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ANTIBACTERIAL EFFECT (*in vitro*) OF *Moringa oleifera* AND *Annona muricata* AGAINST GRAM POSITIVE AND GRAM NEGATIVE BACTERIA

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SUMMARY

Antibacterial effects of aqueous and ethanolic extracts of seeds of moringa (*Moringa oleifera*) and pods of soursop (*Annona muricata*) in the concentration of 1:5 and 1:10 in volumes 50, 100, 150 and 200 µL were examined against *Staphylococcus aureus*, *Vibrio cholerae*, *Escherichia coli* (isolated from the organism and the aquatic environment) and *Salmonella* Enteritidis. Antibacterial activity (inhibition halo > 13 mm) against *S. aureus*, *V. cholerae* and *E. coli* isolated from the whiteleg shrimp, *Litopenaeus vannmaei,* was detected in aqueous and ethanolic extracts of moringa. *E. coli* isolated from tilapiafish, *Oreochromis niloticus*, was sensitive to the ethanolic extract of moringa. The aqueous extracts of soursop showed an antibacterial effect against *S. aureus* and *V. cholerae*, but the antibacterial activity by the ethanol extracts of this plant was not demonstrated.

KEYWORDS: Antibacterial effect; *Moringa oleifera*; *Annona muricata.*

INTRODUCTION

Multiresistant bacterial strains are a growing public health concern worldwide[4,12,22] justifying investments in the search for alternative forms of treatment of infections. As a result, a number of medicinal plants used in indigenous medicine have been tested and found to possess bactericidal properties[11,30,32,35,43].

The moringa plant (*Moringa oleifera*) has been the object of much research due to its multiple uses and well-known bactericidal potential[9,37,17,38]. According to BEZERRA *et al*.[8], the moringa tree is native to northeastern India. It is rich in nutrients and, apart from a range of industrial and medicinal applications, is used to purify water for human consumption. Not surprisingly, as explained by MAKKAR & BECKER[29], the moringa is of economic importance in the production of several commodities, such as oils, foods, condiments and medicines.

Likewise, the soursop (*Annona muricata*) is employed for a number of medicinal purposes[6]. It is found in most of tropical America and, though not native to Brazil, is grown extensively in the northern and northeastern regions of the country[36]. CAVALCANTE *et al*.[10] has shown that the semiarid climate of northeastern Brazil offers the ideal combination of temperature, moisture, sunlight and soil composition for soursop cultivation.

The objective of this study was to evaluate the bactericidal effect of moringa and soursop extracts upon four bacterial species commonly associated with food intoxication: *Staphylococcus aureus*, *Vibrio cholerae*, *Escherichia coli* and *Salmonella* spp.

MATERIALS AND METHODS

Origin of strains: The controls used in the experiment were standard strains of *S. aureus* ATCC25923, *V. cholerae* classic 569B and *Samonella* Enteritidis obtained from the Environment and Seafood Microbiology Laboratory of the Marine Sciences Institute (LABOMAR, Federal University of Ceará). *E. coli* strains were isolated from water samples from a local lake (Lagoa da Fazenda, Sobral, Ceará) and river (Rio Acaraú, Sobral, Ceará) and from aquiculture livestock (Tilapia, *Oreochromis niloticus*, and Pacific white shrimp, *Litopenaeus vannamei*). *E. coli* was isolated with the method described by FENG *et al*.[15] including testing with indole, methyl red, acetoin (Voges-Proskauer), citrate and H_2S, and confirmation by lactose fermentation. All strains were stored in tryptone soy agar (TSA/Difco) until the moment of testing.

Preparation of extracts: To prepare the moringa extracts, 30g of moringa seeds were homogenized with 150 mL distilled water or 150 mL ethanol p.a. in a magnetic stirrer for 30 minutes. The water-based and ethanol-based homogenates were then paper-filtered and spread on Petri dishes in the amounts 50, 100, 150 and 200 µL. Similarly, to prepare the soursop extracts, 10g of soursop peel was homogenized in 100 mL distilled water or 100 mL ethanol p.a. in a magnetic stirrer for 30 minutes, after which the water-based and ethanol-based homogenates were spread

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on Petri dishes in the amounts 50, 100, 150 and 200 µL. All moringa and soursop samples were obtained from the Center for Nutrition and Food Production of the Vale do Acaraú State University (NUNPRA/UVA), and specimens were deposited in the herbarium of the same institution under entry numbers 5823, 5824, 5825 and 5826.

Evaluation of bactericidal effects: The bactericidal effect of the extracts was evaluated with the modified Kirby-Bauer disk diffusion method[7]. The strains were inoculated in TSA and incubated at 35 ºC for 24 hours. Cultures were then adjusted to a concentration of 10^8 CFU/mL by making a suspension in 0.85% saline solution match the 0.5 McFarland turbidity standard[21]. Using a sterilized swab, aliquots from each tube were spread on dishes with Muller-Hinton agar (Difco), extract was added and incubated at 35 ºC for 24 hours. Disks soaked with sterile distilled water and ethanol p.a. were used as negative control. Extracts producing halos of bacterial growth inhibition greater than 13 mm were considered effective[34].

