Marine Sponge *Petrosia* sp. from Maratua Island: A Potential Source of Antibiofilm Agents Against Pathogenic Biofilms

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ABSTRACT

Microbial biofilm-associated infections, particularly those caused by *Pseudomonas aeruginosa* and *Candida albicans*, have emerged as global challenges due to resistance to conventional antimicrobials. This study investigated the antibiofilm activity of ethanol extract from the sponge *Petrosia* sp., collected from the waters of Maratua Island, against both pathogens. Extraction was performed using 96% ethanol maceration, followed by in vitro biofilm inhibition and eradication assays using the broth microdilution method in microtiter plates. Biofilm density was quantified through crystal violet staining and absorbance measurement (OD_{595} nm). The results demonstrated that the extract inhibited both intermediate-stage (24 hours) and mature-stage (48 hours) biofilm formation, with an $MBIC_{50}$ of 0.125% for both microorganisms. At a concentration of 1%, the inhibition percentages reached 73.86% for *P. aeruginosa* and 84.70% for *C. albicans*. The highest mature biofilm eradication activity was observed at 1% concentration, with $MBEC_{50}$ values of 51.98% for *P. aeruginosa* and 80.53% for *C. albicans*, approaching the effectiveness of positive controls (nystatin 82.04% and chloramphenicol 61.72%). The extract exhibited a dose-dependent pattern, with greater efficacy against *C. albicans*, possibly due to differences in biofilm matrix composition. These findings confirm the potential of *Petrosia* sp. as a source of multitarget antibiofilm compounds, particularly for fungal infections, and underscore the significance of Indonesia's marine biodiversity as a basis for innovative therapeutic development.

Keywords: biofilm, *Candida albicans*, Maratua Island, *Petrosia* sp., *Pseudomonas aeruginosa*

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INTRODUCTION

Microbial biofilm-associated infections represent a significant challenge in global healthcare due to resistance to conventional antimicrobials. Biofilms, as microbial communities embedded in a polymeric matrix, exhibit up to 1,000-fold greater resistance to antibiotics and antifungals compared to their planktonic counterparts (Zhang *et al.*, 2020; Di Domenico *et al.*, 2022). This defense mechanism not only prolongs treatment duration but also increases the risk of complications, particularly in immunocompromised patients, with *Pseudomonas aeruginosa* and *Candida albicans* being major causative microorganisms.

P. aeruginosa and *C. albicans* are two clinically significant pathogens known for their ability to form robust biofilms, which are associated with a

wide range of human infections. *P. aeruginosa* is a Gram-negative bacterium frequently implicated in nosocomial infections that are difficult to treat due to its capacity to develop complex biofilm structures. These biofilm architectures contribute to its pathogenicity by promoting immune evasion and therapeutic failure, ultimately resulting in chronic, persistent infections (Tuon *et al.*, 2022). Meanwhile, *C. albicans* is a pathogenic fungus capable of forming biofilms and is involved in mucosal and systemic infections, especially in individuals with compromised immune systems (Kumar and Kumar, 2024).

Natural products have re-emerged as promising candidates for antibiofilm agents due to their multitarget mechanisms of action. The exploration of bioactive compounds from marine organisms, particularly sponges, has gained growing interest because of the diverse secondary metabolites produced by their symbiotic microorganisms. *Petrosia* sp., collected from the waters of Maratua Island—part of the Coral Triangle known for its rich marine biodiversity—represents one such potential source. Marine sponges from the genus *Petrosia* have drawn attention due to their content of various secondary metabolites, including alkaloids, steroids, polyketides, terpenoids, and polyacetylenes, which position them as promising candidates for the development of novel antibiofilm compounds (Lee *et al.*, 2021).

A study by Malaka *et al.* (2021) reports that *Petrosia* sp. extract exhibits strong antioxidant activity, which may contribute to its antibiofilm properties, given that oxidative stress is known to influence biofilm formation and integrity. Furthermore, research by Fahdianto *et al.* (2024) demonstrates that a *Petrosia* sp.-based nanogel formulation displays antibacterial and antibiofilm activities against *Staphylococcus aureus* and shows potential in the treatment of diabetic ulcers.

