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Research Article

Evaluation of Antimicrobial activity of *Moringa oleifera* Leaf extracts against Pathogenic bacteria Isolated from Urinary tract infected Patients

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Abstract: Antibiotic resistance has increased substantially in recent years and is posing an ever-increasing therapeutic problem. One of the methods to reduce the resistance to antibiotics is by using antibiotic resistance inhibitors from plants. The aim of this study is to evaluate the antibacterial properties of aqueous, petroleum ether and methanolic leaf extracts of *Moringa oleifera* plant against pathogenic bacteria isolated from urinary tract infected patients and five standard strains of American type culture collection. The antibacterial activity of *Moringa oleifera* leaf extracts was determined *in vitro*, using Cup plate method, and compared with sensitivity testing of some antibiotic agents using disc diffusion method. The results obtained showed that all concentration of methanolic extracts of *Moringa oleifera* had high inhibitory effects on *S. aureus* ATCC25923, *K. pneumoniae* ATCC35637 standard strains and the *S. aureus, S. saprophyticus and E.coli* isolated from UTI. The three concentration of water extract had inhibitory effects only on *Proteus vulgaris* NCTC8196 strain. The petroleum ether extracts showed no inhibitory activity on any organism. These results were compared with standard antibiotics Amikacin, Ciprofloxacin, and Norfloxacin which showed moderate sensitivity against *S. aureus* and Amikacin was completely resistant to *K. pneumoniae* isolated from UTI. These results provide valuable information that *Moringa oleifera* hold great promise as highly effective antibacterial agents.

Keywords: *Moringa* leaf extracts, Antimicrobial activity, Pathogenic bacteria.

1. Introduction

The increasing prevalence of multidrug-resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raised the specter of 'untreatable' bacterial infections and adds urgency to the search for new infection-fighting strategies (El Astal *et al*., 2005; Rojas *et al*., 2006). Plants are known to produce a variety of compounds to protect themselves against a variety of pathogens. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant pathogens (Sen and Batra, 2012). For a long time, plants have been an important source of natural products for human health. The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives because of their antimicrobial properties (Bugno *et al*., 2007). There are several reports on the presence of antimicrobial compounds in various plant parts like leaves, bark, fruit, root, and flowers (Tsaknis *et al*., 1999). *Moringa* species are well-documented plant herbs due to their extraordinary nutritional and medicinal properties. *Moringa oleifera* Lam. and *Moringa stenopetala* are the most widely cultivated species of the monogenic family, the *Moringaceae* (Walter *et al*., 2011). *Moringa oleifera* is one of the species of family *Moringaceae*, native to, Africa, Arabia, South Asia, South America, Himalaya region, India, Pakistan, the Pacific, and Caribbean Islands. *Moringa oleifera* has been naturalized in many tropic and subtropic regions worldwide, the plant is referred to number of names such as horseradish tree, drumstick tree, Ben oil tree, miracle tree, and "Mothers best friend" (Pinal *et al*., 2014). They have long been known in folk medicine as having value in treating a wide variety of ailments. They are known to be antihelminthic, antibiotic,

