds such as alkaloids, flavonoids, phenolics, and tannins. Antibacterial tests showed that all extracts exhibited antibacterial activity against *S. sonnei*, with *bay leaf* extract at 80% concentration demonstrating the largest inhibition zone of 20.3 mm. Statistical analysis indicated that increasing extract concentration enhanced antibacterial effectiveness. This study suggests that extracts of brotowali, wuluh starfruit, and bay leaf have the potential to be effective natural antibacterial agents against *S. sonnei*. Further research should focus on understanding the mechanisms of action and exploring the potential clinical applications of these plant extracts. Additionally, integrating these findings into science education can enhance students' scientific literacy and provide practical examples of using natural resources in addressing health issues.

Keywords: Antibacterial; *Shigella sonnei; Tinospora crispa; Averrhoa bilimbi; Syzygium polyanthum*

Introduction

Diarrhea is still a health problem in Indonesia, with a high morbidity rate. According to the 2023 Indonesian Health Survey, the prevalence of diarrhea was recorded at 2% across all age groups, 4.9% among toddlers, and 3.9% among infants. Meanwhile, data from the 2018 Sample Registration System indicated that diarrhea remained one of the leading causes of death, contributing to 7% of neonatal deaths and 6% of deaths among 28-day-old infants (Kemenkes RI, 2023). Diarrhea is the second leading cause of death for children under five years old worldwide, with the majority of cases occurring in developing countries (Adria et al., 2023; Djikoloum et al., 2024). Dysentery is a more severe type of diarrhea that is accompanied by blood or mucus in the stool.

Shigella sonnei is one of the bacteria that cause dysentery, is one of the most frequent pathogenic agents causing diarrhea and dysentery in countries with poor

How to Cite:
Yeni, L. F., Nurfatehah, Erma, & Sari, E. E. Antibacterial Activity of Indonesian Medicinal Plant Extracts Tinospora crispa, Averrhoa bilimbi and Syzygium polyanthum against Shigella sonnei. *Jurnal Penelitian Pendidikan IPA*, *11*(4), 355-363. https://doi.org/10.29303/jppipa.v11i4.9378

sanitation and contaminated water (Desalegn et al., 2024; Ohlsen et al., 2024). The bacteria are transmitted through contaminated food and water, as well as direct contact with patients (Berihu et al., 2024; Conti et al., 2024; López-Jiménez et al., 2024). The high prevalence of diarrhea and dysentery in Indonesia, especially those caused by *S. sonnei*, poses a threat to public health.

Although antibiotics have been widely used to treat *S. sonnei* infections, inappropriate and excessive use has led to increasing bacterial resistance to antibiotics. This condition results in a decrease in the effectiveness of standard treatment and increases the risk of complications and death (Asad et al., 2024; Moges et al., 2024). Therefore, effective, and safe alternative treatments are needed, one of which is using medicinal plants that can function as natural antimicrobials.

Indonesia has abundant biodiversity, including yard plants that have potential as antimicrobial agents. Three of them are brotowali (*Tinospora crispa*), wuluh starfruit (*Averrhoa bilimbi*), and bay leaf (*Syzygium polyanthum*). These plants are known to contain active compounds that have the potential to inhibit bacterial growth. Brotowali is known to contain alkaloids, flavonoids, terpenoids and saponins that have antimicrobial and anti-inflammatory activities (Haque et al., 2023; Harahap et al., 2024). Wuluh starfruit leaves contain compounds such as flavonoids, saponins, triterpenoids, sitosterols, phenolic compounds and tannins that have antibacterial properties (Fitria et al., 2023; Sakib et al., 2024; Widiastuti et al., 2024). Meanwhile, bay leaf contains essential oils, flavonoids, alkaloids, phenol compounds, terpenoids, saponins and tannins which are also antimicrobial (Aditya et al., 2023; Pratama et al., 2023).

