



Maqui leaf extract exhibits bactericidal activity against *Vibrio parahaemolyticus*

El extracto de hoja de maqui presenta actividad bactericida frente a *Vibrio parahaemolyticus*

Nicolás Plaza¹ , Camila Manzano¹ , Sebastián Ramírez-Araya² , Constanza Griffiths-Sanhueza¹ , Ítalo M. Urrutia¹ , Diliana Pérez-Reytor¹ , Gino Corsini¹ , Marcelo Ortega³ , Talía del Pozo⁴ , Leonardo Pavez^{5*} , Katherine García^{1*}

¹ Universidad Autónoma de Chile, Instituto de Ciencias Biomédicas, Facultad de Ciencias de la Salud. Santiago de Chile, Chile.

² Masblue Research and Innovation Center, Molecular Biology Laboratory. Iquique, Chile.

³ Universidad Bernardo O'Higgins, Departamento de Ciencias Humanas. Santiago de Chile, Chile.

⁴ Universidad de Las Américas, Facultad de Medicina Veterinaria y Agronomía, Núcleo de Investigación en Sustentabilidad Agroambiental. Santiago de Chile, Chile.

⁵ Universidad de Las Américas, Facultad de Medicina Veterinaria y Agronomía, Núcleo de Investigaciones en Ciencias Biológicas. Santiago de Chile, Chile.

*Corresponding Author: katherine.garcia@uautonoma.cl; lpavez@udla.cl

How to cite this article

Plaza N, Manzano C, Ramírez-Araya S, Griffiths-Sanhueza C, Urrutia IM, Pérez-Reytor D, Corsini G, Ortega M, Del Pozo T, Pavez L, García K: Maqui leaf extract exhibits bactericidal activity against *Vibrio parahaemolyticus*. Rev haban cienc méd [Internet]. 2026 [cited]; 25 Available from: <http://www.revhabanera.sld.cl/index.php/rhab/article/view/6178>

Received: August 29, 2025

Approved: February 02 2026

ABSTRACT

Introduction: *Vibrio parahaemolyticus* is the leading cause of seafood-borne gastroenteritis, particularly in coastal areas with high consumption of raw shellfish. In tropical and subtropical regions, such as the Caribbean, climate change, salinity variations, and antimicrobial resistance have intensified public health risks. In this context, natural antimicrobials derived from agricultural by-products emerge as sustainable, low-cost, and environmentally responsible alternatives.

Objective: To evaluate the antimicrobial activity of leaf extracts of *Aristotelia chilensis* (Molina) Stuntz (Maqui) against clinical and environmental strains of *V. parahaemolyticus*, and to explore their potential application in food safety strategies.

Material and Methods: Crude extracts from maqui leaves, roots, and stems were obtained through methanol and ethanol extraction. Antimicrobial activity was evaluated against cytotoxic strains and environmental isolates resistant to kanamycin or chloramphenicol, determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) using microdilution assays.

Results: The ethanolic extract showed the highest antimicrobial activity, inhibiting the growth of cytotoxic clinical strains and resistant isolates. For the clinical strain VpKX, an MIC of 0.056 mg/mL and an MBC of 0.1125 mg/mL were determined, confirming a bactericidal effect.

Conclusions: Maqui leaves represent a promising source of natural antimicrobial compounds effective against *V. parahaemolyticus*, including antibiotic-resistant strains. Their valorization as a plant by-product contributes to sustainable development and, following validation in food matrices, could be applied to improve food safety in coastal regions of the Caribbean.

RESUMEN

Introducción: *Vibrio parahaemolyticus* es la principal causa de gastroenteritis transmitida por mariscos, especialmente en zonas costeras con alto consumo de moluscos crudos. En regiones tropicales y subtropicales, como el Caribe, el cambio climático, las variaciones de salinidad y la resistencia antimicrobiana han intensificado los riesgos para la salud pública. En este contexto, los antimicrobianos naturales derivados de subproductos agrícolas surgen como alternativas sostenibles, de bajo costo y ambientalmente responsables.

Objetivo: Evaluar la actividad antimicrobiana de los extractos de hojas de *Aristotelia chilensis* (Molina) Stuntz (Maqui) frente a cepas clínicas y ambientales de *V. parahaemolyticus*, y explorar su potencial aplicación en estrategias de inocuidad alimentaria.

Material y Métodos: Se obtuvieron extractos crudos de hojas, raíces y tallos de Maqui mediante extracción por metanol y etanol. La actividad antimicrobiana se evaluó frente a cepas citotóxicas y aislados ambientales resistentes a kanamicina o cloranfenicol, determinando la concentración mínima inhibitoria (CMI) y la concentración mínima bactericida (CMB) mediante ensayos de microdilución.

Resultados: El extracto etanólico mostró la mayor actividad antimicrobiana, inhibiendo el crecimiento de cepas clínicas citotóxicas y aislados resistentes. Para la cepa clínica VpKX se determinó una CMI de 0,056 mg/mL y una CMB de 0,1125 mg/mL, confirmando un efecto bactericida.

Conclusiones: Las hojas de Maqui representan una fuente prometedora de compuestos antimicrobianos naturales eficaces contra *V. parahaemolyticus*, incluidas cepas resistentes a antibióticos. Su valorización como subproducto vegetal contribuye al desarrollo sostenible y, tras validación en matrices alimentarias, podría aplicarse para mejorar la inocuidad en regiones costeras del Caribe.

Keywords:

Foodborne diseases; gastroenteritis; seafood; climate change; antimicrobial resistance; *Vibrio parahaemolyticus*; *Aristotelia chilensis*; shellfish.

Palabras Claves:

Enfermedades transmitidas por alimentos; gastroenteritis; productos del mar; cambio climático; resistencia antimicrobiana; *Vibrio parahaemolyticus*; *Aristotelia chilensis*; moluscos.



