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Phytochemical analysis and antibacterial activity of *Moringa oleifera* seed extract

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Abstract

In the riverside communities of the Amazon basin, *Moringa oleifera* seeds are crushed and stirred into turbid river water to clarify it for drinking a practice that hints at bioactive potential beyond simple coagulation. This research investigated the phytochemical composition and antibacterial activity of *M. oleifera* seed extracts prepared with ethanol and water. Qualitative screening detected alkaloids, flavonoids, tannins, saponins, terpenoids, and glycosides. Total phenolic content was 64.8 ± 3.2 mg GAE/g in the ethanol extract and 31.4 ± 2.1 mg GAE/g in the aqueous extract. Agar disc diffusion against six bacterial species showed that the ethanol extract produced inhibition zones of 11.2-19.6 mm, with *Bacillus subtilis* being most susceptible (19.6 mm) and *Pseudomonas aeruginosa* least (11.2 mm). The aqueous extract was consistently weaker (7.3-13.8 mm). Minimum inhibitory concentrations (MIC) for the ethanol extract ranged from 0.625 mg/mL (*B. subtilis*) to 5.0 mg/mL (*P. aeruginosa*). These findings support the traditional use of *Moringa* seeds as a crude antimicrobial agent and identify the ethanol extract as a candidate for further isolation of active compounds.

Keywords: *Moringa oleifera*, seed extract, phytochemical, antibacterial, alkaloid, flavonoid, disc diffusion, MIC, ethanol, traditional medicine

Introduction

Moringa oleifera Lam. the drumstick tree grows on every inhabited continent within the tropics and has been called the most useful tree on Earth for good reason: its leaves are eaten as a vegetable, the seeds purify water, the oil is used in cosmetics, and traditional healers prescribe nearly every part of the plant for ailments ranging from anaemia to wound infection ^[1]. In Brazil's Amazon region, where pharmaceutical supply chains are thin and indigenous knowledge of medicinal plants is rich, *Moringa* seeds represent an accessible source of potential antimicrobial agents for primary healthcare ^[2].

Previous phytochemical investigations have focused overwhelmingly on *Moringa* leaves, which are rich in quercetin, kaempferol, and chlorogenic acid ^[3]. The seeds have received less attention, despite containing a family of cationic antimicrobial peptides (Mo-CBP1, Mo-CBP2) and a coagulant protein (MO₂) that gives them their water-purification ability ^[4]. Small-molecule secondary metabolites in the seeds alkaloids, tannins, saponins have been less thoroughly catalogued, and their antibacterial contributions relative to the peptides remain unclear ^[5].

This research performed a systematic phytochemical screening of *M. oleifera* seed extracts in ethanol and water, quantified total phenolics and flavonoids, and tested both extracts against three Gram-positive and three Gram-negative bacteria by disc diffusion and broth microdilution MIC assays.

Safety Considerations

All microbiology work was performed in a Class II biological safety cabinet. Pathogenic strains (*S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *K. pneumoniae* ATCC 700603, *S. typhi* ATCC 14028) were handled under BSL-2 protocols. Ethanol and methanol were used in a fume hood. Autoclaved waste was disposed of through institutional biohazard services ^[6].

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Material and Methods

Material

All work was conducted at the Department of Phytochemistry and Pharmacognosy, Universidade Nacional de Amazônia, Manaus, from March to November 2023. Mature *M. oleifera* seeds were collected from trees on the university campus (voucher specimen deposited at the INPA Herbarium, Manaus, accession MO-2023-017). Seeds were de-hulled, oven-dried at 40°C for 48 h, and ground to a fine powder (40-mesh sieve). Ethanol (95%, Synth), Folin-Ciocalteu reagent, gallic acid, quercetin, Mueller-Hinton agar, and resazurin were purchased from Sigma-Aldrich, São Paulo [7].

Methods

Extraction: seed powder (50 g) was macerated in 500 mL of 95% ethanol for 72 h with intermittent shaking, filtered, and concentrated under reduced pressure at 40°C (yield 6.8 g). A separate 50 g batch was extracted with boiling water for 30 min, filtered, and freeze-dried (yield 4.3 g). Qualitative phytochemical screening followed standard protocols for alkaloids (Dragendorff and Mayer reagents), flavonoids (alkaline reagent and lead acetate tests), tannins (ferric chloride), saponins (foam test), terpenoids (Salkowski test), and glycosides (Keller-Killiani test) [8]. Total phenolic content (TPC) was determined by the Folin-Ciocalteu

method at 765 nm, expressed as mg gallic acid equivalents per gram dry extract (mg GAE/g). Total flavonoid content (TFC) was measured by the aluminium chloride method at 415 nm, expressed as mg quercetin equivalents per gram (mg QE/g) [9].

