PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *TENGKAWANG BUKIT* (*Shorea beccariana*) STEM EXTRACT AGAINST *Vibrio parahaemolyticus*

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ABSTRACT

Vibrio parahaemolyticus infection poses a significant challenge in intensive shrimp culture, leading to substantial economic losses. Its common treatments using synthetic antibiotics have been linked to increased risks of antibiotic resistance and residual effects. Therefore, finding environmentally safe and effective natural alternatives is deemed essential. *Tengkawang Bukit* (*Shorea beccariana*) stem extract contains antibacterial compounds, including asiatic acid, oleanolic acid, and lupanone, all classified as terpenoids. This study aims to evaluate the antibacterial potential of *S. beccariana* stem extract through phytochemical screening and a disc diffusion test against *V. parahaemolyticus*. The screening results confirmed the presence of bioactive compounds, including alkaloids, flavonoids, saponins, tannins, and steroids. The disc diffusion test showed an increase in the inhibition zone with increasing extract concentration, with inhibition diameters ranging from 9.04 ± 0.48 mm to 10.75 ± 0.26 mm. The 17% extract yield indicates a high availability of active compounds. These findings suggest that *tengkawang bukit* stem extract has potential as a natural antibacterial alternative for controlling *V. parahaemolyticus* in shrimp culture. This approach could help reduce reliance on synthetic antibiotics and promote sustainable fisheries.

KEYWORDS: antibacterial agents; plant extracts; resistance; *Shorea beccariana*; *Vibrio parahaemolyticus*

ABSTRAK: Skrining Fitokimia dan Uji Aktivitas Antibakteri pada Ekstrak Batang Tengkawang Bukit* (*Shorea beccariana*) *terhadap Vibrio parahaemolyticus

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*Infeksi **Vibrio parahaemolyticus** merupakan salah satu kendala utama dalam budidaya udang yang menyebabkan kerugian ekonomi signifikan. Penggunaan antibiotik sintetis sebagai pengobatan telah menimbulkan kekhawatiran terkait resistensi dan residu, sehingga diperlukan alternatif alami yang aman dan efektif. Batang tengkawang bukit (**Shorea beccariana**) diketahui mengandung senyawa antibakteri seperti asam asiatik, asam oleanolik, dan lupanon yang tergolong ke dalam senyawa terpenoid. Studi ini bertujuan untuk mengevaluasi potensi antibakteri ekstrak batang **S. beccariana** melalui skrining fitokimia dan uji difusi cakram terhadap **V. parahaemolyticus**. Hasil skrining menunjukkan adanya kandungan senyawa bioaktif berupa alkaloid, flavonoid, saponin, tanin, dan steroid. Uji difusi cakram memperlihatkan zona hambat yang meningkat seiring dengan peningkatan konsentrasi ekstrak, dengan diameter hambatan berkisar 9,04 ± 0,48 mm hingga 10,75 ± 0,26 mm. Rendemen ekstrak sebesar 17% menunjukkan ketersediaan senyawa aktif yang melimpah. Hasil riset ini mengindikasikan bahwa ekstrak batang tengkawang bukit berpotensi sebagai alternatif antibakteri alami dalam pengendalian **V. parahaemolyticus** pada budidaya udang, sehingga dapat mengurangi ketergantungan terhadap antibiotik sintetis dan mendukung keberlanjutan perikanan.*

***KATA KUNCI: agen antibakteri; ekstrak tanaman; resistensi;** Shorea beccariana; Vibrio parahaemolyticus*

INTRODUCTION

Mariculture has been considered one of the main pillars of Indonesia's marine and fisheries economic sector, directly contributing to food security and employment. These contributions are driven by increased demand for fish and shrimp in both domestic and international markets. Recently published data from the Ministry of Marine Affairs and Fisheries (MMAF) (2023) suggested that fish consumption in Indonesia increased from 50.69 kg per year in 2018 to 57.61 kg per year in 2023. Surprisingly, mariculture production in Indonesia has shown a fluctuating trend within the five-year period. Mariculture production reached 8.6 million tons in 2019 and decreased slightly to 8.5 million tons in 2020. In 2021, mariculture production declined significantly to 7.3 million tons, rebounded to 7.9 million tons in 2022, and continued to rise in 2023 to 8.3 million tons. These fluctuations reflect that, although the mariculture sector has significant economic potential, various factors and obstacles continue to affect its production stability.

