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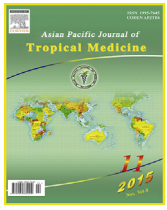

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Vibrio spp. from *Macrobrachium amazonicum* prawn farming are inhibited by *Moringa oleifera* extracts

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ABSTRACT

Objective: To investigate the *in vitro* antimicrobial potential of extracts of stem, leaves, flowers, pods and seeds of *Moringa oleifera* (*M. oleifera*) against *Vibrio* spp. from hatchery water and the prawn *Macrobrachium amazonicum*.
Methods: The ethanol extracts of stem, leaves, pods and seeds and chloroform extract of flowers of *M. oleifera* were tested against *Vibrio cholerae* (*V. cholerae*) serogroups non-O1/non-O139 (n = 4), *Vibrio vulnificus* (n = 1) and *Vibrio mimicus* (n = 1). *Escherichia coli* (*E. coli*) (ATCC® 25922) was used as quality control. *Vibrio* species were obtained from *Macrobrachium amazonicum* prawns and from hatchery water from prawn farming. The Minimum Inhibitory Concentration (MIC) was determined by broth microdilution method.
Results: The best result was obtained with the ethanol extract of pods, which inhibited three strains of the *V. cholerae*, *Vibrio vulnificus, Vibrio mimicus* and *E. coli* (MIC range 0.312–5.000 mg/mL). The chloroform extract of flowers was effective against all *V. cholerae* strains and *E. coli* (MIC range 0.625–1.250 mg/mL). However, the ethanol extracts of stem and seeds showed low effectiveness in inhibiting the bacterial growth.
Conclusions: The extracts of pods, flowers and leaves of *M. oleifera* have potential for the control of *Vibrio* spp. Further studies are necessary to isolate the bioactive compounds responsible for this antimicrobial activity.

1. Introduction

The cultivation of shrimp can be threatened by diseases caused by *Vibrio* species, which can result in up to 100% mortality, 24 h after the appearance of infection [1]. *Vibrio cholerae* (*V. cholerae*), *Vibrio mimicus* (*V. mimicus*) and *Vibrio vulnificus* (*V. vulnificus*) are opportunistic pathogens capable of causing lethal infections in farmed crustaceans when there are stressful environmental conditions, nutritional imbalance and predisposing lesions [2]. Moreover, antimicrobial resistance in these microorganisms has been observed [3].

The emergence of antibiotic resistant bacteria has driven research to find new compounds with antimicrobial properties in plants used in traditional medicine, such as *Moringa oleifera* (*M. oleifera*) (Lam.) [4–10].

M. oleifera is a well-known and widely distributed tree species, belonging to the family Moringaceae [11]. In Brazil it can be found in the Northeast, mainly in the states of Maranhão, Piauí and Ceará [9]. The antimicrobial properties of *M. oleifera* have been attributed to different parts of the plant, such as leaves, flowers, seeds, pods and stems [9,12,13]. The

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literature reports the antimicrobial potential of *Moringa* against bacteria and fungi isolated from shrimp farming [5,9].

Thus, the objective of this study was to evaluate the *in vitro* antimicrobial potential of extracts of stem, leaves, flowers, pods and seeds of *M. oleifera* against *Vibrio* species isolated from hatchery water and *Macrobrachium amazonicum* (*M. amazonicum*) prawn.

2. Materials and methods

2.1. Extracts

The extracts were obtained from specimens of *M. oleifera* grown in Fortaleza, Ceará, Brazil, and provided by the Laboratory of Applied Phytochemistry, Federal University of Ceará. Stem, leaves, pods and seeds were dried in a heated chamber at 40 °C and then subjected to three successive extractions by cold maceration with ethanol at intervals of 24 h, originating the ethanol extracts, while flowers were dried at 40 °C and then subjected to three successive extractions by cold maceration with chloroform at intervals of 24 h, originating the chloroform extract. After filtration, the respective solvents were evaporated under reduced pressure in a rotary evaporator, leaving only the concentrated constituents extracted from the plant parts [7].