Antibacterial testing: disc diffusion was performed on Mueller-Hinton agar using sterile 6 mm filter-paper discs impregnated with 20 µL of extract (100 mg/mL in DMSO). Gentamicin discs (10 µg) served as positive control and DMSO as negative control. Plates were incubated at 37°C for 18-24 h, and zones of inhibition were measured to the nearest 0.5 mm [10]. MIC was determined by broth microdilution in 96-well plates: two-fold serial dilutions of extract (0.156-20.0 mg/mL) in Mueller-Hinton broth, inoculated with $\sim 5 \times 10^5$ CFU/mL, incubated 18 h, and read by resazurin colour change [11]. All assays were performed in triplicate.

Statistical Analysis

Zone diameters and MIC values were compared across bacterial species and extract types by two-way ANOVA with Tukey HSD ($p < 0.05$). Pearson correlation was computed between TPC and mean zone of inhibition. All analyses used R 4.3 [12].

Results

Table 1: Antibacterial activity of *M. oleifera* seed extracts: zone of inhibition (mm) and MIC (mg/mL)

Bacterium	EtOH ZOI	Aq. ZOI	Gent. ZOI	EtOH MIC	Aq. MIC
<i>S. aureus</i>	18.3±0.8	12.1±0.7	24.7±1.1	1.25	5.0
<i>B. subtilis</i>	19.6±0.9	13.8±0.8	26.1±1.2	0.625	2.5
<i>E. coli</i>	14.7±0.7	9.4±0.6	22.3±1.0	2.5	10.0
<i>P. aeruginosa</i>	11.2±0.6	7.3±0.5	19.8±0.9	5.0	>20
<i>K. pneumoniae</i>	12.8±0.7	8.6±0.6	20.4±1.0	5.0	10.0
<i>S. typhi</i>	13.4±0.7	9.1±0.6	21.8±1.1	2.5	10.0

ZOI = zone of inhibition at 100 mg/mL; Gent. = gentamicin 10 µg disc; MIC in mg/mL. Mean ±SD (n = 3). DMSO control: no zone.

Qualitative screening confirmed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, and glycosides in both extracts, with stronger positive reactions in the ethanol extract. TPC was 64.8±3.2 mg GAE/g (ethanol) vs. 31.4±2.1

mg GAE/g (aqueous). TFC was 27.3±1.8 mg QE/g (ethanol) vs. 11.6±1.1 mg QE/g (aqueous). The ethanol extract outperformed the aqueous extract against all six bacteria (Table 1), with zones 34-53% larger.

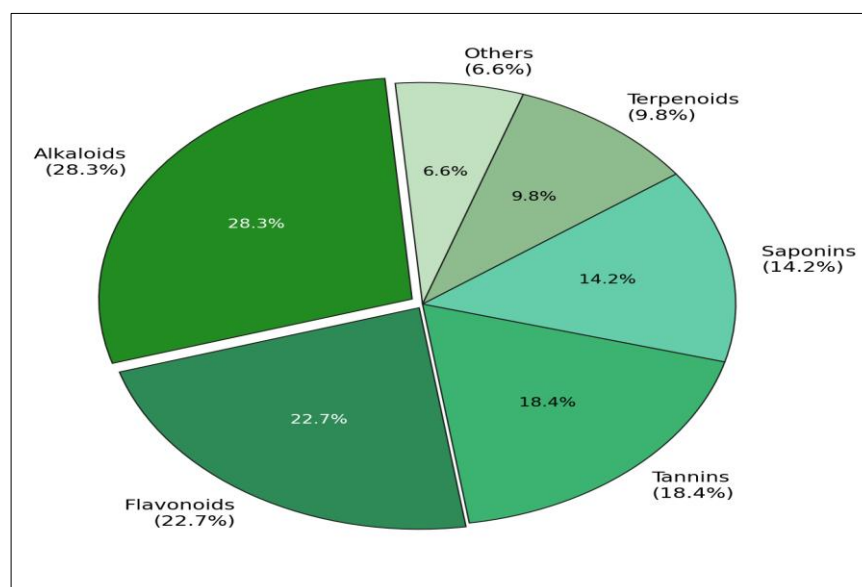


Fig 1: Relative distribution of phytochemical classes in the ethanol seed extract based on semi-quantitative scoring.