INTRODUCTION

Vibrio parahaemolyticus (*V. parahaemolyticus*) is a halophilic bacterium widely distributed in estuarine and coastal environments, where bivalves such as mussels and oysters can accumulate environmental and pathogenic strains.^(1,2) Human consumption of raw or undercooked seafood contaminated with virulent strains is a well-established cause of acute gastroenteritis, characterized by diarrhea, abdominal pain, nausea, and fever.⁽³⁾ In some coastal zones of the Caribbean, where seafood consumption is widespread and cold chain infrastructure is limited, public health risks are heightened as climate change alters sea temperature and salinity, conditions that favor *Vibrio* proliferation.⁽⁴⁾

Historically, *Vibrio cholerae* caused major cholera outbreaks in Cuba, Jamaica, Puerto Rico, and the Dominican Republic during the 19th century.⁽⁵⁾ However, the current expansion of non-cholera *Vibrio* species under changing climatic conditions poses new challenges for food safety and surveillance.^(6,7) In Cuba, raw oyster consumption creates direct exposure to autochthonous *Vibrio*. A survey in southeastern Cuba detected *V. parahaemolyticus* up to 10² MPN/g, with higher counts at lower salinity (~25 psu), underscoring seasonal risks and cold chain weaknesses.⁽⁸⁾ According to the U.S. FDA, regulatory action is recommended when levels exceed 1 × 10⁴ CFU/g in raw shellfish, while post-harvest processed products labeled as “reduced to non-detectable levels” must contain <30 MPN/g.⁽⁹⁾

Post-harvest strategies such as thermal processing, irradiation, high-pressure treatments, and chemical disinfection,^(10,11,12) can reduce bacterial counts below regulatory thresholds but may compromise sensory attributes and marketability.^(13,14) These methods are not always effective against all pathogenic strains and are often less feasible in resource-limited settings.⁽¹⁵⁾ Consequently, natural plant-based antimicrobials are gaining attention as environmentally friendly, low-cost alternatives that preserve food quality. Extracts from agricultural by-products are particularly attractive due to their sustainability and accessibility.⁽¹⁶⁾

Aristotelia chilensis [Mol.] Stuntz (Maqui) is native to southern South America and is naturally distributed in central-southern Chile (Maule to Los Lagos regions) and in northwestern Patagonia in Argentina (Neuquén, Río Negro, and Chubut provinces). In turn, it was first recorded in the USDA Bureau of Plant Industry's Inventory of Seeds and Plants Imported in 1914 and is recognized for its high antioxidant content, including anthocyanins (delphinidins), flavonoids, phenolic acids, stilbenes, and resveratrol.^(17,18) While research has mainly focused on Maqui fruit, its leaves, often discarded as waste, also contain phenolic acids, flavonoids, anthocyanins, stilbenes, and alkaloids, some linked to antimicrobial activity.^(19,20,21) This underutilized plant material may provide a low-cost natural strategy to control foodborne pathogens and could be integrated into post-harvest handling to reduce *Vibrio* loads without compromising seafood quality. Such approaches are especially relevant for Caribbean contexts, where affordable, locally adaptable interventions are needed.

Based on this evidence, this study has the objective to evaluate the antimicrobial activity of Maqui leaf extracts against clinical and environmental strains of *V. parahaemolyticus*, assessing their potential application in seafood safety strategies under conditions of climate variability and emerging marine pathogens. Exploring a native South American plant such as Maqui offers not only a potential antimicrobial source but also a transferable model of resource valorization between Latin American regions sharing similar climatic stressors and public health vulnerabilities.

This research is particularly relevant because Caribbean countries face persistent challenges in seafood safety management due to limited access to cold chain infrastructure and to antimicrobial alternatives that are often prohibitively expensive. Exploring a natural resource native to South America, such as Maqui, offers a sustainable, low-cost option that could be transferred or adapted to similar tropical regions. Furthermore, this work contributes to bridging the gap between plant-based antimicrobial discovery and practical applications in food safety, generating evidence to support biotechnological valorization of agro-industrial by-products and promoting cross-regional collaboration between temperate and tropical ecosystems under shared climate stressors. Ultimately, assessing the antimicrobial potential of Maqui leaves may inform feasible, low-cost interventions for seafood handling and preservation in resource-limited settings. While exploratory, this approach can guide future applied studies and support the gradual incorporation of sustainable, nature-based solutions into regional food safety strategies.

In addition to food safety concerns, increasing reports of reduced susceptibility to commonly used antibiotics raise further challenges for control and treatment. Antimicrobial resistance limits the effectiveness of conventional interventions and underscores the need to explore alternative or complementary antimicrobial approaches. In this context, evaluating plant-derived extracts against strains with variable antibiotic sensitivity allows not only assessment of antibacterial activity but also examination of their potential relevance in scenarios where reduced antibiotic efficacy is observed.

MATERIALS AND METHODS

This study was an experimental, descriptive investigation conducted between March 2019 and December 2020 at the Gastrointestinal Pathology Laboratory at the Universidad Autónoma de Chile (Metropolitan Region, Chile). Clinical reference strains reported in the literature and environmental strains from mussels purchased in open-air markets in the Metropolitan Region of Chile were used. Only isolates confirmed as *V. parahaemolyticus* by multiplex molecular assays were included. For each strain, origin, year of isolation, and genetic characterization were recorded to ensure traceability and representativeness.

Bacterial strains and phenotypic characterization

Twenty-four strains of *V. parahaemolyticus* from diverse origins were used, including the pandemic, clinical and cytotoxic strain VpKX derived from RIMD2210633,⁽²²⁾ the non-pandemic clinical cytotoxic strain PMC53.7,^(23,24) and the non-pandemic environmental cytotoxic strain PMA1.15.⁽²⁵⁾ Additional environmental strains were isolated in 2019 from *Mytilus chilensis* purchased at open-air markets in the Metropolitan Region of Chile. Briefly, ten mussels were homogenized as one sample; 5 g were enriched in 45 mL Alkaline Peptone Water (APW) at 37 °C for 18 h and plated on CHROMagar *Vibrio* (CHROMagar Microbiology, Paris, France). Colonies were tested for *tlh* (species marker), *tdh* and *trh* (virulence factors) genes by multiplex PCR.⁽²⁶⁾ In total, 24 clinical and environmental *V. parahaemolyticus* strains were used. All were maintained in Luria Bertani broth (LB) or agar with 3% NaCl. Two reference strains, *Escherichia coli* BL21 and *Staphylococcus aureus* ATCC25923, were also included.