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detoxifiers, immune builders and have been used to treat malaria (Thilza *et al*., 2010). The Moringa plant (*Moringa oleifera*) has been the object of much research due to its multiple uses and well-known bactericidal potential (Viera *et al*., 2010). Ethanolic extract of *Moringa oleifera* leaves contains niazirin, niazirinin, niazininins A and B (Faizi *et al*., 1994). Benzoic acid, gallic acid, beta benzaldehyde have been isolated from methanolic extract of *Moringa oleifera* leaves (Manguro and Lemmen, 2007). Seeds and leaves (and extracts) show activity against different species of fungi (*Trichophyton rubrum, Trichophyton mentagrophytes, Microsporum canis, Epidermophyton floccosum, Aspergillus flavus, Rhizopus stolonifer, Fusarium solani, Rhizopus solani and Mucor* sp.) (Donli and Dauda, 2003; Jabeen *et al*., 2008). Some of which being strictly anthropophilic dermatophytes. Correspondingly, these extracts have bactericidal and/or bacteriostatic action against *Staphylococcus aureus, Vibrio cholerae, V. parahaemolyticus, Enterococcus faecalis, Salmonella enteritidis, Aeromonas caviae, Pasteurella multocida, Bacillus subtilis, E. coli, Pseudomonas aeruginosa, Enterobacter cloacae, Proteus vulgaris and Micrococcus kristinae* (Jabeen *et al*., 2008). Initially, it was difficult to accurately identify the responsible component(s) for the antimicrobial properties, since majority of studies was performed with seed and leaf crude extracts. However, some authors attributed this effect to the compounds 4-(α-L-rhamnosyloxy)-benzyl isothiocyanate, moringin and 4-(α-L-rhamnosyloxy)phenylacetonitrile synthesized by the plant (Eilert *et al*., 1981; Jahn *et al*., 1986). The objective of this study was to evaluate the antibacterial properties of aqueous, petroleum ether and methanolic leaf extracts of *Moringa oleifera* plant against pathogenic bacteria isolated from urinary tract infected patients and five standard strains of American type culture collection.

2. Materials and Methods

2.1 Preparation of leaf extracts

The plant material used was collected from Omdurman city (Sudan) and fresh leaves were isolated identified, confirmed and dried in shade. One hundred grams of the dried plant leaves were ground to powder by grinder and extracted as follows:

Two amounts of 25g of the powdered leaves were separately extracted in 500ml conical flasks with petroleum ether (petroleum ether extraction) and methanol (methanolic extraction) extracted for 3 hours in soxhlet apparatus. Then the extracts were filtered and evaporated under reduced pressure using Rotavapor. Other 25g of the powdered leaves were separately extracted in 500ml conical flasks with 100ml of sterile distilled water (aqueous extraction), by infusion overnight then it was filtered (through Whatman No.1 filter) and the filtrate was dried and weighted.

Different concentrations (20%, 40%, and 60%) of these extracts (petroleum ether, methanolic and aqueous) were prepared and used in further processes.

2.2 Origin of control strains

Test organisms of five standard strains of American type culture collection were used as control sensitive strains in the study. *Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC35637, *Pseudomonas aeruginosa* ATCC27853 and *Proteus vulgaris* NCTC8196. They were obtained from the Microbiology Laboratory of the National Center for Research (NCR), Khartoum, Sudan. Each of these organisms was subcultured on Luria Broth (LB) medium and maintained on nutrient agar slants at 4°C prior to susceptibility testing.

2.3 Collection of samples of Clinical samples

Fifty urine specimens were collected in sterile urine containers from patients attending Omdurman Teaching Hospital and Khartoum Teaching Hospital. All specimens transported immediately after collection to the microbiology laboratory for culturing and processing, inoculated using CLED agar media then incubated for 24 hours at 37°C for primary isolation.

A series of identification procedures based on microscopical examination of the Gram-stained films, cultural characteristics on selective and differential culture media and biochemical reactions were used to identify the samples according to procedures described by Cowan & Steel's (Barrow and Feltham, 2003).

2.4 Evaluation of antibacterial activity

The crude extracts of *Moringa oleifera* were screened for their antimicrobial activity against some Gram-positive and Gram-negative bacteria isolated from UTI using Cup plate method and compared with sensitivity testing of some antibiotic agents using disc diffusion method.

2.5 Study of antimicrobial activity of *Moringa oleifera* extracts using Cup plate method

The antibacterial activity of the extracts was determined by using the agar-well diffusion method (Cup plate method). Mueller-Hinton agar media was sterilized by autoclaving, cooled, 1ml of 24h old culture of each test organism was added to 19ml of Mueller-Hinton agar media and each plate was properly labeled and allowed to set. Cups were made in each Petri plate using sterile cork borer (10mm diameter and about 2cm apart). About 100µl of different concentrations of plant solvent extracts were added into the wells and allowed to diffuse at room temperature for 2hrs. Then bacterial plates were incubated at 37°C for 24 hours. Each test compound has got three bores (one bore for each concentration (20%, 40%, and 60%)) for which zones of inhibition diameter and mean values was determined

at the end of the period, inhibition zones formed on the medium were evaluated in mm.