Several studies have shown the effectiveness of this plant extract against various types of bacteria. Brotowali extract has potential as an antibacterial agent especially against Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus cereus* (Pham & Nguyen, 2020). Wuluh starfruit has potential as an effective natural antibacterial agent especially against multi-drug resistant (MDR) bacteria, such as *Mycobacterium tuberculosis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* (MRSA), and *Pseudomonas aeruginosa* (Prastiyanto et al., 2020). Bay leaf have potential as a natural antibacterial agent effective against several Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus* (including MRSA), *Streptococcus pyogenes*, *Klebsiella pneumoniae*, and *Salmonella typhimurium* (Abd Wahab & Aqilah Ja'afar, 2021; Afifah et al., 2021; Rochmat et al., 2022).

Research on the inhibitory power of extracts of the three plants against *S. sonnei* is still limited. Therefore, this study aims to test the inhibition of brotowali, wuluh starfruit leaf, and bay leaf extracts against *S. sonnei*. It is hoped that this research can find effective and safe antimicrobial alternatives from nature, which can be used to overcome *S. sonnei* infections and support the development of sustainable and affordable traditional medicine for the community.

This research has significant implications in the context of science education because the findings on the antibacterial activity of Indonesian medicinal plant extracts against *Shigella sonnei* can be integrated into science learning through the development of STEM-based learning materials. This approach not only enriches students' understanding of biological and chemical concepts but also enhances scientific literacy by providing practical insights into the use of natural resources in addressing health issues such as antibiotic resistance.

Method

The raw materials for the extract are brotowali stems (Tinospora *crispa*), wuluh starfruit (*Averrhoa bilimbi*) and bay leaf (*Szygium polianthum*) from Kakap Village, Kubu Raya Regency, West Kalimantan. This research was conducted in July 2022 at the Research Laboratory and Chemical Biotechnology of Tanjungpura University and at the Biology Laboratory of FKIP Untan. The research process includes several stages, namely sample preparation, sample extraction, phytochemical analysis, and inhibition testing using extracts from brotowali stem rhizomes, wuluh starfruit and bay leaf.

Sample Preparation

Brotowali stems, bay leaf, and wuluh starfruit leaves have been collected, washed thoroughly with running water and dried in the shade until completely dry. The dried samples were ground into fine powder using a blender. A total of 100 grams of powder of each sample was put into a glass container and 1000 ml of 70% ethanol was added. The maceration process was carried out with periodic stirring for 24 hours. Filtering was done using whatman paper number 1 to separate the extract from the residue. The maceration process was repeated by adding 500 ml of 70% ethanol for 24 hours. The filtrate was combined and evaporated using a rotary evaporator until a thick extract was obtained (Harborne, 1996).

Phytochemical Test

To test phytochemical compounds, by adding a few drops of Mayer's reagent into 2 ml of extract and observed whether a white precipitate is formed indicating the presence of alkaloids. Next, add 2 ml of the extract into a test tube and a few drops of 10% NaOH; a bright yellow color that fades after the addition of HCl indicates the presence of flavonoids. To detect saponins, shake 2 ml of the extract for 15 minutes and observe the formation of a stable foam that persists for more than 30 seconds. Add a few drops of 1% $FeCl_3$ solution to 2 ml of the extract to test for tannins; a blue-black or green color indicates their presence. For terpenoids, add 2 ml of extract, 2 ml of chloroform, and 3 ml of concentrated H_2SO_4 in a test tube; a red-brown colored layer indicates the presence of terpenoids. Finally, add a few drops of acetic anhydride and concentrated H_2SO_4 to 2 ml of the extract, and a green color will indicate the presence of steroids.

