



Contents lists available at ScienceDirect

## Asian Pacific Journal of Tropical Medicine

journal homepage: [www.elsevier.com/locate/apjtm](http://www.elsevier.com/locate/apjtm)

Document heading doi:

*In vitro* antibacterial effect of aqueous and ethanolic *Moringa* leaf extracts

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## ARTICLE INFO

## Article history:

Received 7 October 2010

Received in revised form 27 January 2011

Accepted 15 February 2011

Available online 20 March 2011

## Keywords:

*Moringa oleifera*

Activity antibacterial

Gram positive and negative bacteria

## ABSTRACT

**Objective:** To evaluate the antibacterial effect of aqueous and ethanolic moringa leaf extracts (*Moringa oleifera*) on the growth of gram-positive and negative bacteria. **Methods:** Paper disks were soaked with 100, 200, 300 and 400  $\mu$  L of extract at 20 g/180 mL and 10 g/190 mL. All extracts were tested against *Escherichia coli* (ATCC25922), *Staphylococcus aureus* (ATCC25923), *Vibrio parahaemolyticus*, *Enterococcus faecalis* (ATCC29212), *Pseudomonas aeruginosa* (ATCC27853), *Salmonella enteritidis* (IH) and *Aeromonas caviae*. The susceptibility tests were performed using the modified disk diffusion method. **Results:** The strains *E. coli*, *P. aeruginosa* and *S. enteritidis* (IH) were resistant to all treatments. In general, disks with 400  $\mu$  L extract were the most efficient against *S. aureus*, *V. parahaemolyticus*, *E. faecalis* and *A. caviae*. **Conclusions:** The study indicates a promising potential for aqueous and ethanolic Moringa leaf extracts as alternative treatment of infections caused by the tested strains.

## 1. Introduction

Much research has been done worldwide to identify and study antibacterial compounds found in medicinal plants[1–3]. According to Ríos and Recio[4], studies using essential oils or isolated compounds such as alkaloids, flavonoids, sesquiterpenes, lactones, diterpenes, triterpenes and naphthoquinones to test antibacterial effects are necessary to validate the use of a range of popular medicines.

The moringa tree (*Moringa oleifera*), a phanerogamous plant native to India, has been the object of extensive study due to its multiple uses as raw material in the production of oils, foods, condiments and drugs[5]. Studies on this plant have revealed promising anti-inflammatory[6], antifungal[7], pro-coagulant[8], flocculating[9] and antibacterial[10] properties. The latter has been attributed to different parts of the plant, such as the leaves, roots, seeds, flowers, fruit

peel and unripe pods[11].

The objective of the present study was to evaluate the antibacterial effect of aqueous and ethanolic moringa leaf extracts on the growth of *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Vibrio parahaemolyticus* (*V. parahaemolyticus*), *Enterococcus faecalis* (*E. faecalis*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Salmonella enteritidis* (*S. enteritidis*) and *Aeromonas caviae* (*A. caviae*)

## 2. Materials and methods

## 2.1. Preparation of extracts

The experiments used leaves of *Moringa oleifera* (*M. oleifera*) Lam. supplied by the Nutrition and Food Production Center (NUNPRA) of the Vale do Acaraú State University (UVA). A specimen was deposited in the herbarium of the same institution under entry number of 5823. To prepare the aqueous extracts, 10 g and 20 g of *Moringa* leaves were homogenized in a magnetic stirrer with 190 mL and 180 mL sterile distilled water, respectively. The ethanolic extracts were prepared by homogenizing 10 g

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e 20 g of *Moringa* leaves in 190 mL and 180 mL solution containing 50% sterile distilled water and 50% ethanol p.a., respectively. Paper disks were soaked with 100, 200, 300 and 400  $\mu$  L filtered homogenate and used immediately for susceptibility testing.

## 2.2. Bacteria

The antibacterial effect of the extracts was tested on the strains *E. coli* (ATCC25922), *S. aureus* (ATCC25923), *V. parahaemolyticus*, *E. faecalis* (ATCC29212), *P. aeruginosa* (ATCC27853), *S. enteritidis* (IH) and *A. caviae* supplied by the microbe bank of the Laboratory of Seafood and Environmental Microbiology (LABOMAR/UFC).

## 2.3. Antibacterial activity

Ten susceptibility tests were performed using the modified disk diffusion method<sup>[12]</sup>. Following adjustment to the 0.5 McFarland turbidity standard ( $10^8$  CFU/mL)<sup>[13]</sup>, the selected strains were seeded in Mueller Hinton agar (Difco). Aliquots were spread on disks soaked with different volumes and concentrations of extract and incubated at 35 °C for 24 hours. Following incubation, the inhibition halos were measured with a caliper. Strains were considered to be susceptible when the diameter of the halo measured  $\geq 13$  mm<sup>[14]</sup>.

