

Antibacterial properties of leaf extracts of *Moringa oleifera* Lam. growing in Sudan

ABSTRACT

Aim: To evaluate the antibacterial activity of different leaf extracts of *Moringa oleifera* growing in Sudan.

Methodology: The antibacterial activity of leaf extracts (Water, Butanol, Ethyl acetate and Chloroform) of *Moringa oleifera* were investigated *in vitro* using Agar- well diffusion method against eight different Gram positive and Gram negative bacteria.

Results: ~~All extracts revealed different degrees of antibacterial activity against some tested microorganisms. However,~~ Ethyl acetate extract recorded the highest active extract against four microorganisms namely, *Staphylococcus epidermidis* ATCC 49461 (16.0±0.5 mm), *Staphylococcus aureus* ATCC 25923 (13.6±0.3mm), *Pseudomonas aeruginosa* ATCC 27853 (13.3±0.3mm) and *Bacillus cereus* ATCC 10876 (10.2±0.7 mm), respectively. Butanol extract was active only against *Staphylococcus epidermidis* (14.0±0.0mm) and *Staphylococcus aureus* (10.3±0.3mm). Water extract was active only against *Staphylococcus epidermidis* (12.3± 0.6mm). Chloroform extract showed antibacterial activity against *Staphylococcus aureus* (11.0±0.5mm).

Conclusion: On the basis of the current findings, *Moringa oleifera* ~~Morenga oleifera~~ leaves could be a good candidate in the search for new antibacterial agents from natural products against different pathogens.

Keywords: antibacterial, *Moringa oleifera* ~~Morenga oleifera~~, Leaf extracts, agar-well diffusion method, Gram negative, Gram positive

1. INTRODUCTION

The growing prevalence incidences of multi-drug resistant microorganisms and the recent appearance of new microbial strains resistant to almost all known antibiotics are alarming and necessitated the international scientific community to apply different strategies and search for new effective drugs from another sources such as plants and natural products [1,2,3,4]. *Moringa* tree has attracted attention last years due to its pharmacological features properties [18]. *Moringa oleifera* ~~Morenga oleifera~~ ~~lam Lam.~~ [14] (*M. oleifera*) is belonging to family Moringaceae. It is a fast-growing tropical edible tree. This plant is a miracle plant utilized from centuries and prescribed extensively in traditional medicine; it was mentioned in ancient Egyptian, Romans and Greeks. It is now distributed and cultivated as a crop in so many African, Asian, Latin America and Caribbean countries [5]. This plant is consumed as a popular food in some countries; it has high nutritional value, being a good source for proteins, vitamins and minerals, so it is used to treat malnutrition in rural regions [5,6, 6]. Leaves of *M. oleifera* have many phytochemical secondary metabolites of great pharmacological properties, ~~studies on it revealed presence of such as -Alkaloids~~ alkaloids, ~~Flavonoids~~ flavonoids, ~~Saponins~~ saponins [7]. All these metabolites were found to have antimicrobial properties [3]. Many medicinal uses were also reported, various parts of this plant are employed as anti-inflammatory, anti-hypertensive, antioxidant, hepato-protective, anti-diabetic and antimicrobial [8]. In Sudan, the rural citizens are traditionally use the

powdered seeds of *M. oleifera* for purifying the drinking water, during this application a decrease in total bacterial count of this water was observed [9]. This study aims to evaluate the antibacterial activity of different leaf extracts of *M. oleifera* growing in Sudan.

2. MATERIAL AND METHODS

2.1 Plant collection

Leaves of *Moringa oleifera* were collected manually from Samrab area, Khartoum North, Sudan. The fresh plant samples were washed and dried in shade for up to 15 days. Then, dried leaves were crushed into fine powders using crushing machine and kept in dark well tight bottles for extraction process.