Antibacterial Test

Mueller-Hinton agar (MHA) medium was prepared and sterilized in an autoclave. *S. sonnei* isolate collected from the Health Laboratory of the West Kalimantan Provincial Health Office was cultured on an inclined medium and incubated for 24 hours at 37°C. Bacterial suspension was prepared by adjusting the turbidity according to 0.5 McFarland standard (approximately 1.5 x 10^8 CFU/ml) (Balouiri et al., 2016). Inhibition was measured using the disc diffusion method. MHA media was poured into sterile petri dishes and allowed to solidify. Suspensions of *S. sonnei* bacteria have been spread evenly on the surface of the media using a sterile swab. Sterile paper discs that had been impregnated with 20 µl of extracts (concentrations of 20%, 40%, 60%, and 80%) were placed on the surface of the media. Each treatment was replicated five times to ensure validity of the results. DMSO was used as a negative control and tetracycline 20% as a positive control. Petri dishes were incubated at 37°C for 24 hours.

The diameter of the inhibition zone around the paper discs was measured using a caliper or ruler. The measurement results were recorded and compared with the positive control. Interpretation of the inhibition strength category based on the size of the inhibition zone formed: weak (< 10 mm); moderate (10-15 mm); strong (15-20 mm) and very strong (> 20 mm) (Balouiri et al., 2016; Bauer et al., 1966; Khante, 2010).

Data Analysis

Inhibition zone measurement data were statistically analyzed to determine the effectiveness of the extract against *Shigella sonnei*. Analysis of variance (ANOVA) was used to compare the zone of inhibition between various concentrations of extracts and controls.

Result and Discussion

This study aims to answer questions regarding the effectiveness of brotowali stem (*T. crispa*), wuluh starfruit (*A. bilimbi*), and bay leaf (*S. polianthum*) extracts in inhibiting the growth of *S. sonnei* bacteria. The results showed that all tested extracts had antibacterial activity against *S. sonnei*.

Phytochemical Test Result

The ethanol extracts of the three plants were tested for phytochemical content at the Chemistry Laboratory of the Faculty of Mathematics and Natural Sciences, Tanjungpura University. The results of the phytochemical test of the three extracts showed variations in the content of secondary metabolites (Table 1).

Table 1. Phytochemical test results of brotowali stem extract (Tc), wuluh starfruit leaf (Ab), and bay leaf (Sp)

Secondary metabolites	Tc	Ab	Sp
Flavonoid	++	+	+++
Tannin	++	+	++
Fenolik	++	+	++
Saponin	-	-	+
Terpenoid	-	-	+
Alkaloid	++	+++	-
Steroid	-	++	-

Description:
(-) : Does not contain
(+) : Low levels
(++) : Sufficient levels
(+++) : High level
Tc: *Tinospora crispa*; Ab: *Averrhoa bilimbi*; Sp: *Syzygium polyanthum*

Phytochemical test results show that bay leaf extract contains flavonoids (+++), tannins (++), phenolics (++), saponins (+), and terpenoids (+). Wuluh starfruit leaf extract contains alkaloids (+++), flavonoids (+), steroids (++), tannins (+), and phenolics (+). Brotowali stem extract contains alkaloids (++), flavonoids (++), phenolics (++), and tannins (+).

Antibacterial Test Results

This study consisted of six treatments, including positive control (tetracycline 20%), negative control (DMSO), and four extract concentrations (20%, 40%, 60%, and 80%). Tests were conducted using the paper disc method to measure the zone of inhibition of *S. sonnei* bacteria (Table, 2, 3, 4, figure 1 and figure 2).

Table 2. Antibacterial activity of brotowali stem extract (*T. crispa*) against *S. sonnei*

Treatment	Mean Zone Inhibition (mm)	Category
Control – (DMSO)	0.00 ± 0.00a	NI
20% extract concentration	15.2 ± 0.75b	Medium
40% extract concentration	15.7 ± 0.90b	Medium
60% extract concentration	19.2 ± 1.03c	Powerfull
80% extract concentration	20.6 ± 1.08c	Very powerfull
Control + (Tetrasiklin 20%)	26.6 ± 1.4d	Very powerfull

Description: the numbers above are the mean values of the five replicates ± standard deviation. Lower case letters indicate significant differences from each treatment at the 95% level; NI: No Inhibition

Table 3. Antibacterial activity of wuluh starfruit leaf extract (*Averrhoa bilimbi*) against *S. sonnei*

