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Comparison of antimicrobial activity of seeds of different *Moringa oleifera* varieties

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Abstract. The antimicrobial activity of seeds of three varieties of *Moringa oleifera* i.e. *M. oleifera* wild local variety of Malawi (Blantyre area), *M. oleifera* variety Mbololo of Kenya (Mtongue area) and *M. oleifera* variety Periyakulam 1 (PKM1) of India, was tested against *Bacillus cereus, Candida albicans, Streptococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli and Aspergillus niger*. Among the varieties examined, the variety from Kenya seems to be more effective against some of the tested microorganisms. Some well-known synthetic compounds used in industry as preservatives were also tested for comparative reasons.

Introduction

A large number of pharmacological investigations have been directed towards the plant kingdom as a source of therapeutic agents. *Moringa oleifera* (LAM) is a tropical plant that grows in Africa and Asia [1]. This plant has been the object of several investigations, many of which led to industrial applications [2-5]. In rural areas of Sudan the powdered seeds of *M. oleifera* are traditionally utilized for water purification because of their strong coagulating properties for sedimentation of suspended mud and turbidity [6,7]. During this procedure a decrease of the total bacterial count of the purified water was observed, indicating that the seeds contain substances with antimicrobial activity [7], and 4 (L-rhamnosyloxy) benzyl isothiocyanate was identified as an active antimicrobial agent from seeds [8]. In continuation of our work [9-11], oriented to obtaining antimicrobial substances from the plants, the seeds of the three above mentioned *M. oleifera* varieties, were tested for their antimicrobial activity. Antimicrobial tests were carried out with entire seeds as well as with the oil extracted from the seeds. Some well-known synthetic compounds such as ethyl paraben, propyl paraben, sorbic acid and sodium benzoate were also used in this work to compare their antimicrobial activity with that of the seeds of *M. oleifera*. This is the first paper describing the antimicrobial activity of the seed extracts of the above *M. oleifera* varieties.

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Materials and Methods

Plant material. The air-dried plant material was kindly provided by KE.F.R.I (Kenya Forestry Research Institute, Nairobi, Kenya). Incubation of the entire seeds was carried out with 10 parts of water normally for two hours and the filtrate was taken for antimicrobial tests.

Oil Extraction. The oil from the seeds was produced by cold pressure [11, 12]. The extraction procedure was performed as follows: the seeds were milled to a fine paste with a Vorwerk Thermomix 3300 (Vorwek France S.A., Paris) at a speed of 12 with the addition of water (in a ratio of 1 seed/2water) prior to extraction which was done with a O.M.F.B. pm 25-S/1 simple hydraulic hand press (Costruz. Mecc. Oleiodinamiche Provaglio D'Ised, Brescia, Italy) with a max pressure of 300 kg/cm^2. The oil was dissolved in dimethylosulfoxide (DMSO) and the above emulsion was taken for antimicrobial tests.

Screening for Antimicrobial Activity. The antimicrobial properties of the entire seeds and the oils were examined according to Gergis et al. [9]. The sensitivity of the following microorganisms was tested: *Bacillus cereus* (ATCC 11778), *Candida albicans* (ATCC 10231), *Streptococcus faecalis* (ATCC 1950), *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis* (ATCC 12228), *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 10536), *Aspergillus niger* (ATCC 6275). Preservatives such as ethyl paraben, propyl paraben, sorbic acid and sodium benzoate (Merck) were used in order to compare their antimicrobial activity with that of the seeds. The parabens were dissolved in DMSO while the other preservatives in water. Standard antibiotics (netilmicine, cefrazidine and ampicillin, Diagnostics Pasteur) were used in order to control the sensitivity of the tested microorganisms. We determined the maximum inhibitory dilution (MID), by the agar dilution method [9]. An aliquot of the above emulsions of seeds, oils or preservatives was mixed with sterile Tryptone Soya Agar or Sabouraud Dextrose Agar (in the case of *C. albicans* and *A. niger*). The final concentrations of the emulsions in the culture medium were between 1/50 and 1/2000. The medium was then inoculated with a loopful from an 18 h broth culture in Tryptone Soya Broth at 37°C or from a 24 h broth culture in Sabouraud Liquid Medium at 30°C or from a 7 days culture in Sabouraud Dextrose Agar at 25°C, for bacteria, yeast and mould respectively. Antimicrobial activity was estimated in all cases after

incubation at the appropriate temperature for 24 h except for *A. niger* which was incubated for 7 days. The total inhibition was estimated by the presence or absence of colonies after the incubation time. At least for the tested microorganisms, DMSO was determined not to be toxic under these experimental conditions.