Preparation of Maqui extracts and screening of their antimicrobial activities over the growth of virulent *V. parahaemolyticus* strains

Vegetative tissue of Maqui was produced via *in vitro* culture in the central zone of Chile in September 2019, following a previously described and validated protocol (Universidad de Las Américas, 2018).⁽²⁷⁾ Roots, stem, and leaves were washed with sterile distilled water, and compounds were extracted individually by solid–liquid extraction (0.5 g tissue in 1 mL solvent: methanol, ethanol, or sterile water as a control).⁽²⁸⁾ Each mixture of vegetative tissue and solvent were stirred for 24 h, centrifuged at 3000 × g for 20 min, filtered, and dried in a Speed-vac (Savant DNA 120, Thermo Scientific) at 35 °C. Extracts were reconstituted in 200 µL sterile water or stored at –20 °C. A total of 28 extracts were obtained. Antimicrobial activity was first screened against cytotoxic *V. parahaemolyticus* strains VpKX, PMC53.7, PMA1.15, and control strains *E. coli* BL21 and *S. aureus* ATCC25923. Using the lawn culture method, 10 µL of each extract was spotted onto LB agar plates and incubated at 37 °C for 24 h. Inhibition zones ≥ 2 mm were considered positive. The best-performing plant part was then subjected to a second screening, varying solvent (methanol, ethanol, water) and maceration time (1, 24 and 72 h). The selected extract was subsequently tested against clinical and environmental *V. parahaemolyticus* strains using 5 µL per spot, and sterile water extracts were included as controls for each maceration time. Kanamycin (KAN) (50 µg/mL) and chloramphenicol (CHL) (20 µg/mL) served as positive controls.

Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC was defined as the lowest concentration of Maqui leaf extract (MLE) inhibiting visible growth after 24 h, and the MBC as the lowest concentration killing ≥99.9% of the initial inoculum. MIC and MBC against the reference strain VpKX were determined by two-fold microdilution in Mueller–Hinton broth (MH), starting at 0.45 mg/mL (calculated from ethanolic extract weight after solvent evaporation). All assays were performed in triplicate. Following CLSI guidelines,⁽²⁹⁾ strains were cultured in MH at 37 °C for 18 h and adjusted to 0.5 McFarland, then diluted 1:100 to ~5 × 10⁵ CFU/mL. MLE was serially diluted two-fold (0.45–0.0002 mg/mL). In 96-well plates, 100 µL of each dilution was combined with 100 µL inoculum and incubated at 37 °C for 24 h. Growth was measured by OD₆₀₀, and the MIC was the lowest concentration inhibiting ≥90% growth relative to controls. MBC was determined from MIC wells and higher concentrations. After 24 h, 100 µL from each was plated on MH agar in triplicate and incubated at 37 °C. Optical density at 600 nm (OD₆₀₀) was used to define MIC values, and bactericidal versus bacteriostatic activity was classified based on MBC/MIC ratios. The MBC was defined as the lowest concentration with no detectable growth. The MBC/MIC ratio classified the effect as bactericidal (≤4) or bacteriostatic (>4).^(30,31)

Statistical Analysis

All experiments included in statistical analyses were conducted using at least three independent biological replicates, with technical replicates averaged prior to analysis. Biological replicates were treated as independent observations.

Inhibition zone diameters (mm) were analyzed using one-way or two-way analysis of variance (ANOVA), depending on the experimental design, considering solvent type and maceration time as fixed factors. Analyses were performed separately for each bacterial strain. Water (H₂O) was included as a negative control for reference purposes and was not considered in inferential comparisons between active treatments.

Normality was assessed using the Shapiro–Wilk test. Due to the limited sample size per condition ($n = 3$), formal assessment of distributional assumptions was constrained. Homogeneity of variance was evaluated by examining variance patterns across groups.

Post hoc comparisons were conducted using Bonferroni's test when significant differences were detected. Statistical analyses were performed using GraphPad Prism, version 8.0 (GraphPad Software, Boston, MA, USA). Differences were considered statistically significant at $p < 0.05$.

Ethical Statement

This study did not involve human or animal subjects. Environmental sampling and bacterial handling followed institutional biosafety and ethical guidelines (Universidad Autónoma de Chile and following the guidelines in the Biosafety Manual (2018 version) of the National Commission of Scientific and Technological Research (ANID, Chile).

RESULTS

Pre-screening evaluation of the antimicrobial activity of different extracts of Maqui on cytotoxic strains of *V. parahaemolyticus*

We tested 28 extracts from three parts of Maqui (stem, leaf, and root) using methanol or ethanol as solvents. After evaporation, extracts were resuspended in sterile distilled water. A rapid pre-screening was conducted to identify which plant part inhibited the growth of the cytotoxic strain VpKX (Figure 1A, red circles).

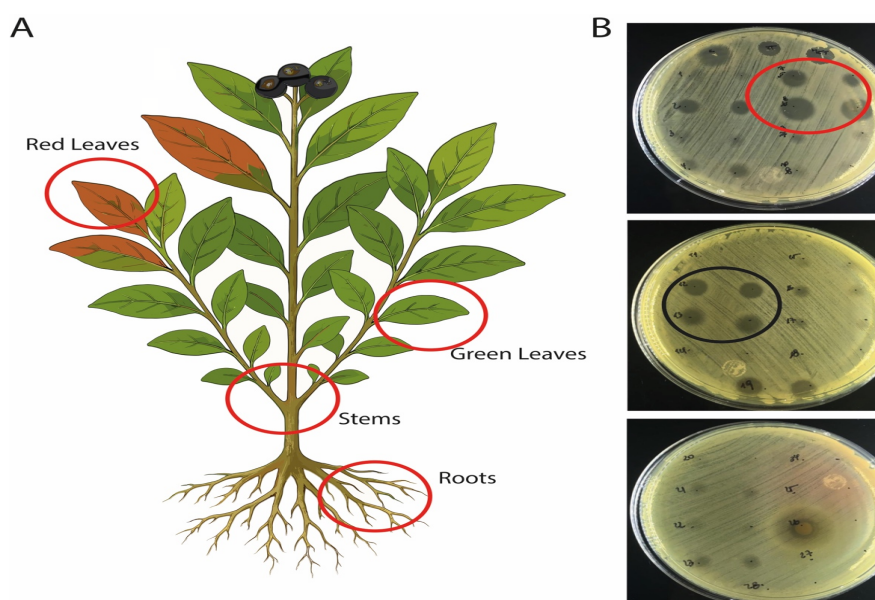


Figure 1. Antimicrobial activity of Maqui against *V. parahaemolyticus* VpKX. (A) Plant illustration showing extract sources: stem, green leaves, red leaves, and roots. (B) Inhibition halos on LB agar; red circles indicate extracts 7–8, black circles are extracts 12–13. Results are representative of two independent experiments.

Pre-screening showed variable inhibition of *V. parahaemolyticus*. For strain VpKX, extracts 7 and 8 (red circle) and extracts 12 and 13 (black circle) produced larger, well-defined inhibition zones (Figure 1B); all were derived from Maqui leaves. Similar assays with PMC53.7, PMA1.15, *E. coli* BL21, and *S. aureus* ATCC25923 are summarized in Table 1.