Although several studies have explored the biological activities of *Petrosia* sp., specific investigations on its antibiofilm activity against *P. aeruginosa*and *C. albicans* , particularly those originating from the waters of Maratua, remain limited. Given the global concern over antimicrobial resistance and the critical role of biofilms in enhancing such resistance, the discovery of new antibiofilm agents from marine biodiversity, such as *Petrosia* sp., holds great promise for the development of innovative therapeutic strategies against biofilm-related infections. Therefore, this study aims to evaluate the antibiofilm activity of *Petrosia* sp. extract from Maratua Island against *C. albicans* and *P. aeruginosa*, with the goal of advancing our understanding of the bioactive mechanisms of marine sponge-derived compounds and identifying effective candidates for novel antibiofilm agents.

METHODS

Materials

The sample used in this study was the sponge *Petrosia* sp., which was collected from the marine waters of Maratua Island, East Kalimantan, Indonesia. Taxonomic identification of the sponge was conducted at the Faculty of Marine Science, Mulawarman University, Samarinda, Indonesia. The tested microorganisms were *P. aeruginosa* ATCC 27853 and *C. albicans* ATCC 10231, both of which are standard biofilm-forming strains. Additional materials used included distilled water, brain heart infusion (BHI), crystal violet, chloramphenicol, 96% ethanol, nystatin, phosphate-buffered saline

(PBS), sabouraud dextrose broth (SDB), and tryptic soy broth (TSB).

Equipment

The equipment used included analytical scales, autoclave, incubator with orbital shaker S1500, laminar airflow cabinet, micropipettes, flat-bottom 96-well polystyrene microplates, microplate reader, and spectrophotometer.

Sample Extraction

The *Petrosia* sp. sponge samples were wet-sorted to remove debris and unwanted organisms, then chopped into smaller pieces and homogenized to increase surface area and extraction efficiency (Pascayantri *et al.*, 2021). The samples were cooled on ice for 24 hours to precipitate salts within the tissues and prevent interference in further processes. Extraction was carried out using maceration with 96% ethanol as the solvent, in a sample-to-solvent ratio of 1:2 (w/v), for three consecutive 24-hour cycles at room temperature, protected from light. The mixture was then filtered using filter paper to separate the filtrate from the residue. The filtrate was concentrated using a rotary evaporator at 50°C and further evaporated using a water bath until a thick extract was obtained.

Microorganism Preparation

P. aeruginosa ATCC 27853 was cultured in TSB medium and incubated at 37°C for 72 hours. *C. albicans* ATCC 10231 was cultured in SDB medium and incubated at 37°C with shaking at 120 rpm for 24 hours. The cell density of each culture was measured using a spectrophotometer at a wavelength of 600 nm (OD_{600}) and adjusted to 0.1, equivalent to a 0.5 McFarland standard (~1.5 × 10^8 CFU/mL). The microbial suspensions were then diluted in fresh medium to achieve an OD_{600} of 0.01 for *P. aeruginosa* and 0.38 for *C. albicans* , according to experimental requirements (Hamzah *et al.*, 2019; Setyowati *et al.*, 2024).

In Vitro Inhibition Assay of Biofilm Formation (Middle and Maturation Phases)

The inhibition of biofilm formation by *C. albicans* ATCC 10231 and *P. aeruginosa* ATCC 27853 was assessed using the broth microdilution method in flat-bottom 96-well polystyrene microplates (Pratiwi and Hamzah, 2020). Microbial suspensions were prepared from overnight cultures grown in BHI medium. The cells were harvested and washed twice with sterile PBS solution, then adjusted to a turbidity equivalent to the 0.5 McFarland standard (~1 × 10^8 CFU/mL). The test extract was added into the microplate wells containing 200 µL of BHI medium, at final concentrations ranging from 0.125% to 1% (w/v). Nystatin (1%) and chloramphenicol (1%) served as positive controls for *C. albicans* and *P. aeruginosa*, respectively. The plates were incubated at 37°C for 24 hours for middle phase biofilm formation and 48 hours for maturation-phase biofilm formation. After incubation, the supernatants were discarded, and planktonic cells were removed by washing with sterile PBS. The remaining biofilms were fixed and stained with crystal violet. The staining intensity was measured using a microplate reader to determine biofilm density (Mochtar *et al.*, 2020).