2.6 Study of antibiotic sensitivity using disc diffusion method

This was performed using the standard disk diffusion method (Kirby-Bauer method) in which the organisms under investigation were cultured in Mueller-Hinton sensitivity testing agar, then different antibiotic disks were placed on the media about two centimeters apart. After overnight incubation at 37°C aerobically the culture was examined for zone of inhibition of bacterial growth around the respective disks which was measured in millimeters. All species isolated were tested for antibiotic sensitivity against commonly used antibiotics: (Ciprofloxacin, Norfloxacin, Amikacin and Nalidixic Acid).

3. Results

3.1 Clinical Isolates

The bacteria isolated from 50 samples were 50 species, 50% were Gram-positive (30% *Staphylococcus saprophyticus* and 20% were *S. aureus*) and 50% were Gram-negative, (24% were *E. coli*, 18% *K. pneumoniae*, 4% *K. oxytoca* and 4% *Pseudomonas aeruginosa*) (Table 1).

3.2 Antimicrobial activity of *Moringa oleifera* leaves extracts against standard organisms

The antibacterial activity of three concentrations (20%, 40%, and 60%) of the aqueous, methanolic and petroleum ether extracts of *M. oleifera* leaves against standard organisms is presented in (Table 2). The results obtained showed that the three concentration of methanolic extracts of *Moringa oleifera* had inhibitory effects of 16, 18, 18mm diameter on *S. aureus* ATCC25923. While the other extracts of water and petroleum ether showed no inhibitory activity against *S. aureus* ATCC strain. Also, the three concentration of methanolic extracts of *Moringa oleifera* had inhibitory effects of 16, 17, 19mm diameter on *K. Pneumoniae* ATCC35637, while water and petroleum ether extracts showed no inhibitory activity.

The three concentration of water extract of *Moringa oleifera* had inhibitory effects of 18, 19, 21mm diameter on *P. vulgaris* NCTC8196 strain, while methanolic and petroleum ether extracts showed no effects. All concentrations of the three extracts of *Moringa oleifera* had no inhibitory effects on *Escherichia coli* and *Pseudomonas aeruginosa* standard strings.

Table 1. Type of bacteria isolated from urine samples (Frequency and percentage).

Bacteria	Number	Percentage (%)
Staphylococcus saprophyticus	15	30%
Staphylococcus aureus	10	20%
E. coli	12	24%
Klebsiella pneumoniae	9	18%
Klebsiella oxytoca	2	4%
Pseudomonas aeruginosa	2	4%
Total	50	100%

3.4 Antibacterial activity of *Moringa oleifera* leaves extracts against clinical isolates

All extracts were examined against clinically isolated bacteria with three concentrations (20%, 40%, 60%). Methanolic extract showed antibacterial activity with variable percentages against *Staphylococcus saprophyticus, S. aureus* and *E. coli,* while *K. pneumoniae, K. oxytoca and Pseudomonas aeruginosa* were not sensitive to the extract. Petroleum ether and water extracts had no antibacterial activity against all tested organisms (Table 3).

3.4 Antibiotic susceptibility of clinical isolates

Four antibiotics were tested against clinical isolates (Ciprofloxacin, Norfloxacin, Amikacin and Nalidixic Acid). Table (4) shows the results of antibiotic sensitivity testing of clinically isolated bacteria.

Table 2. Antibacterial activity of *Moringa oleifera* extracts against standard organisms.