Table 1. Maqui extracts showing antimicrobial effect over cytotoxicity strains of *V. parahaemolyticus*

Extract	Extract Source	VpKX	PMC53.7	PMA1.15	<i>E. coli</i> BL21	<i>S. aureus</i> ATCC25923
1	Methanol/ green stem	+	-	-	-	+
2	Methanol/ red stem	+	+	+	-	+
4	Methanol/ green leaf	-	-	-	+	-
7	Methanol/ red leaf	+	+	+	-	+
8	Methanol/ green leaf	+	+	+	-	+
11	Methanol/ root	+	-	-	-	-
12	Ethanol/ green leaf	+	+	+	-	+
13	Ethanol/ red leaf	+	+	+	+	+
16	Ethanol/ green leaf	+	-	+	+	-
18	Ethanol/ root	+	-	-	+	-

Legend: "+" effective growth inhibition, "-" negative growth inhibition. The table was constructed with two replicates for each extract and strain, as shown in Figure 1B for the VpKX strain.

Based on Table 1, which lists the ten best results, the most effective extracts were obtained from Maqui leaves. Thus, MLE was selected to test antimicrobial activity against *V. parahaemolyticus* under three extraction conditions, assessing whether process variations enhanced growth inhibition.

Screening of antimicrobial effect of Maqui green leaves extract over cytotoxicity strains of *V. parahaemolyticus*

Antimicrobial activity of MLE was tested against *V. parahaemolyticus* strains VpKX, PMC53.7, and PMA1.15 for the three different maceration times with the solvents.

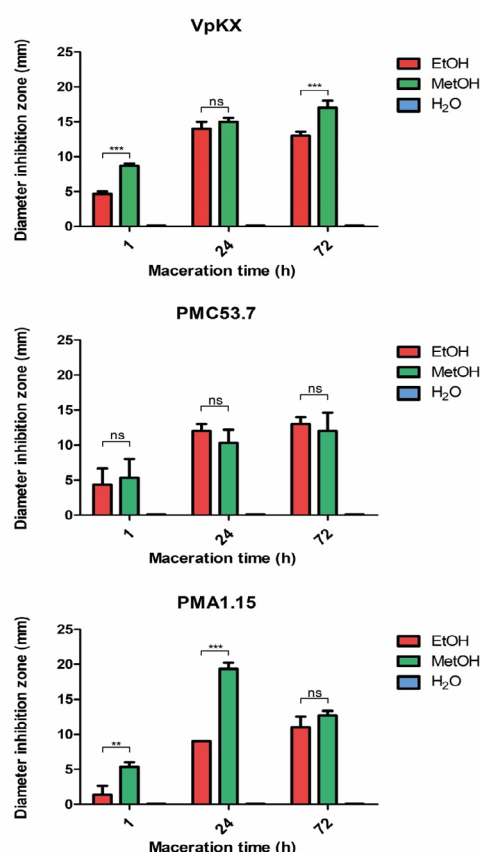


Figure 2. Antimicrobial effect of water-resuspended MLE on *V. parahaemolyticus* strains VpKX, PMC53.7, and PMA1.15. Extracts were macerated for 1, 24, or 72 h with ethanol (EtOH, red), methanol (MetOH, green), or water (H₂O, blue) as a control. Data are presented as mean $\pm$ SD ($n = 3$ biological replicates per condition). Statistical significance between ethanol and methanol within each time point was determined by two-way ANOVA with Bonferroni post hoc test.

Legend: ** $p < 0.01$; *** $p < 0.001$; ns- not significant.

Inhibition zones increased markedly from 1 h to 24 h maceration and remained similar between 24 and 72 h. Methanol extracts generally produced larger inhibition zones than ethanol, particularly for VpKX and PMA1.15 strains. In contrast, no significant differences between solvents were observed for most time points in PMC53.7 (Figure 2). Considering ethanol's lower toxicity and comparable activity at 24 and 72 h, ethanol extracts were selected for further screening against environmental *V. parahaemolyticus* strains.

Antimicrobial activity of Maqui leaf extract on environmental *V. parahaemolyticus* isolated from *M. chilensis*

To evaluate the inhibitory capacity of MLE on the growth of *V. parahaemolyticus*, 21 additional environmental isolates were obtained from mussels purchased at open-air markets across different communes of the Chilean Metropolitan Region. All environmental strains were positive for *tlh* and negative for *tdh* and *trh* (TPc1, TPc3, TPc5, TPc7, MAIc1, MAIc5, MAIc7, MAIc9, PACc2, PACc6, PACc7, PACc8, MACc6, MACc7, MACc8, MACc10, INDc1, INDc2, INDc3, INDc9, INDc10).

Antimicrobial activity assays were performed using MLE resuspended in water (obtained via 24 h ethanolic extraction) (Figure 3). Additionally, KAN (50 µg/mL), CHL (20 µg/mL), and H₂O were included as growth controls (Figure 3).

The results showed that some environmental strains exhibited smaller inhibition zones (Table 1S); however, at least six strains (MACc8, PACc8, TPc1, MACc7, MACc10, and MAIc5) displayed clear inhibition zones, with differential responses to control antibiotics compared to the areas treated with MLE (Figure 3). Strains MACc8, PACc8, and TPc1 showed lower sensitivity to the extract, presenting smaller inhibition zones relative to the antibiotics KAN or CHL (Figure 3A). In contrast, strain MACc7 showed a greater inhibition with MLE than with KAN, while strains MACc10 and MAIc5, which showed minimal to no inhibition when exposed to CHL (an antibiotic to which pandemic strains are typically sensitive; Figure 1S), exhibited greater inhibition in response to MLE (Figure 3B).