Treatment	Mean Zone Inhibition (mm	Category
Kontrol – (DMSO)	0.00 ± 0.00a	NI
20% extract concentration	16,6±1.29b	Powerfull
40% extract concentration	17,8±1.04b	Powerfull
60% extract concentration	18,9±0.96b	Powerfull
80% extract concentration	19,9±1.19b	Powerfull
Control + (Tetrasiklin 20%)	29,1± 1.29c	Very powerfull

Description: the numbers above are the mean values of the five replicates ± standard deviation. Lower case letters indicate significant differences from each treatment at the 95% level; NI: No Inhibition

Table 4. Antibacterial activity of bay leaf extract (*Syzygium polyanthum*) against *S. sonnei* bacteria

Treatment	Mean Zone Inhibition (mm	Category
Kontrol – (DMSO)	0.00 ± 0.00a	NI
20% extract concentration	17,8 ±1,61b	Powerfull
40% extract concentration	18,3 ±1,61b	Powerfull
60% extract concentration	19,8 ±1,92b	Powerfull
80% extract concentration	20,3 ±1,44b	Very powerfull
Control + (Tetrasiklin 20%)	29,1 ±1,67c	Very powerfull

Description: the numbers above are the mean values of the five replicates ± standard deviation. Lower case letters indicate significant differences from each treatment at the 95% level; NI: No Inhibition

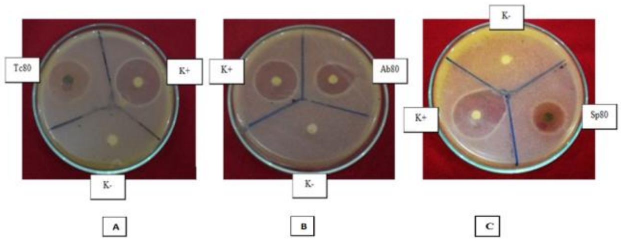

Figure 1. Zone of inhibition at 80% concentration of each extract compared to positive control and negative control. A. Tc80: 80% concentration of *T. crispa* extract. B. Ab80: 80% concentration of *A. blimbi* extract. C. Sp80: *S. polyanthum* extract 80% concentration; K+: Positive control (tetracycline 20%); K-: Negative control (DMSO)

The largest zone of inhibition of brotowali stem extract was found at 80% concentration with a diameter of 20.6 mm categorized as very strong. The 60% concentration had an inhibition zone of 19.2 mm (strong), 40% concentration of 15.7 mm (medium), and 20% concentration of 15.2 mm (medium) (Table 2). The zone of inhibition of wuluh starfruit leaf extract at 80% concentration was 19.9 mm, which was also categorized as very strong. The 60% concentration had an inhibition zone of 18.9 mm (strong), the 40% concentration was 17.8 mm (strong), and the 20% concentration was 16.6 mm (strong) (Table 3). The largest inhibition zone of bay leaf extract was found at 80% concentration with an average diameter of 20.3 mm, 60% concentration had an inhibition zone of 19.8 mm (strong), 40% concentration of 18.3 mm (strong), and 20% concentration of 17.8 mm (strong) (Table 4). These results indicate that the higher the concentration of the extract, the greater the inhibition (Figure 2). Statistical analysis of One-way ANOVA test showed that there were significant differences between treatments (p value <0.05). Duncan's further test showed that the higher concentration of the extract had a significant difference compared to the lower concentration and negative control.

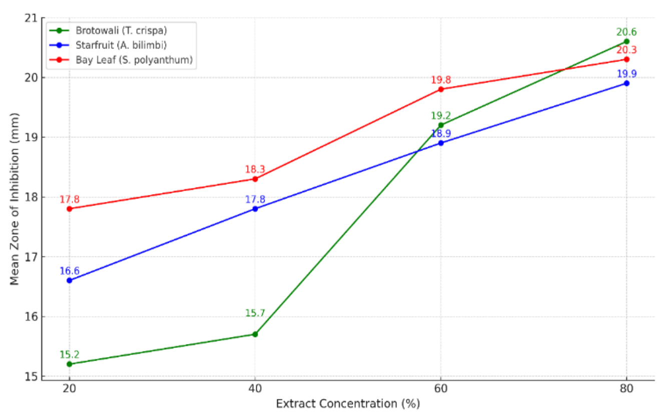

Figure 2. The concentration of the extract and the mean zone of inhabation (MZI)