## 3. Results

The strains *E. coli*, *P. aeruginosa* and *S. enteritidis* (IH) were resistant to all treatments (Table 1). Table 1 shows the

results of the antibiogram for aqueous and ethanolic extracts at the concentration of 20 g/180 mL. The most promising results were those for disks with 400  $\mu$  L extract, producing halos measuring on the average 23.3 mm (*S. aureus*), 19.4 mm (*E. faecalis*), 23.8 mm (*A. caviae*) and 21.9 mm (*V. parahaemolyticus*). The corresponding values for aqueous extracts were 25.4 mm, 17.8 mm, 22.3 mm and 20.7 mm.

At the concentration of 10 g/190 mL (Table 2), ethanolic extracts produced halos measuring 9 – 23mm. Disks with 400  $\mu$  L extract displayed the largest halos: 22.3 mm (*S. aureus*), 17.0 mm (*E. faecalis*), 21.2 mm (*A. caviae*) and 17.8 mm (*V. parahaemolyticus*).

Aqueous extracts produced halos measuring 9–26 mm. Disks with 400  $\mu$  L extract displayed the largest average halos for *S. aureus* (22.0 mm), *A. caviae* (21.4 mm) and *V. parahaemolyticus* (20.7 mm). However, the largest average halos for *E. faecalis* (16.3 mm) were observed for disks with 300  $\mu$  L.

The variance analysis (Table 3) revealed a statistically significant relation between the variables (cells, extract, concentration and volume) and the size of the inhibition halo at the level of 1% and 5% probability for the species *V. parahaemolyticus*, *A. caviae* and *E. faecalis*. The relation between the variable 'extract' and halo size was not significant for *S. aureus* ( $P>0.05$ ).

## 4. Discussion

The strains *E. coli*, *P. aeruginosa* and *S. enteritidis* (IH) were resistant to all treatments. The observed resistance of *E. coli* matches findings from a study on the antibacterial

**Table 1**

Susceptibility of *E. coli*, *S. aureus*, *V. parahaemolyticus*, *E. faecalis*, *P. aeruginosa*, *S. enteritidis* and *A. caviae* to aqueous and ethanolic *Moringa* leaf extracts (*M. oleifera* Lam.) at a concentration of 20 g/180 mL.

Species	$\mu$ L/disc	Halo (mm)					
		Ethanolic			Aqueous		
		Min	Max	Mean	Min	Max	Mean
<i>S. aureus</i>	100	15.0	20.0	17.9	12.0	25.0	16.5
	200	19.0	21.0	19.9	15.0	30.0	19.8
	300	20.0	27.0	22.3	16.0	35.0	22.9
	400	22.0	27.0	23.3	19.0	38.0	25.4
<i>E. faecalis</i>	100	14.0	16.0	14.4	12.0	15.0	30.0
	200	15.0	18.0	16.7	13.0	17.0	14.9
	300	16.0	19.0	18.2	14.0	20.0	16.4
	400	18.0	21.0	19.4	16.0	20.0	17.8
<i>A. caviae</i>	100	16.0	24.0	20.2	16.0	17.0	16.4
	200	18.0	25.0	21.9	16.0	20.0	18.4
	300	20.0	26.0	23.4	17.0	22.0	20.4
	400	20.0	26.0	23.8	21.0	25.0	22.3
<i>V. parahaemolyticus</i>	100	11.0	19.0	15.5	15.0	18.0	15.9
	200	12.0	22.0	18.7	16.0	21.0	18.3
	300	20.0	22.0	20.8	17.0	22.0	20.0
	400	20.0	23.0	21.9	17.0	24.0	20.7

**Table 2**

Susceptibility of *E. coli*, *S. aureus*, *V. parahaemolyticus*, *E. faecalis*, *P. aeruginosa*, *S. enteritidis* and *A. caviae* to aqueous and ethanolic *Moringa* leaf extracts (*M. oleifera* Lam.) at a concentration of 10 g/190 mL.

Species	$\mu$ L/disc	Halo (mm)					
		Ethanolic			Aqueous		
		Min	Max	Mean	Min	Max	Mean
<i>S. aureus</i>	100	15.0	20.0	17.3	11.0	20.0	15.2
	200	17.0	21.0	18.5	15.0	23.0	18.6
	300	19.0	23.0	21.3	16.0	25.0	20.2
	400	20.0	24.0	22.3	18.0	26.0	22.0
<i>E. faecalis</i>	100	9.0	12.0	10.8	8.0	17.0	12.1
	200	12.0	15.0	13.9	11.0	18.0	13.4
	300	15.0	17.0	16.1	13.0	21.0	16.3
	400	16.0	19.0	17.0	13.0	20.0	16.1
<i>A. caviae</i>	100	14.0	18.0	16.3	13.0	19.0	15.6
	200	17.0	21.0	19.5	18.0	21.0	18.8
	300	18.0	22.0	20.2	10.0	24.0	19.1
	400	19.0	23.0	21.2	17.0	25.0	21.4
<i>V. parahaemolyticus</i>	100	13.0	15.0	14.3	15.0	17.0	15.7
	200	15.0	23.0	16.7	17.0	19.0	17.6
	300	16.0	24.0	17.5	17.0	20.0	19.0
	400	17.0	20.0	17.8	18.0	22.0	20.7

**Table 3**

Variance analysis of susceptibility of *E. coli*, *S. aureus*, *V. arahaemolyticus*, *E. faecalis*, *P. aeruginosa*, *S. enteritidis* and *A. caviae* to aqueous and ethanolic *Moringa* leaf extracts.