As cultivation intensification increases to meet demand, maintaining a sustainable production is also becoming more complex. One of the main challenges is the high infection rate of diseases caused by pathogenic bacteria (Pramadani, 2022). Diseases caused by pathogenic bacteria are among the main limiting factors in fish and shrimp farming, especially in intensive systems that have a higher risk of disease spread. Bacterial infections not only inhibit growth and reduce the survival rate of cultured organisms but also directly reduce productivity and cause considerable economic losses for farmers (Semwal *et al*., 2023). One of the causative agents in bacterial diseases in marine fish and shrimp is *Vibrio parahaemolyticus* (Evan *et al*., 2021).

V. parahaemolyticus is a Gram-negative bacterium causing vibriosis in fish and shrimp. In shrimp farming, *V. parahaemolyticus* infection is often associated with acute hepatopancreatic necrosis disease (AHPND), which causes severe damage to the hepatopancreas and can lead to mass mortality (Evan *et al*., 2021). Cases of *V. parahaemolyticus* infection have been reported in shrimp farming centers across Indonesia, including Sumbawa, East Java, and Lampung,

resulting in drastic decreases in production and even total harvest failures (MMAF, 2023). This bacterial infection causes aquatic organisms to become sick, with symptoms including a swollen, hard abdomen, a brown anus, a pale liver, swollen intestines, slow movement, decreased appetite, swollen kidneys, hemorrhagic intestines, and skin wounds (Khairiyah *et al*., 2022). Given the magnitude of the impact, it is imperative to control this bacterial infection, which until now has relied mainly on synthetic antibiotics in aquaculture practices.

The use of synthetic antibiotics is one of the steps to prevent bacterial infections. However, the use of synthetic antibiotics is currently limited because it can lead to bacterial resistance and leave residues that pose a serious global health risk (Alamsjah *et al*., 2020). To overcome this issue, alternatives to synthetic antibiotics are needed, such as natural ingredients with antibiotic properties. One potential plant is *Tengkawang Bukit* (*Shorea beccariana*), whose stems contain terpenoid compounds, including asiatic acid, oleanolic acid, and lupanon, which are known to be antibacterial (Musa *et al*., 2024). Until now, no research has specifically examined the antibacterial activity of the *tengkawang bukit* plant stems using qualitative phytochemical screening and disc diffusion tests.

This study aimed to evaluate the antibacterial potential of *tengkawang bukit* extract using phytochemical screening and a disc diffusion test to assess the inhibition zone of the extract against *V. parahaemolyticus*. Phytochemical screening was conducted to identify the presence of bioactive compounds, including flavonoids, alkaloids, saponins, tannins, and steroids, known to have antibacterial activity. Furthermore, the disc diffusion test was used to assess the extract's ability to inhibit the growth of the target bacterium (*V. parahaemolyticus*). Therefore, the identification and development of natural materials, especially *tengkawang bukit* extract as an alternative antibacterial agent, are highly relevant to supporting disease control efforts in shrimp culture in a sustainable manner. By finding effective antibacterial agents from local natural resources, it is expected that dependence on synthetic antibiotics can be reduced, thus preventing the emergence of antimicrobial resistance and maintaining the sustainability of the fisheries sector.

MATERIALS AND METHODS

This research was conducted from June to August 2025. Extract preparation activities were carried out at the Organic Chemistry Laboratory, Department of Chemistry, Faculty of Science and Technology, Airlangga University, Surabaya, Indonesia. The antibacterial activity test was conducted at the Microbiology Laboratory of the Faculty of Fisheries and Marine Sciences, Airlangga University, Surabaya.

In this study, *V. parahaemolyticus* ATCC isolate was obtained from the Indonesian Agricultural Quarantine Agency. Whereas, *tengkawang bukit* stems were taken from the forest in Madurejo, South Arut, West Kotawaringin Regency, Central Kalimantan, Indonesia. These materials were then transported to Airlangga University for further analysis.