This finding confirms that the higher the concentration of the extract, the greater the inhibition against bacterial growth. The inhibitory activity of brotowali extract is attributed to its phytochemical constituents, such as alkaloids, flavonoids, and diterpenoids, which have been identified in various previous studies (Pham & Nguyen, 2020). For instance, the alkaloid fraction of *T. crispa* is known to exhibit effective antibacterial activity against Gram-positive bacteria, such as *Staphylococcus aureus* and *Bacillus cereus* (Wahyuningrum et al., 2018). Additionally, other studies have demonstrated that the ethanolic extract of *T. crispa* is capable of inhibiting the growth of *Helicobacter pylori*, a bacterium that also exhibits high resistance to many conventional antibiotics (Pham & Nguyen, 2020). This activity is thought to result from the complex interaction between the phytochemical components in the extract and the bacterial cell wall, affecting cell permeability and causing structural damage.

The data showed that the higher the concentration of wuluh starfruit leaves extract, the larger the inhibition zone produced. This trend suggests that the phytochemical content in the extract works more effectively at higher concentrations in inhibiting the growth of *S. sonnei*. This study is in line with previous literature which shows that star fruit leaf extract has phytochemical components that contribute to antibacterial activity, including *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Shigella dysenteriae*, *Shigella boydii*, *Pseudomonas aeruginosa* and *Vibrio cholerae* (Fitria et al., 2023; Sakib et al., 2024; Widiastuti et al., 2024). The phytochemical content in bay leaf extract, namely flavonoids, tannins, phenolics, saponins, and terpenoids, collectively contributes significantly to antibacterial activity, including *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Vibrio cholerae* and *Vibrio parahaemolyticus* (Aditya et al., 2023; Harahap et al., 2024; Pratama et al., 2023; Ramli et al., 2023). The formation of a clear zone around the disc containing the extract indicates the death or inhibition of bacterial cell growth around the application area of the phytochemical compound on agar media. This clear zone is an indicator of the effectiveness of the antibacterial compound in inhibiting bacterial growth, with a larger zone size indicating stronger antibacterial activity.

The combination of various inhibitory mechanisms by alkaloids, flavonoids, tannins, phenolics, saponins, terpenoids, and steroids make plant extracts effective antibacterial agents. Each of these classes of phytochemicals works through their own specific mechanisms, leading to the disruption of various bacterial cellular processes, from membrane disruption to inhibition of DNA and protein synthesis (Radulović et al., 2013; Yixi et al., 2015).

Alkaloids are known to have various antibacterial mechanisms, including intercalation with DNA, which interferes with DNA replication and bacterial protein synthesis. Some alkaloids, such as berberine, are able to interact directly with DNA through the intercalation process, causing disruption of the normal function of DNA and inhibiting bacterial growth (Haque et al., 2023; Yan et al., 2021). Flavonoids exhibit antibacterial activity through several mechanisms. One of the main mechanisms is the disruption of bacterial cell membranes, including the outer and cytoplasmic membranes, which disrupts the transportation of nutrients and metabolites and inhibits the supply of energy to bacteria (favonoids). Flavonoids can also inhibit peptidoglycan synthesis and β-lactamase enzyme activity in antibiotic-resistant bacteria (Yan et al., 2024; Yixi et al., 2015; Yuan et al., 2021). Tannin works by disrupting the bacterial cell membrane and inactivating important enzymes or proteins. Tannins can interact with bacterial cell membranes, cause disruption of membrane structure and function, and inhibit protein synthesis through binding to enzymes that are essential for bacteria (Farha et al., 2020; Hossain et al., 2021; Javed et al., 2020).