In Vitro Biofilm Eradication Assay

To assess biofilm eradication ability, monospecies biofilms were first grown without exposure to antimicrobial compounds (Krislee *et al.*, 2019). Biofilms were cultivated in 96-well flat-

bottom polystyrene microplates as described in the previous method. The biofilms were formed by incubation at 37°C for 48 hours. After incubation, the supernatants were discarded, and planktonic cells were removed by washing with sterile PBS. The established biofilms were then exposed to various concentrations of the test extract, ranging from 0.125% to 1% (w/v). The plates were incubated again at 37°C for 48 hours. Nystatin (1% w/v) and chloramphenicol (1% w/v) were used as positive controls for *C. albicans* and *P. aeruginosa*, respectively. After incubation, the contents of each well were discarded, and the wells were washed three times with 200 µL of PBS to remove non-adherent cells. Biofilm reduction was determined based on the decrease in crystal violet staining intensity, quantified using a microplate reader at an appropriate wavelength (Pratiwi and Hamzah, 2020).

Data Analysis

All experiments were conducted in triplicate. Optical density (OD) readings were performed using a microplate reader at 595 nm. The resulting data were used to calculate the percentage of biofilm inhibition or eradication at each test extract concentration, using the following formula (Hamzah *et al.*, 2019):

$$\%\ \text{Inhibition OR}\ \%\ \text{Eradication} = \frac{OD\ control - OD\ samples}{OD\ control} x100\%$$

The minimum concentration of the test extract that inhibited biofilm formation by ≥50% was defined as the Minimum Biofilm Inhibitory Concentration ($MBIC_{50}$), while the concentration that eradicated ≥50% of mature biofilm was defined as the Minimum Biofilm Eradication Concentration ($MBEC_{50}$).

RESULT AND DISCUSSION

The results of this study showed that the *Petrosia* sp. extract exhibited significant antibiofilm activity against both tested microorganisms, in terms of inhibiting biofilm formation as well as eradicating established biofilms. This activity demonstrated a clear dose-dependent pattern in both target microorganisms, with varying levels of efficacy depending on the biofilm growth phase.

Biofilm Formation Inhibition Activity

The inhibition of *P. aeruginosa* biofilm formation by *Petrosia* sp. extract increased in parallel with rising extract concentrations (Figure 1). In the intermediate phase (24 h), the inhibition percentage ranged from 63.20% (0.125%) to 73.86% (1%), while chloramphenicol 1% as the positive control exhibited 77.86% inhibition. A similar profile was observed in the maturation phase (48 h), with inhibition ranging from 63.09% (0.125%) to 69.71% (1%). These results indicated that *Petrosia* sp. extract had the ability to inhibit biofilm formation in both growth phases, with the $MBIC_{50}$ occurring at the lowest tested concentration (0.125%).

The inhibition of *C. albicans* biofilm formation by *Petrosia* sp. extract (Figure 2) showed a similar pattern, although the inhibition percentages were generally higher in the maturation phase compared to the intermediate phase. At a 1% concentration, the extract showed 70.88% inhibition during the intermediate phase, which increased to 84.70% in the maturation phase. These findings indicated that *Petrosia* sp. extract was effective in inhibiting *C. albicans* biofilm formation, with comparable activity to 1% nystatin (positive control), which showed 79.96% inhibition (intermediate phase) and

90.70% (maturation phase), with the $MBIC_{50}$ also occurring at 0.125%.

The high inhibitory activity at low concentrations (0.125%) against both microorganisms indicated the potential of *Petrosia* sp. extract as an effective antibiofilm agent. This finding was consistent with the report by Ta and Arnason (2015), which stated that secondary metabolites from marine sponges possess significant antibiofilm activity through quorum sensing (QS) inhibition mechanisms. According to Duplantier *et al.* (2021), QS inhibition is a promising strategy to combat *P. aeruginosa* biofilms, as it can reduce virulence factor expression without directly affecting bacterial growth.