2.2. Strains of Vibrio spp.

V. cholerae serogroups non-O1/non-O139 (n = 4), *V. mimicus* (n = 1) and *V. vulnificus* (n = 1), belonging to the bacterial collection of the Laboratory of Emerging and Reemerging Pathogens of Ceará Federal University, were used in this study. These strains were obtained through the collection of specimens of ovigerous *M. amazonicum* females from Sapiranga Lake (3°48′3.46″ S and 38°27′30.83″ W), Fortaleza, Ceará, Brazil, and samples of hatchery water from *M. amazonicum* farming, during the larval development stage, at the Laboratory of Shrimp Farming of the State University of Ceará.

2.3. In vitro susceptibility test

The *in vitro* susceptibility test with extracts of *M. oleifera* was performed following the method described by Rocha [7] in 2011, with some modifications. Initially, each extract was dissolved in dimethyl sulfoxide (DMSO) (LGC Biotecnologia Ltda, São Paulo, Brazil) and then diluted in Müeller-Hinton broth (Difco™, São Paulo, Brazil). In previous tests with strains of *Vibrio* spp., we verified that DMSO alone at concentrations up to 5% was not able to inhibit the growth of these strains. Thus, the concentration of DMSO in the susceptibility test did not exceed 5%, to assure that results refer to the action of each extract tested [7].

Minimum inhibitory concentration (MIC) of *M. oleifera* extracts against strains of *Vibrio* spp. was determined through the broth microdilution method as standardized by the Clinical Laboratory Standards Institute, based on the document M07-A9 [14]. The MIC was considered the lowest concentration of the extracts able to inhibit 50% of bacterial growth compared to the control growth [9]. The strain *Escherichia coli* (*E. coli*) (ATCC® 25922) was included as quality control. The initial concentration of each extract used was 20 mg/mL and the range of concentrations evaluated in the susceptibility test was from 0.01 to 5.00 mg/mL. The microdilution assays were performed in 96 well plates with a final volume of 200 μL, incubated at 35 °C and read after 20 h, according to the document M45-A2 [15]. The inocula were prepared at a turbidity of 0.5 on McFarland scale (108 CFU/mL), and then diluted with Müller-Hinton broth so that each well after inoculation presented approximately 5 × 105 CFU/mL. All assays were performed in duplicate, and for each strain growth control and sterility control of the culture medium were included [14]. The reading was performed with a spectrophotometer (BioteK®, Winooski, United State) at 590 nm and the obtained absorbance values were corrected by the absorbance obtained for each tested extract alone. Only extracts that inhibited the control strain (*E. coli* ATCC® 25922) were considered to have antimicrobial activity against *Vibrio* spp. Chloramphenicol (Sigma–Aldrich® Brazil Ltda, São Paulo, Brazil) was used as standard antibiotic, as recommended by the document M100-S22 [16].

2.4. Research licensing

This study was previously approved by the Chico Mendes Institute for Conservation of Biodiversity/Biodiversity Authorization and Information System – SISBIO, under the number 28175-1.

3. Results

The ethanol pod extract showed the best inhibitory activity against isolates from the hatchery water, with MIC values ranging from 0.3125 to 1.250 mg/mL against three strains of *V. cholerae* non-O1/non-O139 (3/4) and an MIC of 5 mg/mL against *V. vulnificus*. This extract also showed inhibitory effect against *V. mimicus* from the digestive tract of *M. amazonicum* and *E. coli* (ATCC® 25922), with MIC values of 1.25 mg/mL and 2.50 mg/mL, respectively (Table 1).

Table 1
MIC values of *Moringa oleifera* extracts against *Vibrio* spp. recovered from hatchery water and *Macrobrachium amazonicum* prawn.

Source	Species	MIC (mg/mL)					MIC (μg/mL)
		Ethanol stem	Ethanol leaves	Chloroform flowers	Ethanol pods	Ethanol seeds	Chloramphenicol
Hatchery water	*V. cholerae*	n.i	0.078	1.25	0.312 5	5	0.5
	V. cholerae	n.i	0.625	0.625	n.i	n.i	0.5
	V. cholerae	n.i	n.i	0.625	1.25	n.i	0.5
	V. cholerae	2.5	n.i	0.625	0.312 5	2.5	0.5
	V. vulnificus	n.i	n.i	n.i	5	n.i	0.5
Prawn	*V. mimicus*	1.25	5	n.i	1.25	n.i	0.5
	E. coli ATCC®M25922	n.i	5	1.25	2.5	n.i	4