		Diameter of inhibition zones of test organisms/mm				
Solvent extract	Conc.	*S. aureus*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*P. vulgaris*
Petroleum ether	20%	-	-	-	-	-
	40%	-	-	-	-	-
	60%	-	-	-	-	-
Methanol	20%	16	-	16	-	-
	40%	18	-	17	-	-
	60%	18	-	19	-	-
water	20%	-	-	-	-	18
	40%	-	-	-	-	19
	60%	-	-	-	-	21

*Less than 14mm: resistant; *More than 14mm: Sensitive

Table 3. Antibacterial activity of *Moringa oleifera* leaves extracts against clinical isolates.

		Percentage of affected organisms (Diameter of inhibition zones more than 14mm)					
Solvent extract	Conc.	*S. aureus*	*E. coli*	*K. pneumoniae*	*K. oxytoca*	*P. aeruginosa*	*S. saprophyticus*
Methanol	20	20%	33%	0	0	0	26%
	40	20%	33%	0	0	0	40%
	60	30%	33%	0	0	0	40%

Table 4. Results of antibiotic sensitivity testing of clinically isolated bacteria.

Antibiotic	Sensitivity test	*S. aureus*	*S. saprophyticus*	*E. coli*	*K. pneumoniae*	*K. oxytoca*	*P. aeruginosa*
AK	S	60 %	64 %	42%	0 %	100 %	100%
	R	40%	36%	58%	100%	0%	0%
Cip	S	70% %	64%	25%	100 %	100 %	0 %
	R	30 %	36 %	75%	0 %	0 %	100%
Na	S			58%	50%	0%	100%
	R			42%	50%	100%	0%
No	S	40 %	36%	50%	100%	0%	100%
	R	60%	64%	50%	0%	100%	0%

AK = Amikacin; Cip = Ciprofloxacin; No = Norfloxacin; Na = Nalidixic acid

4. Discussion

Concern has been expressed about the rising prevalence of pathogenic microorganisms, which are resistant to the newer or modern antibiotics that have been produced in the last three decades (Valarmathy *et al*., 2010). Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials. *M. oleifera* is highly valued plant, with impressive range of medicinal uses and high nutritional value (Tamanna, 2010).

In this study, we evaluated the antimicrobial activity of *Moringa oleifera* leaf extracts against pathogenic bacteria isolated from urinary tract infections and five standard strains of American type culture collection. The results obtained showed that all concentrations of methanolic extracts of *Moringa oleifera* had high inhibitory effects on *S. aureus, K. pneumoniae* standard strains and the *S. aureus, S. saprophyticus and E.coli* isolated from UTI. The three concentration of water extract had inhibitory effects only on *P. vulgaris* standard strain. The petroleum ether extracts showed no inhibitory activity on any organism. These results were compared with standard antibiotics Amikacin, Ciprofloxacin, and Norfloxacin which showed moderate sensitivity against *S. aureus* and Amikacin was completely resistant to *K. pneumoniae* isolated from UTI. But the methanolic extracts of *Moringa oleifera* showed high inhibitory effects on *S. aureus and K. pneumoniae* than the given standard antibiotics. These varying antimicrobial activity of *Moringa oleifera* leaf extracts showed on different microorganisms was also reported by other workers. Devendra *et al*., (2011) mentioned that chloroform extract of *Moringa oleifera* Lam. plant leaves showed antibiotic property against wide range of pathogens like *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 424), *Staphylococcus aureus* (MTCC3160) and *Streptococcus pyogenes* (MTCC 442). Eleyinmi (2007) and Bukar *et al.*, (2010) reported antimicrobial activities of *Moringa oleifera* leaves plant extracts on foodborne pathogens. Thus, *M. oleifera* Lam. could become promising natural antimicrobial agent with potential applications in pharmaceutical industry for controlling the pathogenic bacteria. However, if plant extracts are to be used for medicinal purposes, issues of safety and toxicity will always need to be considered.

In conclusion, this study has shown that the water and methanolic extracts of *Moringa oleifera* leaf possess some degree of antimicrobial activity and these results provide valuable information that *Moringa oleifera* hold great promise as highly effective antibacterial agents. More and further researchers will achieve that *Moringa oleifera* can be used to discover antibacterial agent for developing new pharmaceuticals.

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