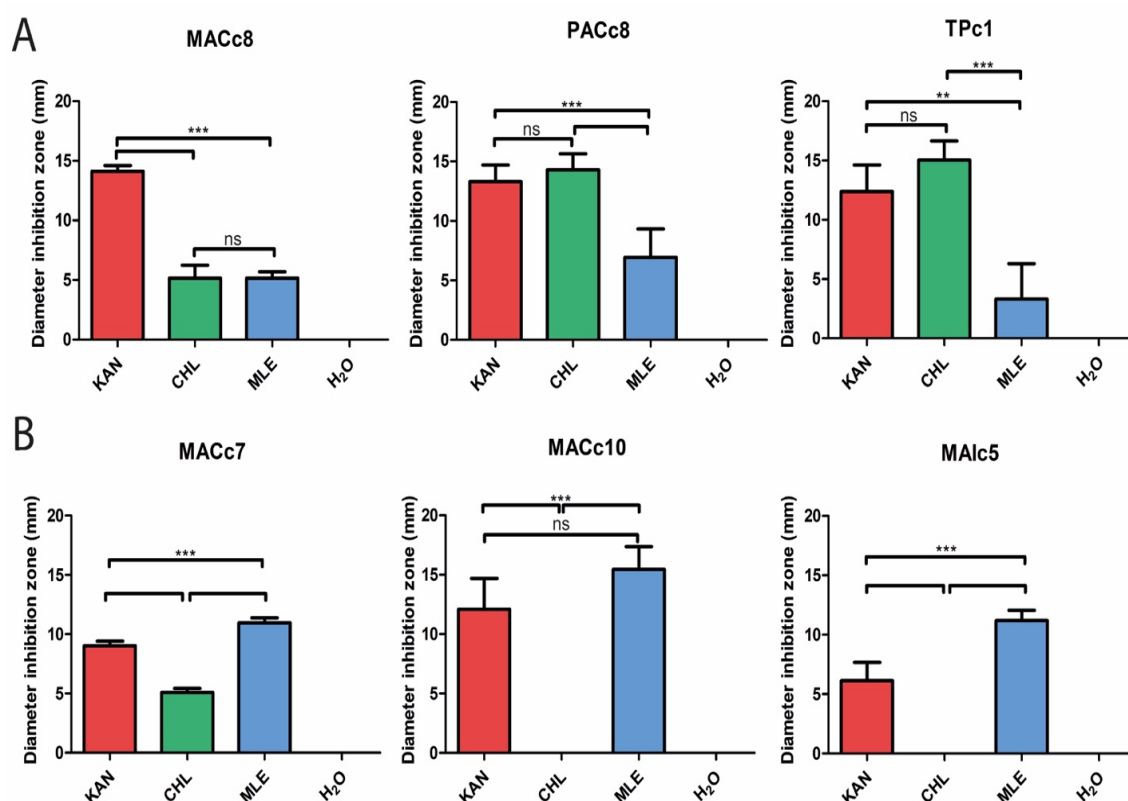


Figure 3. Antimicrobial activity of MLE or traditional antibiotics against environmental *V. parahaemolyticus* strains. (A) KAN and CHL showed higher activity than MLE. (B) MLE was more effective in strains with reduced sensitivity to KAN or CHL. For both panels, H₂O was used as a control. Data are presented as mean $\pm$ SD ($n = 3$). Statistical significance among active treatments within each strain was determined by one-way ANOVA followed by Bonferroni post hoc test.

Legend: ** $p < 0.01$; *** $p < 0.001$; ns- not significant.

Control assays with evaporated solvents resuspended in water showed no inhibition of VpKX (Figure 4), confirming that antimicrobial effects were not due to residual solvent.

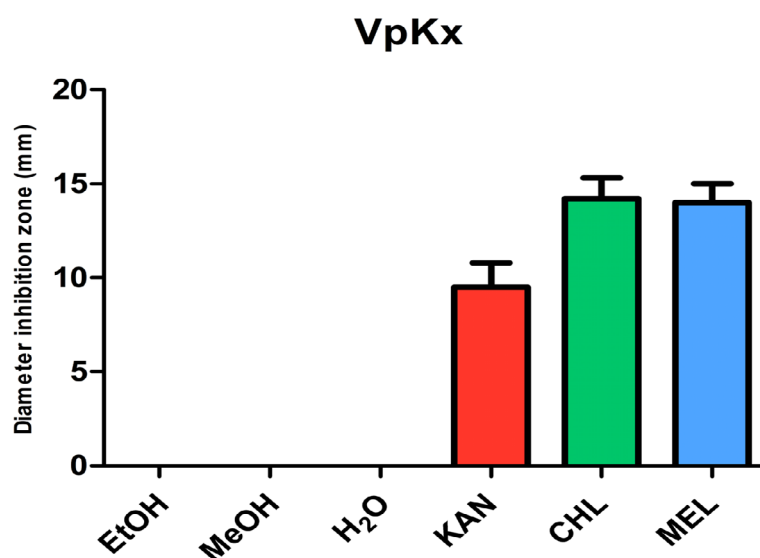


Figure 4. Antimicrobial activity of residual solvents removed by evaporation and antibiotics. The test was performed using the *V. parahaemolyticus* VpKX strain and samples containing only evaporated solvent resuspended in distilled water (EtOH and MeOH)

Determination of MIC and MBC of MLE over a pandemic strain of *V. parahaemolyticus*

The antimicrobial activity of MLE against the pandemic strain VpKX was evaluated by MIC and MBC. MIC assays showed a dose-dependent reduction in viability, with 0.056 mg/mL (1/8 dilution) reducing growth to <10% after 24 h (Figure 5A). At 0.00088 mg/mL (1/512 dilution), viability recovered to ~80%. The MBC, defined as the lowest concentration with no detectable growth, was 0.1125 mg/mL (2×MIC; Figure 5B). The MBC/MIC ratio of 2 indicates bactericidal activity.

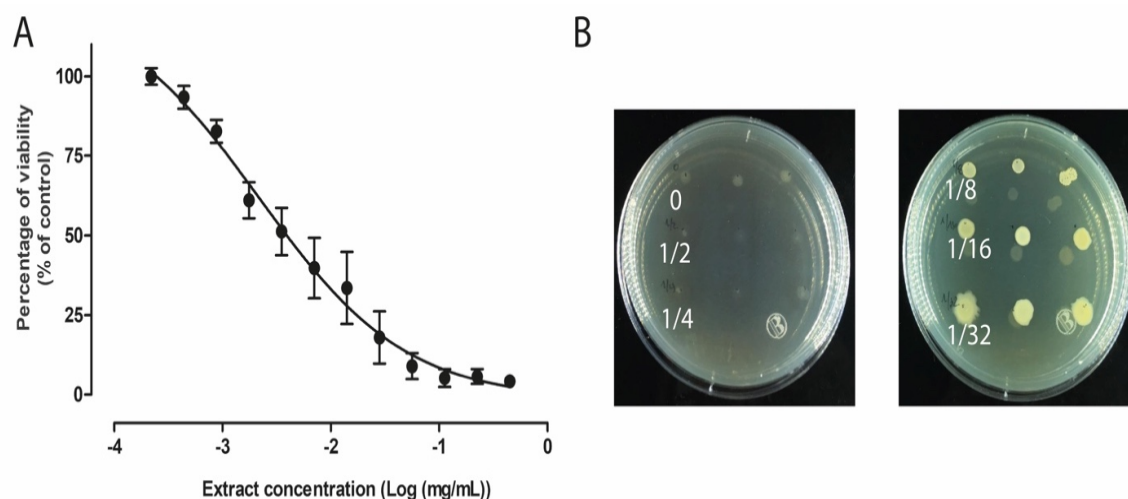


Figure 5. MIC and MBC evaluation of MLE against *V. parahaemolyticus* VpKX. (A) Growth inhibition curve at different extract concentrations (log scale; log 10⁻⁴ = most diluted). (B) Bacterial growth at each dilution used to determine MBC. Data are mean ± SD (n = 3).