2.2 Plant extraction

Extraction was performed as described elsewhere with minor modification [10], the powdered *M. oleifera* leaves was percolated in 500ml chloroform in one liter capacity conical flasks stopper and kept for two weeks with intermittent shaking. The percolates were filtered with Whatman no.1 filter paper. Then, it was concentrated at 40°C under reduced pressure using rotary evaporator which yields semi-solid residues. The same quantity of the powdered plant material was again percolated with Ethyl-ethyl acetate, butanol and water following the same method in order to obtain four extracts namely, chloroform, ethyl acetate, butanol and water extracts. The semi-solid residues were left in Incubator at 40°C until totally dried (about two days). Dried extracts were reconstituted in 10% DMSO to make a concentration 200 mg/ml and kept in refrigerator in dark well tight bottles until used in the antibacterial testing.

2.3 Tested bacteria

Eight referenced bacterial strains purchased from Watin-Biolife, KSA, representing five Gram negative bacteria (*Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 49132, *Klebsiella pneumonia* ATCC 27736, *Salmonella enterica* ATCC 5174 and *Pseudomonas aeruginosa* ATCC 27853) and three Gram positive bacteria (*Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 49461 and *Bacillus cereus* ATCC 10876) were used in this study.

2.4 Preparation of inoculum

Referenced bacterial strains were sub-cultured from stock cultures in sterile bottles containing Nutrient broth incubated over-night at 37 °C. Directly, prior to the experiment, fresh microbial cultures were adjusted to 0.5 McFarland to be equivalent to about $1-2 \times 10^8$ CFU/ml.

2.5 Antibacterial testing

The antibacterial activity of different *M. oleifera* leaf extracts were determined based on Agar-well diffusion method [11], with minor modification, 100 µl of previously prepare inoculum was loaded to a sterile Petri-dish (100 × 15 mm). Then, 25 ml sterile-molten Mueller Hinton Agar (Watin-Biolife, KSA) was loaded on the Petri-dish and left at room temperature^[15] to solidify. After that, wells were punched into the agar with a sterile cork

borer (6 mm in diameter). 100 µl from each of the four extracts of *M. oleifera* leaves (200 mg/ml) was loaded into the wells, 100 µl from 10% DMSO (as a negative control) and 100 µl of 5 mg/ml chloramphenicol as a positive control (Riyadh Pharma, KSA) were loaded in another well ~~(as a positive control)~~. All plates were kept in the incubator for 24 h at 37 ° C. Tests were repeated three times and the mean zone of inhibition was calculated.

3. RESULTS AND DISCUSSION

Little is known about the bioactive properties of *Moringa oleifera* [16] cultivated in Sudan. The current studies revealed that there are significant variations in antimicrobial activities between *Moringa oleifera* [17] cultivars from different geographical localities all over the globe [21]. As shown in ~~Table 1 and Figure 1~~ Tables 1, 2 and Figure 1, almost all extracts showed different degrees of antibacterial activity at concentration of 200mg/ml against gram positive and gram negative bacteria. The inhibition zone above 10 mm is considered as good antibacterial activity [11]. In light of this, ~~Ethyl-ethyl~~ acetate extract revealed the highest antibacterial activity against *Staphylococcus epidermidis* (16.0±0.5mm), *Staphylococcus aureus* (13.6±0.3mm), *Pseudomonas aeruginosa* (13.3±0.3mm) and *Bacillus cereus* (10.2±0.7 mm), respectively. Butanol extract was active only against *Staphylococcus epidermidis* (14.0±0.0mm) and *Staphylococcus aureus* (10.3±0.3mm). Water extract was active only against *Staphylococcus epidermidis* (12.3± 0.6mm). Chloroform extract showed antibacterial activity against *Staphylococcus aureus* (11.0±0.5mm). This activity against both gram negative and gram positive bacteria may be attributed to presence of some broad-spectrum antibacterial compounds. These compounds are claimed to be from bioactive secondary metabolites such as ~~Alkaloids~~alkaloids, ~~Flavonoids~~flavonoids, ~~Saponins~~saponins and ~~Tannins~~tannins [7]. The results of this investigation in harmony with Numerous previous studies on ~~*Moringa*–*M. oleifera*~~ leaves from different geographical regions with different extraction methods and different solvents such as aqueous, ethanol, methanol, chloroform and many more reported interesting antibacterial activity against different-wide spectrum microorganisms, from both gram positive and gram negative bacteria [12,13, 14,15,8,7, 16, 17]. Interestingly, different parts from *M. oleifera* -showed antibacterial activity against multi-drug resistant bacteria (MDR). For example, seed extracts from *M. oleifera* exhibited antibacterial activity against Methicillin resistant *Staphylococcus aureus* (MRSA) [19]. Recently, MRSA is fast becoming an international problem [20]. Moreover, the non-toxic antimicrobial properties can be used as food preservative. Presently, a study on tomato preservation showed that the powder from the leaf and stem bark of *M. oleifera* preserved tomatoes from early spoilage and enhanced the shelf life of fresh tomatoes [22]. ~~Figure 1 presenting the variation between *M. oleifera* extracts and the positive control which is the antibiotic chloramphenicol (5mg/ml). This result is axiomatic. Whereas,~~ Although, in the current study the antibiotic chloramphenicol recorded the highest antibacterial effect against tested reference bacterial strains. However, the ~~testes~~ tested extracts are crude, the nature and quantity of the antibacterial substances are still unknown compared to the isolated and the pure formula of Chloramphenicolchloramphenicol[18]. Qualitative and quantitative phytochemical analysis, Fractionationfractionation, separation and isolation studies on these antibacterial compound(s) are required besides other chemical and pharmacological studies.