Extract Preparation

The extraction of compounds from *tengkawang bukit* stems was carried out according to the method described by Musa *et al.* (2024b). The initial stage began with the preparation of raw materials, specifically fresh *tengkawang bukit* stems weighing up to 12 kg. The fresh material was washed with clean water, then placed in a drying room to dry at room temperature, out of direct sunlight. This drying method was chosen to maintain the stability of the bioactive compounds in the material, especially phenolic compounds, flavonoids, and tannins, which are easily degraded by heat and sunlight (Pujiastuti & Ma'rifah, 2022). Drying was performed by evenly spreading the material on a mesh

tray and flipping it daily to ensure good air circulation and prevent microbial growth. *Tengkawang bukit* stems, as a hardwood material, are estimated to take 14-21 days to reach dry moisture content. The material was considered dry once its weight had remained constant for several days, it no longer felt cool to the touch, it no longer emitted a damp odor, and it showed characteristic textural changes, such as the trunk becoming harder and lighter. (Supriningrum *et al*., 2018).

After complete drying, all materials are ground into a fine powder or small shavings using a crusher (grinder). This process increased the material's surface area in contact with the solvent, thereby maximizing the efficiency of active compound extraction in the next stage. Each type of plant powder material will be extracted separately through the maceration method. The dried powder was soaked separately in 30 L of methanol. Methanol was chosen as the extraction solvent in this study because it is a universal solvent that can attract non-polar, polar, and semi-polar compounds, has a low boiling point (65°C), and is easily evaporated (Supriningrum *et al*., 2018). This soaking lasted for 72 hours at 25-28 °C in room conditions, under protected light conditions. To maximize extraction and minimize solvent saturation, the methanol solvent will be renewed or replaced three times, every 24 hours. During the maceration stage, intermittent stirring will be applied to ensure even dispersion of the sample (Musa *et al*., 2024b).

After the maceration process, the entire volume of the methanol filtrate obtained from the *tengkawang bukit* sample was collected into a single container. The methanol extract was then concentrated using a rotary vacuum evaporator at 65°C, 150 mbar, and 120 rpm. This procedure aimed to evaporate methanol to produce a more concentrated extract efficiently. This concentrated extract was evaporated again using a water bath at 40°C for 2 hours to produce a crude extract. The crude extract resulting from this process was stored under suitable conditions before being used in a series of further antibacterial activity tests (Musa *et al*., 2024b).

Yield Calculation

The yield of *tengkawang bukit* stem extract was calculated by weighing the dried extract, dividing by the initial weight of the sample (initial simplicia), and multiplying by 100% (Widyastuti *et al*., 2021). The equation (1) is used to calculate the extract yield.

$$\% \text{ Yield} = \frac{\text{Weight of Extract (g)}}{\text{Weight of Simplisa (g)}} \text{ x100} \quad \text{.....(1)}$$

Preparation of Test Bacteria

The test bacteria used were *V. parahaemolyticus*. The bacterial preparation method is described in the research by Huyyirnah and Fitriyani (2020). Bacteria were inoculated onto tryptic soy agar (TSA) + NaCl 3% and propagated in tryptic soy broth (TSB) + NaCl 3%. Bacterial inoculation was carried out using a test tube containing 10 mL of media (TSB + NaCl 3%), followed by the addition of 500 μL (0.5 mL) of bacteria, and vortexed until homogeneous. The test tube was placed in an incubator (Incucell-MMM, Germany) at 37°C for 18 to 24 hours.

Qualitative Phytochemical Analysis

Phytochemical analysis included qualitative tests for alkaloids, flavonoids, saponins, tannins, and steroids. Testing of alkaloid compounds was carried out by reacting 2 mL of *tengkawang bukit* stems extract with 1 mL of HCl in three test tubes. Each tube was then added three drops of Mayer, Wagner, and Dragendorff reagents. A positive alkaloid reaction is characterized by the presence of a cloudy white or yellowish white precipitate with Mayer's reagent, brown color with Wagner's reagent, and orange-red or reddish brown with Dragendorff's reagent (Affandy *et al*., 2021).

The flavonoid test of *tengkawang bukit* stem

extract was performed by mixing 0.5 mL of the extract with 10 mL of hot water, then adding 0.25 g magnesium powder and five drops of concentrated HCl. The formation of an orange or bright red color indicates the presence of flavonoids in the extract (Yeti & Yuniarti, 2021).