DISCUSSION

V. parahaemolyticus is a major cause of seafood-borne illness, particularly where raw shellfish are consumed.⁽³²⁾ In Latin America and the Caribbean, foodborne diseases remain a concern due to limited surveillance, with pathogens like *Salmonella spp.* and *E. coli* posing risks.⁽³³⁾ Likewise, *V. parahaemolyticus* is naturally present in estuarine environments and associated with shellfish, emphasizing the need for surveillance and prevention.^(4,6) Plant extracts from agricultural by-products offer promising antibacterial properties against foodborne pathogens, meeting consumer demand for sustainable alternatives and the urgent need for novel strategies against antimicrobial resistance.⁽³⁴⁾

In this study, 28 Maqui extracts were screened, with leaves showing the strongest inhibitory effect against cytotoxic and pathogenic strains. Since leaves are usually discarded, their use represents a low-cost by-product valorization strategy with food safety potential.⁽¹⁶⁾ Pérez et al. profiled Maqui leaves, identifying phenolic acids, flavonoids, anthocyanins, and stilbenes.⁽²¹⁾ Reported metabolites include indole alkaloids, terpenes, and polyphenols such as ethyl caffeate and quercetin, compounds with recognized antimicrobial activity.⁽²¹⁾ Quercetin inhibits diverse bacteria including *S. aureus*, *Pseudomonas aeruginosa*, *Shigella sonnei*, Methicillin-resistant *Staphylococcus aureus* (MRSA),⁽²⁰⁾ *Serratia marcescens*,⁽³⁵⁾ *Salmonella enterica*, and *E. coli*.⁽³⁶⁾ It affects metabolism, inhibits extracellular polysaccharide synthesis, disrupts bacterial membranes,⁽³⁵⁾ and interferes with ATP activity.⁽³⁷⁾ Polyphenols from Maqui fruit also display antibacterial effects through membrane disruption and metabolic interference.⁽³⁸⁾ These findings support that Maqui leaf compounds underlie the observed inhibition, though our study did not isolate specific molecules.

Future work should assess extraction yield, pH-matched controls, and chemical profiling (UHPLC-MS, NMR) to identify active constituents. Extraction conditions influenced efficacy: ethanol and methanol extracts were active, while aqueous extracts were not, suggesting lipophilic bioactives. Longer extraction (>24 h) did not enhance activity, indicating sufficiency of shorter periods. Ethanolic extracts were chosen for their lower toxicity and suitability for food applications. Testing against strains from Chilean mussels showed variable inhibition, including in chloramphenicol-resistant isolates, suggesting mechanisms distinct from conventional antibiotics. This supports MLE as a candidate for combating antimicrobial resistance, especially in low-resource regions.⁽³⁹⁾ Quantitative assays confirmed bactericidal activity against VpKX (MIC 0.056 mg/mL; MBC 0.1125 mg/mL), comparable to or better than other plant antimicrobials such as punicalagin (MIC 0.15–0.20 mg/mL),⁽³⁴⁾ and berberine (MIC 0.03 mg/mL).⁽⁴⁰⁾ Solvent controls ruled out residual ethanol effects. In vivo studies and food matrix applications are needed, but activity against both clinical and environmental strains, including resistant isolates, is relevant for Caribbean contexts. In Cuba, where raw oyster consumption and weak cold-chain infrastructure elevate risks,⁽⁸⁾ preventive post-harvest interventions are necessary.

Current methods like thermal or high-pressure treatments may be inaccessible to artisanal fisheries. Locally sourced plant extracts could serve as rinsing or dipping agents to reduce bacterial loads without affecting sensory quality. Broader applications include testing against *Salmonella spp.*, *E. coli*, and *L. monocytogenes*, key pathogens in Latin America and the Caribbean, where foodborne diseases remain a persistent concern and public health challenges.⁽³³⁾ Integrating such strategies could reduce gastroenteritis burden, strengthen food safety in artisanal seafood supply chains, and promote circular economy practices by valorizing agro-industrial by-products.

This study was limited by its *in vitro* design, the absence of compound identification, and the evaluation of a single plant species without comparison to other natural extracts. Future work should include chemical characterization, toxicity assessment, and validation in real seafood matrices to confirm the practical applicability of these findings. Even within these **limitations**, this study highlights the potential of native South American plants as sustainable sources of bioactive compounds for food safety applications.

CONCLUSIONS

This study demonstrates that ethanolic MLE inhibits clinical and environmental *Vibrio parahaemolyticus*, including antibiotic-resistant isolates. These findings support MLE as a low-cost, sustainable plant by-product aligned with sustainability objectives, and as a feasible alternative or complement to antibiotics in resource-limited regions affected by *Vibrio* infections.

ACKNOWLEDGMENTS

We thank Victoria Villegas and Alequis Pavon for their excellent technical assistance.

