

ANTIMICROBIAL ACTIVITY OF *MORINGA OLEIFERA* LAM. SEEDS EXTRACT

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Abstract

Phytochemical properties of the seeds of *Moringa oleifera* were carried out using methanolic, lead acetate and aqueous extracts. The phytochemical analyses showed the presence of alkaloids, saponins, tannins and anthraquinones in appreciable quantities. All the extracts exhibited various levels of antimicrobial activities. The extracts inhibited the growth of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Candida albicans* as comparable to the chloramphenicol control drug.

Introduction

Moringa oleifera Lam is a short, slender deciduous, perennial tree that grows to about 10m tall. It has drooping branches with brittle stems, corky bark; leaves are feathery, pale green, compound, tripinnate, 30-60cm long with many small leaflets (Duke, 1978). The plant belongs to the family Moringaceae. The plant has been found to be useful in water treatment (Berger *et al*, 1984), the leaves are ground and used for scrubbing utensils and for cleaning walls. Its seeds yield about 40% of non-drying oil known as Ben oil, used in the arts and for lubricating watches and other delicate machinery. The oil is clear, sweet and odourless. The leaves and young branches are eaten by livestock. The bark can serve for tanning.

The flowers, leaves, seeds and roots are used as folk remedies for tumors. Leaves are applied as a poultice to sores, they are rubbed on the temples for headaches. The barks, leaves and roots are taken to facilitate digestion. The bark is regarded as an antiscorbutic, and exudes a reddish gum sometimes used for diarrhoea. The roots are bitter, act as tonic to the body and lungs and are expectorant. They are a mild diuretic and are used as a stimulant in paralytic affections, epilepsy and hysteria (George & Audrey, 2005).

Materials and Methods

250g of the seeds were collected for the methanolic, lead acetate and water extract preparation. They were dried in the oven at 70°C for 4 hours and ground into powder. 50g each of the ground powder was wrapped in a filter paper and inserted into a tumbler. 250ml of the solvents were separately poured into the Soxhlet extractor. The extraction was done for 6 hours with continuous flows. These were then evaporated under reduced pressure and dried using a rotary evaporator at 55°C.

For the aqueous extraction, 50g of the seed powder was weighed and poured into 250ml Erlenmeyer flask and 50ml of distilled water was added. This was boiled for 1 hour using the hot plate with constant stirring. It was then filtered using No. 1 Whatman filter paper. The filtrate was also filtered using a membrane filter with 0.45 cm diameter. The extracts were then concentrated in a hot water bath at 80°C for 6 hours.

Phytochemical Screening

Alkaloids.

0.5g of the ground seeds were stirred with 1% aqueous hydrochloric acid on a steam water bath. A few drops of Dragendorff's reagent were used to treat 1ml of the filtrate. Precipitation indicated the presence of alkaloids.

Saponins

0.5g of the ground seeds were dissolved in distilled water in a 250ml beaker. Persistent frothing on warming was an indication for saponins.

Anthraquinone

0.5g of the powder was shaken with benzene and 10% ammonium solution added. A pink coloration indicated the presence of anthraquinone. These methods were adopted after

Adegoke & Bukola, 2009.

Microorganisms.

The microorganisms were 24 hours cultures Isolated from the Department of Microbiology Kaduna State University, Kaduna. They were Identified using standard biochemical tests of Cowan & steel (2004).

Antimicrobial Sensitivity Test

A loopful of twenty four hours old culture of the organisms was spread over uniformly on Muller-Hilton agar with a sterile bent rod. The ground flour extracts was diluted to obtain different concentrations of 500,250,125 and 65.5mg/ml using peptone water. Various concentrations of diluted samples were used to fill the holes bored by 5mm cork borer in the already inoculated agar. The plates were made in triplicates with chloramphenicol as the standard drug. The Inoculated plates were incubated at 37°C for 24 hours. The zones of inhibition were taken by calculating the diameters cleared. The microbial activity was calculated as the division of zone of inhibition of extract by that of chloramphenicol. The methods of Ochei & Kolhatkar (2000) were used-

Results and Discussion

Table 1 shows the results of the phytochemical analyses of the plant seeds. It shows the presence of alkaloids; tannins, saponins and antraquinones in substantial quantities.