The saponin test of *tengkawang bukit* stem extract was performed by mixing 1 mL of the extract with 5-10 mL of warm distilled water, shaking vigorously for 10 seconds, and standing for 15 minutes. The formation of stable foam up to 1-10 cm in height, which does not disappear after adding one drop of HCl, indicates a positive result for saponins (Kusumawati *et al*., 2017).

The tannin test of *tengkawang bukit* stem extract was performed by adding 2 mL of the extract to a test tube, then adding 10 drops of 1% $FeCl_3$ solution. Changes in color to dark blue or greenish black indicate a positive reaction to tannins (Kusumawati *et al*., 2017).

A steroid test of *tengkawang bukit* stem extract was carried out by mixing 1 mL of the extract with Liebermann-Burchard reagent, a mixture of 2 mL chloroform, 10 drops of acetic anhydride, and three drops of concentrated sulfuric acid, then shaking gently and allowing it to stand. The formation of a green color indicates a positive result for steroids (Yeti & Yuniarti, 2021).

Disc Diffusion Test

The antibacterial activity test using the Kirby-Bauer disc diffusion method was conducted in a completely randomized design with six treatments and three replicates. This test procedure is based on the research by Ngamsurach and Praipipat (2022). The test was begun with the preparation of extract concentrations according to the treatment given, namely P1 (1 mg per disc), P2 (1.5 mg per disc), P3 (2 mg per disc), P4 (2.5 mg per disc), positive control in the form of ampicillin antibiotics, and negative control using sterile distilled water. Treatments P1, P2, P3, and P4 were prepared by dissolving 100, 150, 200, and 250 mg of dry extract, respectively, in 1 mL of distilled water. Subsequently, 10 μL of each extract solution was applied to a blank disc paper. Furthermore, pure cultures of bacteria were inoculated into TSB + 3% NaCl. The bacterial inoculum was diluted with 0.85% NaCl to the equivalent of ×a 0.5 McFarland standard turbidity or 1.5 x 10^8 CFU mL^{-1} using a turbidimeter (DL Medcall, China).

The diluted bacterial inoculation was spread onto Mueller-Hinton agar (MHA) + 3% NaCl using a sterile cotton swab. The next step was placing an antimicrobial disc (a paper disc) for each treatment. Furthermore, the petri dish was incubated at 37°C for 24 hours until an inhibition zone was observed. The size of the inhibition zone was measured using a digital caliper in millimeters (mm) (Ngamsurach & Praipipat, 2022).

Data Analysis

Inhibition zone diameter data were analyzed using one-way ANOVA in IBM SPSS Statistics version 25 at a 95% confidence level. If the one-way ANOVA test showed a significant effect of treatments (p-value $<$ 0.05), the analysis was continued with the Tukey test. Observations on the results of qualitative phytochemical tests were analyzed descriptively after data collection.

RESULTS AND DISCUSSION

The results showed that the yield value of *tengkawang bukit* stem extract was 17%. Phytochemical screening tests were qualitatively carried out on *tengkawang bukit* stem extract to determine the presence of secondary metabolite compounds with potential as antibacterial agents. The results of the qualitative phytochemical screening tests are shown in Table 1. *Tengkawang bukit* is a tall dipterocarp tree reaching up to 60 m, with prominent buttress roots and grey-brown bark that becomes scaly in mature trees (Randa *et al*., 2022). Its leaves are stiff and elliptic with a bluish underside that turns brown when

dried (Vallahayil *et al*., 2024). The species also produces pink inflorescences and ovoid fruits about 4 cm long (Barstow *et al*., 2019). The appearance of the *tengkawang bukit* tree is shown in Figure 1.

The antibacterial activity of the extract from *tengkawang bukistems* was demonstrated by the formation of an inhibition zone around the paper disc. The measurement results indicated that the extract could inhibit the growth of *V. parahaemolyticus*. The results of the disc diffusion test using the Kirby-Bauer method are presented in Figure 2.