RESULTS AND DISCUSSION

Water-based moringa extracts efficiently inhibited (halo > 13 mm) the growth of *S. aureus*, *V. cholerae* and *E. coli* isolated from shrimp samples, especially at 150 and 200 µL/dish. Similar strains isolated from tilapia and shrimp samples were inhibited by ethanol-based moringa extracts at all the volumes tested (Table 1).

Likewise, all volumes of soupsop extract were bactericidal to *S. aureus* and *V. cholerae*. The greatest halos (16 and 23 mm) were observed at 200 µL/dish (Table 2).

In the present study moringa extracts were found to be more bactericidal than soursop extracts (Fig. 1). Nevertheless, in an investigation of the active substances contained in moringa seeds, EILERT *et al*.[13] satisfactorily inhibited the growth of *Mycobacterium phlei* with 40 µM extract/L and that of *Bacillus subtilis* with 56 µM extract/L, compared to the greater volumes used in our study (10g/100 mL).

JAHN *et al*.[24] identified the bactericidal substances in moringa seeds as pterygospermin, moringine and the glycosides 4-(α-L-rhamnosyloxy)-benzylisothiocyanate and 4-(α-L-rhamnosyloxy)-phenylacetonitrile. These substances have been shown to inhibit mainly *Bacillus subtilis*, *Mycobacterium phei*, *Serratia marcescens*, *E. coli*, *Pseudomonas aeruginosa*, *Shigella* and *Streptococcus*. According to GALLÃO *et al*.[16], moringa seeds consist of mostly proteins (approximately 40%),

Table 1
Bactericidal effect of water- and ethanol-based moringa extracts (*Moringa oleifera)*

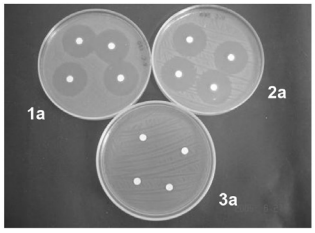
Extract	Volume µL/dish	Diameter of inhibitory halo (mm)						
		SA	VC	SAL	EC_1	EC_2	EC_3	EC_4
Water-based	50	19	21	-	-	16	-	-
	100	22	22	-	-	17	-	-
	150	25	24	-	-	20	-	-
	200	25	25	-	-	23	-	-
Ethanol-based	50	26	26	-	22.5	21	-	-
	100	26	28	-	23	24	-	-
	150	27	28	-	24	25	-	-
	200	28	29.5	-	27	27	-	-

* -; no activity; SA (*Staphylococcus aureus* ATCC25923); VC (*Vibrio cholerae* classic 569B); SAL (*Salmonella* Enteritidis); EC_1 (*Escherichia coli* - fish); EC_2 (*E. coli* - shrimp); EC_3 (*E. coli* - river); EC_4 (*E. coli* - lake).

Table 2
Bactericidal effect of water- and ethanol-based soursop extracts (*Annona muricata*)

Extract	Volume µL/dish	Diameter of inhibitory halo (mm)						
		SA	VC	SAL	EC_1	EC_2	EC_3	EC_4
Water-based	50	14	17	-	-	-	8	-
	100	14	18	-	-	-	8	-
	150	15	19	-	-	-	9	-
	200	16	23	-	-	-	10	-
Ethanol-based	50	-	-	-	-	-	-	-
	100	-	-	-	-	-	-	-
	150	-	-	-	-	-	-	-
	200	-	-	-	-	-	-	-

* -; no activity; SA (*Staphylococcus aureus* ATCC25923); VC (*Vibrio cholerae* classic 569B); SAL (*Salmonella* Enteritidis); EC_1 (*Escherichia coli* - fish); EC_2 (*E. coli* - shrimp); EC_3 (*E. coli* - river); EC_4 (*E. coli* - lake).

a) 

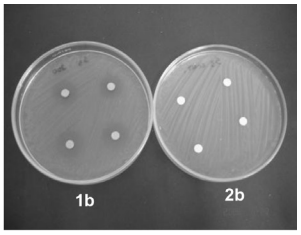
b) 

Fig. 1 - a) Antibacterial activity of water moringa extracts against *Escherichia coli* (**1a** and **2a**) and disks soaked with sterile distilled (negative control) (**3a**). **b)** Antibacterial activity of water soursop extracts against *Escherichia coli* (**1b**) and disks soaked with sterile distilled (negative control) **(2b).**

followed by lipids (18.8%), starch (6.02%), oligosaccharides (3.31%) and soluble sugars (3.14%).