The methanolic, lead acetate and aqueous extracts of the seeds of *Moringa oleifera* showed varied degrees of antimicrobial activities on the test microorganisms. The results of the antimicrobial activity (Table2) indicates that the zones of inhibition were wider on *E. coli*, *Bacillus siibtilis* and *Candida albicans* and less on *Staphylococcus aureus* using the matlianolic extract at 500mg/ml compared to the chloramphenicol drug. The microbial activity was also higher on *E. coli*, *Staph. aureus* and *Candida albicans* than in *Bacillus subtilis*(Tab\e 3)

Lead acetate extract had the highest antimicrobial activity (9.1 mm/per 500mg/ml) on *E. coli* as compared to *Staph. aureus* (8.2mm), *Candida, albicans* (8.2mm) and *Bacillus siibtilis* (7.6mm). The microbial activity was equally similar on the *E. coli* than the other three microorganisms. The aqueous extract had limited antimicrobial activity with the highest zone of inhibition on *Candida albicans* followed by *E.coli*, *Staph. aureus* and *Bacillus subtilis*. This trend was similar in the other concentrations (250mg/ml, 125mg/ml and 65.5mg/ml).

The results obtained from this research showed that the seeds of *Moringa oleifera* have potency on the microorganisms investigated. These were the alkaloids, tannins, saponins and the antraqiinones. The antimicrobial properties of the plant seeds could inhibit the growth of the organisms to a large degree. The medicinal property of the plant seed has been corroborated with the research of Caceras *et al* (1991) where their disk diffusion method also inhibited the growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

The phytochemicals produced by *Moringa oleifera* affords the opportunity to examine the range of unique compounds that have antimicrobial activities. Similar compounds also observed in *Moringa oleifera*. by Jed (2005) that had antibacterial activity included benzyl isothiocynate, niazimiciii, pterygospermin and benzyl glucosinolate. The results obtained in this research further confirm the antimicrobial activities of *Moringa oleifera* in a similar study by Dahot (1998) where a small protein/peptide exhibited antibacterial activity against *E. coli*, *Klebsiela aeruginosa*, *Staphylococcus aureus* and *Bacillus substillis*. These attributes have made *Moringa oleifera* unique in the science of medicine

Table 1 Phytochemical Properties of *Moringa Oleifera* Seeds

Phytochemical	Occurrence
Alkaloids	++
Tannins	+
Sapronins	++
Anthraquinone	++

++ = Present in high quantity

+ = Present in low quantity

Table 2. Antimicrobial Properties of *Moringa Oleifera* Seed Extracts using the Agar Diffusion Method (mm)

Isolate	Conc. (µg/ml)	500µg/ml						250µg/ml						125µg/ml						62.5µg/ml					
		A		B		C		A		B		C		A		B		C		A		B		C	
		MzI	AI	MzI	AI	MzI	AI	MzI	AI	MzI	AI	MzI	AI	MzI	AI	MzI	AI	MzI	AI	MzI	AI	MzI	AI	MzI	AI
<i>E. coli</i>	7.5	9.0	1.2	9.1	1.1	9.0	0.9	0.7	0.1	0.6	1.0	2.2	0.5	0.4	0.7	0.6	0.4	2.0	0.4	0.1	0.4	2.9	0.5	2.2	0.4
<i>S. aureus</i>	9.0	0.8	1.2	0.2	1.0	4.5	0.8	0.3	0.9	2.8	0.5	4.5	0.7	0.0	1.0	0.9	0.5	0.1	0.4	4.2	0.3	2.0	0.8	2.8	0.3
<i>B. subtilis</i>	7.7	7.4	0.8	7.0	0.8	3.5	0.7	0.0	0.7	7.8	0.4	4.4	0.6	5.4	1.0	0.8	0.3	5.8	0.5	4.0	0.4	2.8	1.0	2.3	0.4
<i>C. alb</i>	8.9	0.4	1.0	0.1	1.0	5.8	0.0	0.1	0.0	0.3	0.3	0.5	0.5	0.1	0.7	0.3	0.4	0.9	0.3	4.5	0.5	3.7	0.5	2.4	0.5

Key

- A = JVFethanolic extract
- B = Lead acetate extract
- C = Aqueous extract
- MzI = mean zone of Inhibition
- AI - Microbial Activity.

Table 3. Antimicrobial Properties of *Moringa Oleifera* Seed Extracts using the Tube Dilution Method

Isolate	J0fmgW			250µg/ml			125µg/ml			62.5µg/ml		
	A	a	C	A	n	C	A	ti	C	A	li	C
<i>C. coli</i>	11W	[70	13	1.4	1.3	1.3	1.0	1.3	1.0	0.e	10	118
<i>S. aureus</i>	240	3.4	7.1	2.3	24	2.5	2.4	2.5	13	2.1	2.W	U.7
<i>B. subtilis</i>	2.30	3.3	34	3J}	3.1	3.0	2.7	1.0	24	2.0	2.1	2.0
<i>Candida alb</i>	3.5	44	46	4.7	4.1	35	30	3.4	1.1	2.R	2.8	2.1
<i>S. aureus</i>	4.2	4.3	4.4	S.11	S.1	5.3	4.7	4.8	1.1	5.1	5.3	5.4

- A = Methanolic extract
- B = Lead acetate extract
- C = Aqueous extract
- * These values are in the Nephelometric Units (NU)

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