Results and Discussion

The results of the antimicrobial activity screening of the aqueous seed extracts of *M.oleifera* varieties, the preservatives and the standard antibiotics are summarized in Tables 1, 2 and 3 respectively.

Table 1. MID'S of the aqueous seed extracts of *Moringa oleifera* varieties.

Microorganisms	1	2	3
B. cereus	1/500	1/750	1/500
C. albicans	< 1/125	< 1/125	< 1/125
Str. faecalis	1/125	1/250	1/125
St. aureus	1/500	1/500	1/500
St .epidermidis	1/500	1/750	1/500
B. subtilis	1/250	1/500	1/250
Ps. aeruginosa	< 1/125	1/125	N.T
E. coli	< 1/125	1/125	< 1/125
A. niger	< 1/250	1/250	N.T

1: variety of Malawi 2: variety Mbololo
3: variety PKM1 N.T: not tested

The sensitivity of the tested microorganisms to the seed extracts showed that Gram-negative bacteria (*E. coli and Ps. aeruginosa*) and yeast (*C. albicans*) were more resistant than Gram-positive bacteria (*B. cereus*, *Str. faecalis*, *St. aureus*, *St. epidermidis* and *B. subtilis*). The MID values of the preservatives used in this study, ranged from <1/50 to 1/500. Sodium benzoate was also active only against *A. niger* (MID = 1/500).

Table 2. MID'S of the preservatives

Microorganisms	1	2	3	4
B. cereus	1/500	1/500	1/500	< 1/50
C. albicans	1/750	1/500	1/500	1/50
Str. faecalis	1/500	1/500	1/500	< 1/50
St. aureus	1/500	1/500	1/250	< 1/50
St. epidermidis	1/500	1/250	1/500	< 1/50
B. subtilis	1/250	1/250	1/500	< 1/50
Ps. aeruginosa	1/500	1/500	1/250	< 1/50
E. coli	1/500	1/500	1/250	< 1/50
A. niger	1/250	1/500	< 1/250	1/500

1: ethyl paraben, 2: propyl paraben, 3: sorbic acid, 4: sodium benzoate

Table 3. MID'S of the standard antibiotics.

Microorganisms	N	C	A
B. cereus	$1/10^4$	$1/10^5$	$1/10^4$
C. albicans	N.T	N.T	N.T
Str. faecalis	$1/10^4$	$1/10^4$	$1/10^7$
St. aureus	$1/10^5$	$1/10^5$	$1/10^4$
St. epidermidis	$1/10^4$	$1/10^5$	$1/10^5$
B. subtilis	$1/10^5$	$1/10^7$	$1/10^5$
Ps. aeruginosa	$1/10^5$	$1/10^5$	$1/10^6$
E. coli	$1/10^6$	$1/10^4$	$1/10^5$
A. niger	N.T	N.T	N.T

N: Netilmicine, C: Cefrazidine, A: Ampicillin, N.T: not tested.

It is interesting to note that the variety Mbololo of Kenya in comparison with the other varieties, is more active against *B. cereus*, *Str. faecalis*, *St. epidermidis* and *B. subtilis*, while the MID values of the above variety are comparable with most of the preservatives used in this study.

The oil extracts from the seeds were completely inactive against all the microorganisms tested.

To our knowledge the antimicrobial activity of seeds the above *M.oleifera* varieties as well as the comparison of their activity with that of some preservatives commonly used in industry, has not been reported up to day.

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