REFERENCES

1. Pruzzo C, Gallo G, Canesi L. Persistence of vibrios in marine bivalves: the role of interactions with haemolymph components. *Environ Microbiol* [Internet]. 2005;7(6):761–72. Available from: <http://doi.org/10.1111/j.1462-2920.2005.00792.x>
2. Flores-Villaseñor H, Velázquez-Román J, León-Sicaire N, Angulo-Zamudio UA, Lira-Morales C, Martínez-García JJ, et al. Serodiversity, antibiotic resistance, and virulence genes of *Vibrio parahaemolyticus* in oysters collected in coastal areas of northwestern Mexico between 2012 and 2020. *Food Microbiol* [Internet]. 2024;123:104567. Available from: <http://doi.org/10.1016/j.fm.2024.104567>
3. Letchumanan V, Chan KG, Lee LH. *Vibrio parahaemolyticus*: a review on the pathogenesis, prevalence, and advanced molecular identification techniques. *Front Microbiol* [Internet]. 2014;5:705. Available from: <http://doi.org/10.3389/fmicb.2014.00705>
4. Gugnani HC, Chopra Q, Currie A, Ostrer T, Wiegersma W, Ray P. Occurrence of *Vibrio* species in marine sources surrounding Bonaire, Dutch Caribbean. *Eur J Med Health Sci* [Internet]. 2022;4(5):153–62. Available from: <http://doi.org/10.34104/ejmhs.022.01530162>
5. Jenson D, Szabo V. Cholera in Haiti and other Caribbean regions, 19th century. *Emerg Infect Dis* [Internet]. 2011;17(11):2130–5. Available from: <http://doi.org/10.3201/eid1711.110958>
6. Raszl SM, Froelich BA, Vieira CR, Blackwood AD, Noble RT. *Vibrio parahaemolyticus* and *Vibrio vulnificus* in South America: water, seafood and human infections. *J Appl Microbiol* [Internet]. 2016;121(5):1201–22. Available from: <http://doi.org/10.1111/jam.13246>
7. Campbell AM, Gavilan RG, Abanto Marin M, Yang C, Hauton C, van Aerle R, et al. Evolutionary dynamics of the successful expansion of pandemic *Vibrio parahaemolyticus* ST3 in Latin America. *Nat Commun* [Internet]. 2024;15(1):7828. Available from: <http://doi.org/10.1038/s41467-024-52159-y>
8. Martínez-Alfonso Y, Lantero-Abreu MI. Presencia de *Vibrio parahaemolyticus* en masa de ostión comercializada en localidades al sureste de Cuba. *Ciencia y Tecnología de Alimentos* [Internet]. 2019;29(1):21–6 [cited 04/02/2026]. Available from: <https://revcitecal.iiia.edu.cu/revista/index.php/RCTA/article/view/8>
9. US Food and Drug Administration. Appendix 5: FDA and EPA safety levels in regulations and guidance. In: *Fish and fishery products hazards and controls guidance*. 4 ed [Internet]. EE.UU: Silver Spring; 2021 [cited 04/02/2026]. Available from: <https://www.fda.gov/media/80400/download>
10. Calik H, Morrissey MT, Reno PW, An H. Effect of high-pressure processing on *Vibrio parahaemolyticus* strains in pure culture and Pacific oysters. *J Food Sci* [Internet]. 2002;67(4):1506–10. Available from: <http://doi.org/10.1111/j.1365-2621.2002.tb10313.x>
11. Ye M, Huang Y, Chen H. Inactivation of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in oysters by high-hydrostatic pressure and mild heat. *Food Microbiol* [Internet]. 2012;32(1):179–84. Available from: <http://doi.org/10.1016/j.fm.2012.05.009>
12. Al-Garadi MA, Aziz RN, Almashhadany DA, Al Qabili DMA, Abdullah Aljoborey AD. Validity of cold storage and heat treatment on the deactivation of *Vibrio parahaemolyticus* isolated from fish meat markets. *Ital J Food Saf* [Internet]. 2024;13(1):11516. Available from: <http://doi.org/10.4081/ijfs.2024.11516>
13. Wang W, Li M, Li Y. Intervention strategies for reducing *Vibrio parahaemolyticus* in seafood: a review. *J Food Sci* [Internet]. 2015;80(1):R10–9. Available from: <http://doi.org/10.1111/1750-3841.12727>
14. Ma Y, Wang R, Zhang T, Xu Y, Jiang S, Zhao Y. High hydrostatic pressure treatment of oysters (*Crassostrea gigas*)—impact on physicochemical properties, texture parameters, and volatile flavor compounds. *Molecules* [Internet]. 2021;26(19):5731. Available from: <http://doi.org/10.3390/molecules26195731>
15. DePaola A. Managing *Vibrio* risk in oysters. *Food Prot Trends*. 2019;39(4):338–47.
16. Enciso-Martínez Y, Zuñiga-Martínez BS, Ayala-Zavala JF, Domínguez-Avila JA, González-Aguilar GA, Viuda-Martos M. Agro-industrial by-products of plant origin: therapeutic uses as well as antimicrobial and antioxidant activity. *Biomolecules* [Internet]. 2024;14(7):762. Available from: <http://doi.org/10.3390/biom14070762>
17. US Department of Agriculture, Bureau of Plant Industry. *Inventory of seeds and plants imported*. Washington (DC): Government Printing Office; 1914.
18. García-Milla P, Peñalver R, Nieto G. A review of the functional characteristics and applications of *Aristotelia chilensis* (maqui berry), in the food industry. *Foods* [Internet]. 2024;13(6):838. Available from: <http://doi.org/10.3390/foods13060838>
19. Vidal L, Avello L, Loyola C, Campos J, Aqueveque P, Dungan SR, et al. Microencapsulation of maqui (*Aristotelia chilensis* Molina Stuntz) leaf extracts to preserve and control antioxidant properties. *Chil J Agric Res* [Internet]. 2013;73(1):17–23. Available from: <http://doi.org/10.4067/S0718-58392013000100003>
20. Nguyen TLA, Bhattacharya D. Antimicrobial activity of quercetin: an approach to its mechanistic principle. *Molecules* [Internet]. 2022;27(8):2494. Available from: <http://doi.org/10.3390/molecules27082494>