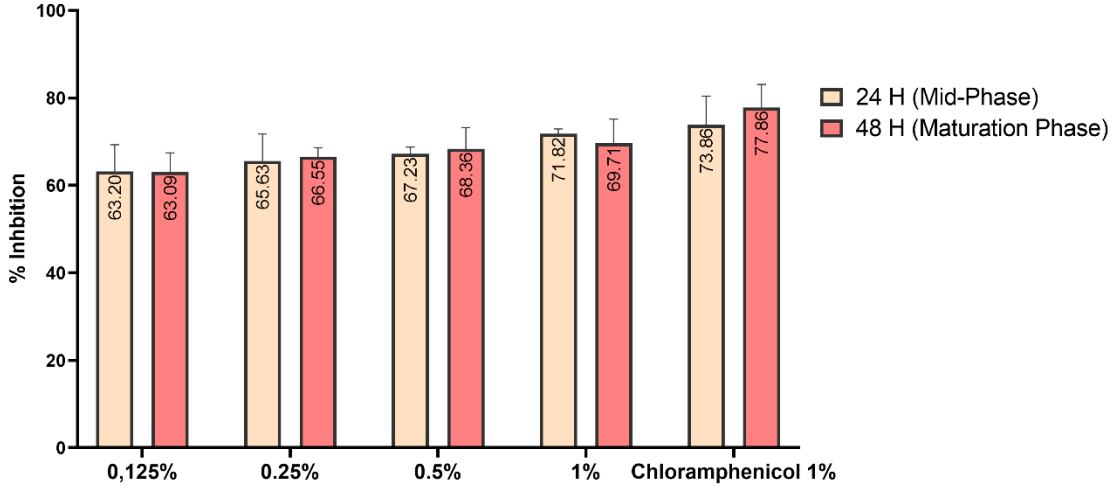

Figure 1. Inhibitory Activity of *Petrosia* sp. Extract on Biofilm Formation of *P. aeruginosa* at the Middle Phase (24 Hours) and Maturation Phase (48 Hours).

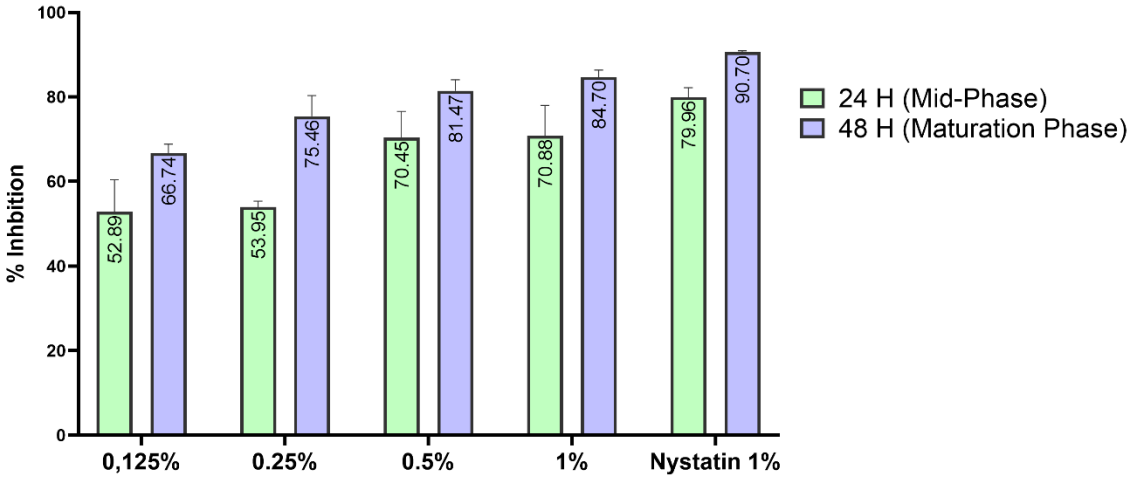

Figure 2. Inhibitory Activity of *Petrosia* sp. Extract on Biofilm Formation of *C. albicans* at the Middle Phase (24 Hours) and Maturation Phase (48 Hours).

