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

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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 μL 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 μL 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 naphtoquinones 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 μL 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[12]. Following adjustment to the 0.5 McFarland turbidity standard (10^8 CFU/mL)[13], 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 ℃ 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 ⩾13 mm[14].

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 μL 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 μL 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 μL 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 μL.

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	μL/disc	Halo (mm)					
		Ethanolic			Aqueous		
		Min	Max	Mean	Min	Max	Mean
S. aureus	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
E. faecalis	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
A. caviae	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
V. parahaemolyticus	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	μL/disc	Halo (mm)					
		Ethanolic			Aqueous		
		Min	Max	Mean	Min	Max	Mean
S. aureus	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
E. faecalis	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
A. caviae	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
V. parahaemolyticus	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	*S. aureus*					*V. parahaemolyticus*					*A. caviae*					*E. faecalis*				
	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]. Rajendrhan *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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