21. Pérez R, Figueredo C, Burgos V, Cabrera-Pardo JR, Schmidt B, Heydenreich M, et al. Natural compounds purified from the leaves of *Aristolelia chilensis*: makomakinol, a new alkaloid and the effect of aristoteline and hobartine on NaV channels. *Int J Mol Sci* [Internet]. 2023;24(21):15504. Available from: <http://doi.org/10.3390/ijms242115504>
22. Makino K, Oshima K, Kurokawa K, Yokoyama K, Uda T, Tagomori K, et al. Genome sequence of *Vibrio parahaemolyticus*: a pathogenic mechanism distinct from that of *V. cholerae*. *Lancet* [Internet]. 2003;361(9359):743–9. Available from: [http://doi.org/10.1016/S0140-6736\(03\)12659-1](http://doi.org/10.1016/S0140-6736(03)12659-1)
24. Pérez-Reytor D, Pavón A, Lopez-Joven C, Ramírez-Araya S, Peña-Varas C, Plaza N, et al. Analysis of the zonula occludens toxin found in the genome of the Chilean non-toxigenic *Vibrio parahaemolyticus* strain PMC53.7. *Front Cell Infect Microbiol* [Internet]. 2020;10:482. Available from: <http://doi.org/10.3389/fcimb.2020.00482>
25. Castillo D, Pérez-Reytor D, Plaza N, Ramírez-Araya S, Blondel CJ, Corsini G, et al. Exploring the genomic traits of non-toxigenic *Vibrio parahaemolyticus* strains isolated in southern Chile. *Front Microbiol* [Internet]. 2018;9:161. Available from: <http://doi.org/10.3389/fmicb.2018.00161>
26. Bej AK, Patterson DP, Brasher CW, Vickery MC, Jones DD, Kaysner CA. Detection of total and hemolysin-producing *Vibrio parahaemolyticus* in shellfish using multiplex PCR amplification of *tlh*, *tdh* and *trh*. *J Microbiol Methods* [Internet]. 1999;36(3):215–25. Available from: [http://doi.org/10.1016/s0167-7012\(99\)00037-8](http://doi.org/10.1016/s0167-7012(99)00037-8)
27. Lillo Valenzuela JE. Evaluación de metabolitos secundarios de interés comercial en tejido vegetativo de maqui (*Aristolelia chilensis*) mediante su crecimiento en biorreactores [thesis]. Santiago: Universidad de Las Américas; 2018.
28. Rivera-Tovar PR, Torres MD, Camilo C, Mariotti-Celis MS, Domínguez H, Pérez-Correa JR. Multi-response optimal hot pressurized liquid recovery of extractable polyphenols from leaves of maqui (*Aristolelia chilensis* [Mol.] Stuntz). *Food Chem* [Internet]. 2021;357:129729. Available from: <http://doi.org/10.1016/j.foodchem.2021.129729>
29. Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically standard M07. 11 ed. Pennsylvania: CLSI; 2018.
30. Pankey GA, Sabath LD. Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of Gram-positive bacterial infections. *Clin Infect Dis* [Internet]. 2004;38(6):864–70. Available from: <http://doi.org/10.1086/381972>
31. Bury-Moné S. Minimal inhibitory and bactericidal concentrations *in vitro*. In: Antibacterial therapeutic agents: antibiotics and bacteriophages [Internet]. Philadelphia: Elsevier; 2014. Available from: <http://doi.org/10.1016/B978-0-12-801238-3.00244-0>
32. Rezny BR, Evans DS. *Vibrio parahaemolyticus* infection. [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 [cited 04/02/2026]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK459164>
33. Severino N, Reyes C, Fernandez Y, Azevedo V, Francisco LED, Ramos RT, et al. Bacterial foodborne diseases in Central America and the Caribbean: a systematic review. *Microbiol Res* [Internet]. 2025;16(4):78. Available from: <http://doi.org/10.3390/microbiolres16040078>
34. Liu H, Zhu W, Zou Y, Xia X. Antimicrobial activity and mechanisms of punicalagin against *Vibrio parahaemolyticus*. *Foods* [Internet]. 2024;13(9):1366. Available from: <http://doi.org/10.3390/foods13091366>
35. Kannan S, Balakrishnan J, Govindasamy A, Arunagiri R. New insights into the antibacterial mode of action of quercetin against uropathogen *Serratia marcescens* *in-vivo* and *in-vitro*. *Sci Rep* [Internet]. 2022;12(1):21912. Available from: <http://doi.org/10.1038/s41598-022-26621-0>
36. Qi W, Qi W, Xiong D, Long M. Quercetin: its antioxidant mechanism, antibacterial properties and potential application in prevention and control of toxipathy. *Molecules* [Internet]. 2022;27(19):6545. Available from: <http://doi.org/10.3390/molecules27196545>
37. Plaper A, Golob M, Hafner I, Oblak M, Šolmajer T, Jerala R. Characterization of quercetin binding site on DNA gyrase. *Biochem Biophys Res Commun* [Internet]. 2003;306(2):530–6. Available from: [http://doi.org/10.1016/S0006-291X\(03\)01006-4](http://doi.org/10.1016/S0006-291X(03)01006-4)
38. Genskowsky E, Puente LA, Pérez-Álvarez JA, Fernández-López J, Muñoz LA, Viuda-Martos M. Determination of polyphenolic profile, antioxidant activity and antibacterial properties of maqui [*Aristolelia chilensis* (Molina) Stuntz] a Chilean blackberry. *J Sci Food Agric* [Internet]. 2016;96(12):4235–42. Available from: <http://doi.org/10.1002/jsfa.7628>

39. Iskandar K, Molinier L, Hallit S, Sartelli M, Hardcastle TC, Haque M, et al. Surveillance of antimicrobial resistance in low- and middle-income countries: a scattered picture. *Antimicrob Resist Infect Control* [Internet]. 2021;10(1):63. Available from: <http://doi.org/10.1186/s13756-021-00931-w>

40. Zheng H, Liu Y, Cai J, Zhang M, Wen Y, Guo L. The exploration of anti-*Vibrio parahaemolyticus* substances from *Phellodendri Chinensis* cortex as a preservative for shrimp storage. *Front Microbiol* [Internet]. 2022;13:1004262. Available from: <http://doi.org/10.3389/fmicb.2022.1004262>

Funding statement

This work was supported by Internal Project Genera UA1703 of Universidad Autónoma de Chile (KG), Competitive Funds of Universidad de Las Américas, Chile, Project UDLAPI2018026 (LP), Fondecyt postdoctoral 3130660 ANID (LP), Fondecyt de Iniciación 11231192 of ANID (NP), and Research Direction grant DIUA 313-2025 of VRID Autonomous University of Chile (IMU).

Conflict of interests

The authors declare that they have no conflicts of interest in the research.

Authors' contributions

Nicolás Plaza: Conceptualization; data curation; analysis; writing - original draft; drafting-revision and editing.

Camila Manzano: Data curation; methodology; validation.

Sebastián Ramírez-Araya: Data curation; validation; drafting - revision and editing.

Constanza Griffiths-Sanhueza: Data curation; analysis; drafting - revision and editing.

Ítalo M. Urrutia: Analysis; drafting - revision and editing.

Diliana Pérez-Reytor: Analysis; drafting - revision and editing.

Gino Corsini: Acquisition of funds; research; methodology.

Marcelo Ortega: Research; methodology.

Talía del Pozo: Research; methodology.

Leonardo Pavez: Conceptualization; acquisition of funds; research; project administration; resources; supervision.

Katherine García: Conceptualization; acquisition of funds; research; project administration; resources; supervision; writing - original draft; drafting - revision and editing.

All the authors have participated in the discussion of the results and have read, reviewed, and approved the final text of the article.