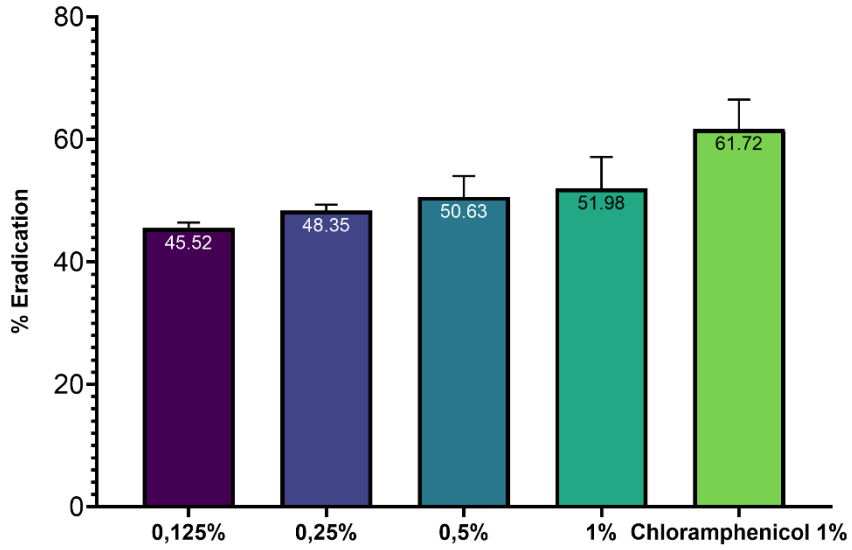

Figure 3. Eradication Activity of *Petrosia* sp. Extract on Biofilm Formed of *P. aeruginosa*.

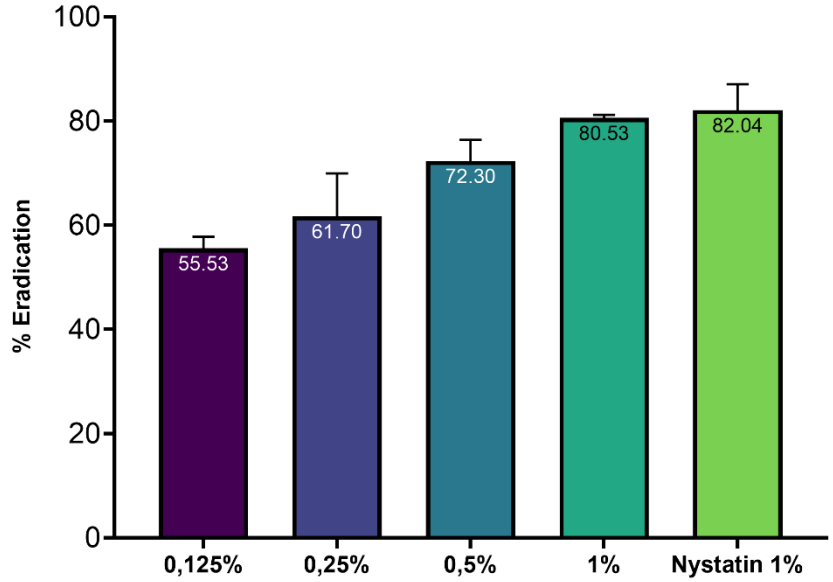

Figure 4. Eradication Activity of *Petrosia* sp. Extract on Biofilm Formed of *C. albicans*.

The ability of *Petrosia* sp. extract to interfere with biofilm formation in both early and mature phases suggested that its active metabolites could act at various stages of biofilm development. This is likely related to the presence of several secondary metabolites in marine sponges that are capable of disrupting initial cell attachment and preventing the synthesis of extracellular polymeric substance (EPS) matrix.

Biofilm formation is influenced by several factors and mechanisms specific to each microorganism and host organism. Each microorganism forms biofilms over different time spans, such as *C. albicans*, which takes approximately 38–72 hours, and *P.*

aeruginosa, which takes about 24–72 hours. This is a complex process that consists of multiple stages.

The first stage involves attachment to either living or non-living surfaces. The next is the intermediate phase, which includes cell proliferation and the production of extracellular polymeric substances (EPS). This is followed by the biofilm maturation phase. The final step in biofilm development is the dispersion stage, during which some microbial cells detach from the biofilm and spread into the environment, where they may undergo a reversible process to reform new biofilms (Chen *et al.*, 2020; Malinovská *et al.*, 2023).

Biofilm Eradication Activity

The eradication activity of established biofilms exhibited greater variation between the two tested microorganisms. *Petrosia* sp. extract showed increased *P. aeruginosa* biofilm eradication with increasing concentrations (Figure 3), ranging from 45.52% (0.125%) to 51.98% (1%), slightly lower than 1% chloramphenicol (61.72%), with a calculated $MBEC_{50}$ of 0.5%.