The lack of bactericidal effects in our ethanol-based soursop extracts matches findings published by LIMA *et al*.[27], who reported *S. aureus*, *E. coli* and *Salmonella* to be resistant to all soursop extracts based on 95% ethanol. On the other hand, bioactivity of water-based soursop extracts against *S. aureus* and *V. cholerae* may be related to the chemical structure of the active substances. In an investigation of the bactericidal properties of eight species of annonaceae, TAKAHASHI *et al*.[39] isolated and confirmed the ability of trachylobanoic acid to inhibit *B. subtilis* and *S. aureus*. Annonaceae contain other bioactive substances, including a range of acetogenins with a wide spectrum of action, including antibiotic effects. Structurally, annonaceous acetogenins are series of C-35/C-37 natural products derived from C-32/C-34 fatty acids and combined with a 2-propanol unit[2].

Three of the four extracts tested in this study (the water- and ethanol-based moringa extracts and the water-based soursop extract) inhibited the growth of two Gram-negative bacterial cultures (*V. cholerae* and *E. coli*) and one Gram-positive (*S. aureus*) bacterial culture. According to KOSTOVA & DINCHEV[25], the observation of both Gram-negative and Gram-positive effects in the same plant extract may be explained by the presence of a wide spectrum of bactericidal substances, or by the action of toxins produced by the plant.

As shown by GONÇALVES *et al*.[18] and NASCIMENTO *et al*.[31], *S. aureus* has been inhibited by extracts prepared from a variety of phanerogams. Likewise, VIEIRA *et al*.[42] found *S. aureus* to be sensitive to guava leaf extracts in a study testing medicinal plants against bacteria causing diarrhea in children.

The susceptibility of *V. cholerae* to vegetable extracts was also tested by THAKURTA *et al*.[40] who found serotypes O1, O139, non-O1 and non-O139 to be inhibited by extracts of the neem tree (*Azadirachta indica*). Moreover, AKINSINDE & OLUKOYA[1] observed vibriocidal effects of medicinal plants from Nigeria, and in a study testing the bactericidal effect *in vitro* of 14 plant species upon *V. cholerae*, GUEVARA *et al*.[19] proposed using extracts of pomegranate peel (*Punica granatum*), the most efficient of the samples, as an alternative treatment for cholera.

The susceptibility of *E. coli* strains to moringa extracts in our study is supported by a study by JABEEN *et al*.[23], in which moringa seed extracts were observed to produce halos of bacterial inhibition measuring 18-20.5 cm.

In contrast with the extracts used by HEINRICH *et al*.[20], our water-based extracts of soursop peel did not inhibit *E. coli* effectively (halos < 13 mm).

Also, while MADSEN *et al*.[28] observed *Salmonella* Enteritidis cultures to be susceptible to water treated with moringa seed extract, in our own study none of the extracts was capable of inhibiting this species (Table 1). *Salmonella* has however been shown to be susceptible to medicinal plant extracts before, as in the studies by AL-BAYATI & AL-MOLA[3] and by EJA *et al*.[14].

The fact that the only strains resistant to all our extracts (*Salmonella* and *E. coli*, sampled at the lake "Lagoa da Fazenda") were Gram-negative, may be related to cell wall structure. According to TORTORA *et al*.[41], the cell wall of Gram-negative bacteria acts as a barrier to a number of substances, including antibiotics. This would also explain why medicinal plants tend to be more effective against Gram-positive than Gram-negative cultures[5,26,33].

The results of the present study confirm the importance of laboratory-testing medicinal plants used in indigenous medicine in search of new substances capable of inhibiting *S. aureus*, classic *V. cholerae* and *E. coli*.

RESUMO

Efeito antibacteriano (*in vitro*) de *Moringa oleifera* (moringa) e *Annona muricata* (graviola) frente a bactérias Gram-negativas e Gram-positiva

Para avaliação do efeito bactericida frente à *Staphylococcus aureus*, *Vibrio cholerae*, *Escherichia coli* (isolada de pescados e ambiente aquático) e *Salmonella* Enteretidis, foram testados extratos aquosos e etanólicos de sementes de moringa (*Moringa oleifera*) e casca de graviola (*Annona muricata*) na concentração de 1:5 e 1:10, nos volumes de 50, 100, 150 e 200 µL. Os resultados mostraram efeito antibacteriano (halo de inibição > 13mm) dos extratos aquosos e etanólicos de moringa frente a *S. aureus*, *V. cholerae* e *E. coli* isoladas de camarão cinza *Litopenaeus vannmaei*. A cepa de *E. coli* isolada do pescado *Oreochromis niloticus* apresentou sensibilidade frente ao extrato etanólico de moringa. Os extratos aquosos de graviola apresentaram efeito bactericida frente a *S. aureus* e *V. cholerae*, entretanto, os extratos etanólicos dessa planta não mostraram atividade antibacteriana.

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