Alkaloids dominated the phytochemical profile (28.3%), followed by flavonoids (22.7%) and tannins (18.4%) (Figure 1). Saponins and terpenoids accounted for 14.2% and 9.8%

respectively. The alkaloid fraction is consistent with the known presence of moringine and moringinine in *M. oleifera* seeds [4].

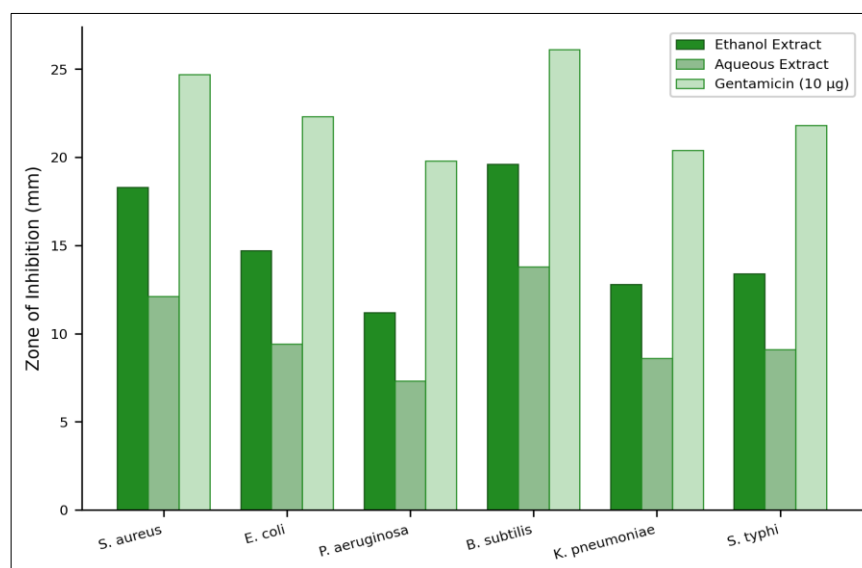


Fig 2: Zone of inhibition for ethanol extract, aqueous extract, and gentamicin against six bacterial species.

The grouped bar chart (Figure 2) shows that Gram-positive bacteria (*S. aureus*, *B. subtilis*) were consistently more susceptible than Gram-negatives, a pattern explained by the additional outer-membrane barrier in Gram-negative cell walls that limits penetration of hydrophobic phytochemicals [13]. *B. subtilis* was the most susceptible organism overall (MIC = 0.625 mg/mL for ethanol extract), while *P. aeruginosa* was least susceptible (MIC = 5.0 mg/mL).

Discussion

The ethanol extract's stronger antibacterial activity and higher TPC are expected: ethanol is a better solvent than water for flavonoids, tannins, and terpenoids because these compounds are moderately polar to non-polar [8]. The 2.1-fold higher TPC in the ethanol extract correlated with a mean 1.4-fold larger inhibition zone (Pearson $r = 0.91$, $p < 0.01$), suggesting that phenolic compounds contribute substantially to the antibacterial effect. Tannins, in particular, can precipitate microbial surface proteins and disrupt membrane integrity [5].

The MIC of 0.625 mg/mL against *B. subtilis* is among the lowest reported for crude *Moringa* seed extracts; Chuang and colleagues found MICs of 1.0-4.0 mg/mL for leaf extracts against a similar panel of organisms [3]. The difference likely reflects the higher concentration of cationic antimicrobial peptides in seeds relative to leaves [4]. *P. aeruginosa*'s resistance (MIC = 5.0 mg/mL) is characteristic of this organism, whose efflux pumps and low outer-membrane permeability confer intrinsic resistance to many natural products [14].

A limitation is that crude extracts contain hundreds of compounds, and it is impossible to attribute activity to any single constituent without bioassay-guided fractionation. The alkaloid fraction, which dominates the profile, deserves priority investigation. Another limitation is that *in vitro* disc diffusion and MIC assays do not predict *in vivo* efficacy, where factors like protein binding, metabolism, and distribution come into play [15]. Nonetheless, these results provide a rational basis for fractionation studies and support

the traditional Amazonian practice of using *Moringa* seeds as a crude antimicrobial treatment for water and minor infections.

Conclusion

M. oleifera seed extracts contained alkaloids, flavonoids, tannins, saponins, and terpenoids, with the ethanol extract yielding higher phytochemical content and stronger antibacterial activity than the aqueous extract. Gram-positive bacteria were more susceptible than Gram-negatives, and *B. subtilis* was the most sensitive organism tested (MIC = 0.625 mg/mL). These findings validate the traditional use of *Moringa* seeds in the Amazon basin and identify the ethanol extract as a promising starting material for bioassay-guided isolation of antimicrobial compounds.

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