The eradication activity against *C. albicans* biofilms (Figure 4) showed more promising results, with significant eradication percentages even at the lowest concentration. At 0.125%, the extract eradicated 55.53% of the biofilm, which increased to 80.53% at 1%, approaching the efficacy of 1% nystatin (82.04%), with the $MBEC_{50}$ also occurring at the lowest concentration (0.125%). These findings suggested that *Petrosia* sp. extract had higher potential to eradicate *C. albicans* biofilms compared to *P. aeruginosa*.

The differing responses of the two microorganisms in biofilm eradication could be explained by the structural and compositional differences in their biofilms. *P. aeruginosa* biofilms are known to possess a more complex EPS matrix composed of alginate, Psl, and Pel polysaccharides, which form a highly protective structure (Gheorghita *et al.*, 2023). Meanwhile, although *C. albicans* biofilms also have a complex EPS matrix, their composition, dominated by β-1,3-glucans, may be more susceptible to the active metabolites in *Petrosia* sp. extract (Sandai *et al.*, 2016; Pierce *et al.*, 2017). The ability of *Petrosia* sp. extract to eradicate mature biofilms is a significant finding, considering that mature biofilms are generally highly resistant to most conventional antimicrobial agents.

Potential Mechanisms and Significance of Antibiofilm Activity

Malaka *et al.* (2021) reported that *Petrosia* sp. extract possesses strong antioxidant activity, which may contribute to its antibiofilm effect. Oxidative stress is known to influence biofilm formation and integrity, and compounds with antioxidant activity may disrupt the redox balance necessary for optimal biofilm development (Ong *et al.*, 2018).

The stronger antibiofilm activity observed against *C. albicans* compared to *P. aeruginosa* may be explained by differences in cell wall structure and biofilm matrix composition between the two microorganisms. *P. aeruginosa* has a complex outer membrane typical of Gram-negative bacteria, which can serve as an additional barrier against antibiofilm compound penetration (Lee and Yoon, 2017; Cooper *et al.*, 2018). Moreover, *P. aeruginosa* possesses efficient efflux pump systems that can expel various antimicrobial compounds from the cell (Koo, 2015; Usman *et al.*, 2023).

The lowest tested concentration (0.125%) already demonstrated

significant biofilm inhibition for both microorganisms, indicating the potential of *Petrosia* sp. extract to be further developed as a low-dose, effective antibiofilm agent. This is in line with the findings of Fahdianto *et al.* (2024), who reported that a nanogel formulation of *Petrosia* sp. extract effectively inhibited S. aureus biofilm formation and showed potential for diabetic ulcer healing.

This study demonstrates that *Petrosia* sp. extract from the waters of Maratua exhibits significant antibiofilm activity against *P. aeruginosa* and *C. albicans*, with stronger effects against *C. albicans*. The extract inhibited biofilm formation during both intermediate and maturation phases, and eradicated established biofilms, showing activity comparable to that of positive controls at several concentrations. These findings suggest that *Petrosia* sp. extract has potential as a new source of antibiofilm compounds that could be developed to address biofilm-related infections, particularly those caused by *C. albicans*.

CONCLUSION

Petrosia sp. extract from the waters of Maratua is effective in inhibiting the formation of *P. aeruginosa* and *C. albicans* biofilms during both intermediate and maturation phases at a minimum concentration of 0.125% ($MBIC_{50}$). Moreover, the extract is also capable of eradicating mature biofilms of both microorganisms, showing higher efficacy against *C. albicans* ($MBEC_{50} \leq$ 0.125%) compared to *P. aeruginosa* ($MBEC_{50}$ approximately 1%). These findings indicate the potential of *Petrosia* sp. extract as a promising antibiofilm agent for the development of innovative therapeutic strategies to combat biofilm-associated infections, particularly those caused by *C. albicans* and *P. aeruginosa*.

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Author Contribution

Chaerul Fadly Mochtar: Conceptualization, Methodology, Data Curation, Writing- Original Draft Preparation. Hasyrul Hamzah: Writing-Reviewing and Editing. Muhammad Tursina Bakti: Visualization, Investigation. Abdul Rozak: Software, Validation.

Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical Approval

None.

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