The results of the one-way ANOVA test showed that different extract concentrations had a significant effect on the diameter of the inhibition zone formed ($p < 0.05$). The *S. beccariana* stem extract at a concentration of 2.5 mg per disc (P4) produced the highest inhibition zone diameter (10.75 ± 0.26 mm). These results were not significantly different from the positive control (Ampicillin), which had an inhibition zone of 10.94 ± 0.26 mm. A concentration of *tengkawang bukit* of 2 mg per disc (P3) produced an inhibition zone of 9.75 ± 0.24 mm, while P2 and P1 treatments showed

Figure 1. Tree of *Shorea beccariana*
Source: Musa *et al.* (2024a)

Table 1. Results of qualitative phytochemical analysis of *tengkawang bukit* (*S. beccariana*) stems extract

Parameter	Result	Description
Alkaloid	+	There is a yellowish-white precipitate with Mayer's reagent, a brown precipitate with Wagner's reagent and a brownish-red precipitate on Dragendorff's reagent
Flavonoid	+	There is a red or orange color change
Saponin	+	Forms a stable foam
Tannin	+	There is a color change to blackish green
Steroid	+	There is a color change to intense green

Note: (+) There is a precipitate or color change, (-) There is no precipitate or color change.

lower values (9.17 ± 0.43 mm and 9.04 ± 0.48 mm, respectively) (Table 2).

The diameter of the inhibition zone in the disc diffusion test indicated that the *tengkawang bukit* stem extract had strong antibacterial activity against *V. parahaemolyticus*. The diameter of the inhibition zone increased with increasing extract concentration. The extract yield obtained from the maceration process was 17%, a high value, indicating the abundance of bioactive compounds that support its antibacterial effectiveness. Based on research by Musa *et al*. (2024b), *tengkawang bukit* stems contain terpenoid compounds, including asiatic acid, oleanolic acid, and lupanon. Terpenoid compounds are effective at disrupting bacterial cell membranes, inhibiting quorum sensing (QS) to reduce communication between bacterial cells and reduce virulence, and inhibiting protein and ATP synthesis. Terpenoid compounds cause ion leakage and direct membrane damage, mainly by penetrating and disrupting the lipid bilayer of bacterial cells (Ergüden, 2021).

This mechanism is also consistent with the finding that membrane-penetrating

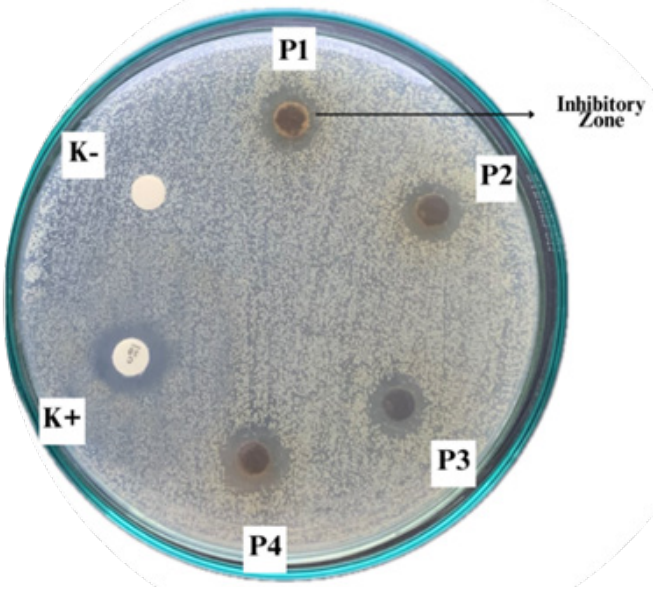

Figure 2. Results of the disc diffusion test of *tengkawang bukit* (*S. beccariana*) stems extract on *V. parahaemolyticus*. P1: 1 mg per disc; P2: 1.5 mg per disc; P3: 2 mg per disc; P4: 2.5 mg per disc; K+: positive control (Ampicillin); and K-: negative control (sterile distilled water)

Table 2. Inhibition zone of *tengkawang bukit* (*S. beccariana*) stems extract against *V. parahaemolyticus*

Treatment	Concentration	Inhibition Zone Diameter (mm)
P1	1 mg per disc	9.04 ± 0.48^{b}
P2	1.5 mg per disc	9.17 ± 0.43^{b}
P3	2 mg per disc	9.78 ± 0.26^{b}
P4	2.5 mg per disc	10.75 ± 0.26^{c}
K+	Ampicillin 10 mcg	10.94 ± 0.26^{c}
K-	Sterile water	0.00 ± 0.00^{a}