SV	<i>S. aureus</i>					<i>V. parahaemolyticus</i>					<i>A. caviae</i>					<i>E. faecalis</i>				
	SS	DF	MS	F	P	SS	DF	MS	F	P	SS	DF	MS	F	P	SS	DF	MS	F	P
Cells	1152.98	15	76.86	5.291	<0.01	743.89	15	49.59	23.104	<0.01	1001.18	15	66.74	18.853	<0.01	819.49	15	54.64	22.407	<0.01
Extract	3.03	1	3.03	0.208	>0.05	13.81	1	13.81	6.432	<0.05	184.90	1	184.90	52.228	<0.01	26.41	1	26.41	10.830	<0.01
Concentration	99.22	1	99.22	6.831	<0.05	97.66	1	97.66	45.495	<0.01	202.50	1	202.50	57.199	<0.01	142.51	1	142.51	58.447	<0.01
Volume	982.13	3	327.38	22.537	<0.01	553.37	3	184.46	85.932	<0.01	445.48	3	148.49	41.944	<0.01	599.57	3	199.86	81.969	<0.01
Interaction	68.60	10	6.86	0.472	>0.05	79.06	10	7.91	3.683	<0.05	168.30	10	16.83	4.753	<0.05	51.00	10	5.10	2.092	<0.05
Error	2091.80	144	14.53			309.10	144	2.15			509.80	144	3.54			351.10	144	2.44		

\* SV: Sources of variation. SS: Sum of squares. DF: Degree of freedom. MS: Mean square.

properties of Indian plants showing *Moringa* extracts to be ineffective against *E. coli*[15]. Rajendran et al.[16] also reported *E. coli* to be resistant to *Moringa* extracts.

Doughari et al.[10] observed inhibition halos up to 8 mm when challenging *Salmonella* with aqueous and ethanolic *Moringa* leaf extracts. The authors attributed the antibacterial effect to the presence of saponine, tannic, phenolic and alkaloid phytoconstituents. However, due to the absence in our study of antibacterial activity against *Salmonella*, their findings cannot be compared to ours.

The antibacterial activity of the extract was greater against gram-positive species (*S. aureus* and *E. faecalis*) than against gram-negative strains (*E. coli*, *Salmonella*, *P. aeruginosa*, *V. parahaemolyticus* and *A. caviae*). Similar effects have been reported for other medicinal plant extracts in several studies[17–19].

Cáceres et al.[20] found *Moringa* leaf extracts (*M. oleifera*) can inhibit the growth of *S. aureus* and *P. aeruginosa*. Likewise, in a study by Valsaraj et al.[21] evaluating the antibacterial effect of 78 plants used in India to treat infectious diseases, *P. aeruginosa* and *S. aureus* were

inhibited by extracts of *Moringa* peel. In the present study, aqueous and ethanolic *Moringa* leaf extracts had an antibacterial effect on *S. aureus*, but not on *P. aeruginosa*.

*E. faecalis* was susceptible to all the extracts tested, contrary to findings published by Suarez et al.[22–26] who found *Moringa* extracts to be ineffective against *E. faecalis* isolated from clinical samples. On the other hand, their extracts produced bacteriostatic and bactericidal effects on *S. aureus*.

The susceptibility of *V. parahaemolyticus* to aqueous and ethanolic *Moringa* leaf extracts suggests the presence of vibriocidal compounds. In a study testing 60 medicinal plants (including *Moringa pterygosperma*) for activity against vibrios, Sharma et al.[27] only identified three species with antivibrio properties: *Syzygium cumini*, *Lawsonia inermis* and *Terminalia bellerica*. The authors attributed the antibacterial effect on *V. cholerae* and *V. parahaemolyticus* to the presence of gallic acid and tannin.

Obi et al.[28] evaluated the susceptibility profiles of aeromonad bacteria to medicinal plant extracts and found the species *A. hydrophila*, *A. sobria* and *A. caviae* to be

sensitive to extracts of *Pterocarpus angolensis*, *Syzygium cordatum* and *Zornia milneana*. This indicates a potential alternative use for medicinal plants in the treatment of infections involving *Aeromonas*—a possibility supported by the susceptibility of *A. caviae* to Moringa extracts observed in the present study.

In conclusion, aqueous and ethanolic Moringa leaf extracts were shown to contain compounds with wide-spectrum antibacterial activity, capable of inhibiting the growth of gram-positive and negative bacteria.

### Conflict of interest statement

We declare that we have no conflict of interest.

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