Phenolic compounds, such as chlorogenic acid, can disrupt bacterial cell membranes, increasing permeability and causing leakage of intracellular components. They can also inhibit key enzymes in bacterial metabolism and DNA/RNA synthesis, resulting in inhibition of bacterial growth (Radulović et al., 2013). Saponins are known for their ability to interact with sterols in bacterial cell membranes, causing pore formation and loss of membrane integrity. This results in increased membrane permeability, leading to leakage of ions and other small molecules from the bacterial cell, as well as disruption of electrochemical gradients (Jubair et al., 2021; Nguyen et al., 2020).

Terpenoids, such as thymol, interact with bacterial cytoplasmic membranes, insert themselves between phospholipid acyl chains, increase membrane fluidity, and cause disruption of membrane function. Terpenoids can also affect ATP synthesis and citrate metabolism in bacteria, resulting in inhibition of bacterial growth (Ergüden, 2021; Wang et al., 2019).

Steroids such as steroid glycosides function by inhibiting bacterial protein synthesis and damaging cell membrane integrity. They can cause coagulation of cytoplasmic components and disruption of bacterial intercellular communication, such as the quorum sensing system, which is important for virulence regulation and biofilm formation (Dogan et al., 2017).

The results of this study are in line with the literature showing that flavonoids, tannins, phenolics, and alkaloids have antibacterial activity. The advantage of this study lies in the use of three types of local plants that have not been widely studied before to inhibit *S. sonnei*, which is a bacterium that causes diarrhea. This study provides new information about the antibacterial potential of Indonesian local plant extracts, which can be further developed as an alternative to natural medicine.

The significance of this study shows that bay leaf, wuluh starfruit, and brotowali stem extracts have the potential to be an effective natural antibacterial source against *S. sonnei*. Potential applications of the results of this study include the development of herbal medicines or health supplements for the prevention and treatment of bacterial infections.

This research has significant implications in the context of science education, particularly in the development of relevant and applicable learning materials. The findings on the antibacterial activity of Indonesian medicinal plant extracts against *Shigella sonnei* can be integrated into science education through the development of student worksheets (LKPD) or thematic modules focusing on bacterial resistance and phytochemistry. For instance, teachers can design STEM-based practical activities that involve students researching and observing how extracts from plants such as brotowali, wuluh starfruit, and bay leaf can inhibit bacterial growth. Through these activities, students not only grasp fundamental concepts in biology and chemistry but also gain insights into the importance of Indonesia's biodiversity and its applications in developing natural remedies. Additionally, discussions on the mechanisms of phytochemical action can enhance students' scientific literacy by providing a deeper understanding of how natural compounds can be utilized to address health issues like antibiotic resistance. Thus, this research not only contributes to scientific knowledge but also offers educational value that can improve the quality of science education in schools.

Conclusion

This study successfully showed that brotowali stem extract, wuluh starfruit leaves, and bay leaves have significant antibacterial activity against *S. sonnei* bacteria. Bay leaf extract at 80% concentration showed the largest inhibition zone, followed by wuluh starfruit and brotowali extracts. Increasing the concentration of the extracts was shown to increase the antibacterial effectiveness. The results of this study support the use of the three plants as an alternative natural treatment for diarrhea, with the potential to be developed into a safe and effective herbal medicine. Further research is needed to further explore the mechanism of action and potential clinical applications of these plant extracts.

This study achieved its main objective by showing that bay leaf, wuluh starfruit and brotowali stem extracts have significant antibacterial activity against *S. sonnei*. However, there are some results that require further research, such as variations in effectiveness between concentrations and types of extracts. Further research is needed to understand the mechanism of action and potential clinical applications of these plant extracts. Additionally, integrating these findings into science education can enhance students' scientific literacy and provide practical examples of using natural resources in addressing health issues.

Author Contributions

All authors have made a real contribution to completing this manuscript.

Funding

This research did not receive external funding from any party.

Conflicts of Interest

The authors declare that there is no conflict of interest in this study.

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