Note: Different lowercase letters (a, b, c) indicate significant differences ($p < 0.05$) among treatments. Values are based on mean ± SD (n=3).

hygroscopic terpenoid compounds can inhibit the quorum-sensing system, an important pathway for virulence coordination and biofilm formation in *V. parahaemolyticus* (Yu *et al*., 2022). Terpenoid compounds are also effective antibacterials because they enhance the activity of other antibacterials. In addition to terpenoid content, the results of phytochemical tests on *S. beccariana* stem extract showed the presence of five classes of active compounds, namely alkaloids, flavonoids, saponins, tannins, and steroids.

Alkaloids inhibit bacteria by damaging bacterial cell walls that interact with DNA, inhibiting protein synthesis, and preventing the formation of strong cell walls, thus causing cell lysis or destruction (Marbun & Situmorang, 2020). Flavonoids, a major group of phenolic compounds, are effective against viruses, bacteria, and fungi by damaging cell walls, inhibiting bacterial motility, and disrupting energy metabolism and bacterial respiration (Bontjura *et al*., 2015). Flavonoids and alkaloids are widely reported as the most effective compounds in antibacterial activity with strong mechanisms of action (Huang *et al*., 2022).

Steroid compounds act by penetrating the membrane's lipophilic layer, reducing membrane integrity and disrupting cell morphology, leading to bacterial cell rupture (Kumalasari *et al*., 2020). Saponins increase the permeability of the cell membrane by directly interacting with membrane sterols, leading to the leakage of proteins and enzymes from the cell and, eventually, the lysis of bacteria (Elsa *et al*., 2023). Tannins are bactericidal by binding to cell walls and essential proteins, disrupting microbial adhesion and inactivating vital enzymes within the cell (Isnaini *et al*., 2018). Steroids have a strong mechanism of action by directly damaging the integrity of bacterial lipid membranes, so the absence of steroid compounds in *tengkawang bukit* extract can reduce the synergy between bioactive compounds (Batubara *et al*., 2021).

Thus, the higher the extract concentration, the more active compounds diffuse into the media, expanding the inhibition zone and increasing its antibacterial effect. This is consistent with the findings of Wanda *et al*. (2023), who reported that the higher the extract concentration, the larger the inhibition zone diameter. Previous research reported that a mangrove stem extract produced an inhibition zone of 5.4-6.5 mm at 1-4 mg per disk against *V. parahaemolyticus*. In contrast, this study showed a larger inhibition zone, ranging from 9.04 ± 0.48 mm to 10.75 ± 0.26 mm, at lower concentrations. This difference suggests that the *tengkawang bukit* stem extract has greater antibacterial activity and could be a natural candidate for the development of new antimicrobial agents.

CONCLUSIONS

Based on the results of the study, *tengkawang bukit* stem extract showed potent antibacterial activity against *V. parahaemolyticus*, as indicated by a wider zone of inhibition as the extract concentration increased in the disc diffusion test. The high extract yield (17%) indicated the presence of significant amounts of bioactive compounds. Phytochemical screening revealed the presence of five major classes of active compounds, namely alkaloids, flavonoids, saponins, tannins, and steroids, in addition to the main content of terpenoid compounds such as asiatic acid, oleanolic acid, and lupanon. This approach could help reduce reliance on synthetic antibiotics and promote sustainable fisheries. To obtain more comprehensive results and support the development of this extract as an antibacterial agent, further studies are recommended. A quantitative phytochemical analysis of *tengkawang bukit* extract is needed to determine the levels of the main bioactive compounds responsible for its antibacterial activity. In addition, *in vivo* testing on target organisms, such as shrimp or fish susceptible to *V. parahaemolyticus,* is important to evaluate

the biological effectiveness and the potential direct application of the extract in aquaculture systems.

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AUTHOR CONTRIBUTION

HA: conceptualization, methodology, investigation, data management, formal analysis, and initial draft writing; ATM: supervision, methodology, and manuscript review and editing; GM: supervision, validation, and manuscript review and editing; MA: conceptualization, methodology, supervision, validation, and review and editing of the manuscript; NSA: conceptualization, methodology, and review.

DECLARATION OF COMPETING INTEREST

The authors declare no conflict of interest.

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