Table 1. Antibacterial activity of different extracts of *Moringa oleifera* leaves compared to chloramphenicol against gram positive bacteria.

Extract	Mean zone of inhibition (mm) of tested bacteria (Mean±SEM)*[19]		
	SA	SE	BC
Water	7.3	12.3	7.7
200mg/ml	±0.3	±0.6	±0.4
Butanol	10.3	14.0	9.0
200mg/ml	±0.3	±0.0	±0.6
Ethyl acetate	13.6	16.0	10.2
200mg/ml	±0.3	±0.5	±0.7
Chloroform	11.0	9.0	7.4
200mg/ml	±0.5	±0.5	±0.3
Chloramphenicol	33.0	36.6	32.0
5mg/ml	±1.5	±0.3	±1.2
DMSO 10%	0.0	0.0	0.0

* Mean±standard error of means, – = No inhibitory activity. SA = *Staphylococcus aureus* ATCC 25923, SE = *Staphylococcus epidermidis* ATCC 49461, ~~KP = *Klebsiella pneumonia* ATCC 27736~~, ~~EC = *Escherichia coli* ATCC 25922~~, ~~SL = *Salmonella enterica* ATCC 5174~~, ~~PR = *Proteus vulgaris* ATCC 49132~~, ~~PS = *Pseudomonas aeruginosa* ATCC 27853~~, Bc = *Bacillus cereus* ATCC 10876.

Table 2. Antibacterial activity of different extracts of *Moringa oleifera* leaves compared to chloramphenicol against gram negative bacteria.

Extract	Mean zone of inhibition (mm) of tested bacteria (Mean±SEM)*				
	KP	EC	SL	PR	PS
Water	=	=	=	=	9.6
200mg/ml					±0.3
Butanol	6.3	=	=	=	11.6
200mg/ml	±0.3				±0.3
Ethyl acetate	6.6	7.0	6.6	6.3	13.3
200mg/ml	±0.3	±0.0	±0.3	±0.3	±0.3
Chloroform	=	=	=	=	=
200mg/ml					
Chloramphenicol	31.3	37.0	34.0	32.3	18.0
5mg/ml	±0.8	±0.5	±0.5	±0.6	±2.0
DMSO 10%	0.0	0.0	0.0	0.0	0.0

* Mean±standard error of means, – = No inhibitory activity. KP = *Klebsiella pneumonia* ATCC 27736, EC = *Escherichia coli* ATCC 25922, SL = *Salmonella enterica* ATCC 5174, PR = *Proteus vulgaris* ATCC 49132, PS = *Pseudomonas aeruginosa* ATCC 27853.

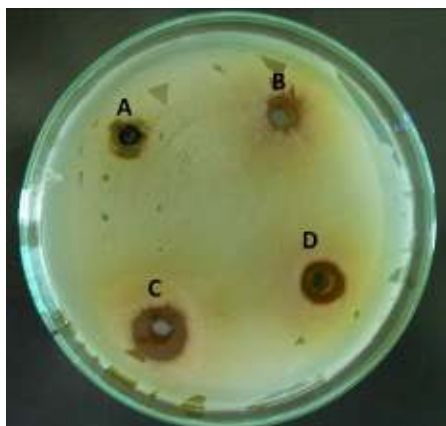
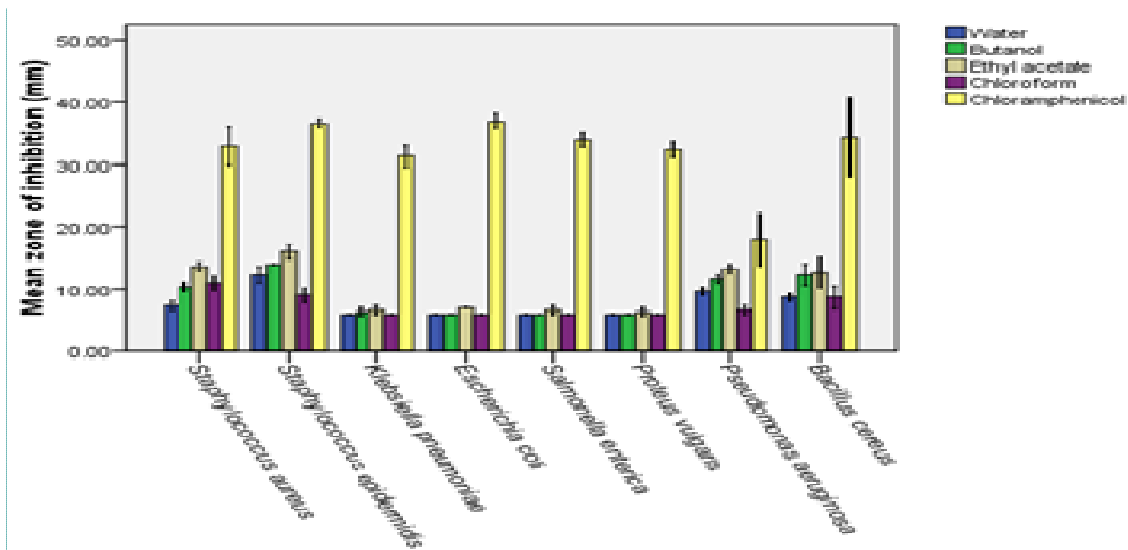


Figure 1. Representative photo showing the antibacterial activity of different leaves extracts of *Moringa oleifera* against *Bacillus cereus* ATCC 10876

A= Water (aqueous extract), B= Butanol, C= Ethyl acetate and D= Chloroform.



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Fig. 1. Mean zone of inhibitions of different extracts compared to the antibiotic chloramphenicol*.

*Mean±standard error of means. 6.0±0.0mm = No inhibitory activity.

4. CONCLUSION

~~Morenga oleifera is a miracle medicinal plant prescribed in traditional medicine since antiquity against different ailments. In this study, Leaves of *M. oleifera* cultivated in Sudan was found to have antibacterial activity against some microorganisms. Too many studies on *M. oleifera* from different localities around the world support this claim. It is time now for the scientific community to take positive steps in order to isolate these antibacterial substances, to innovate new natural antibiotics derived from such medicinal plants to face the recent disastrous spread of multi-drug resistant bacteria.~~

The current investigation has revealed the potential of the leaves of *M. oleifera*^[111] cultivated in Sudan, as antibacterial agent against the tested pathogens, supporting the application of their leaves in Sudanese folk medicine for some microbial ailments. Further studies are needed to isolate these antibacterial substances, to innovate new natural antibiotics derived from such medicinal plants to face the recent disastrous spread of multi-drug resistant bacteria.

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