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Original article

Antibacterial Activity of *Moringa oleifera* Leaf Extract on Some Enteric Pathogens

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ABSTRACT

Background: The antimicrobial activities of *Moringa oleifera* leaf extract against enteric pathogens have been documented. The present study was designed to evaluate the antibacterial activity of *M. oleifera* leaf extract and its phytochemical constituents against clinical isolates of enteric origin. **Materials and methods:** The leaf extract of *M. oleifera* was prepared by cold maceration using methanol and distilled water. The antibacterial activity and minimum inhibitory concentration (MIC) were determined for the crude leaf extract by agar well diffusion method. **Results:** The results indicate that the aqueous leaf extract of *M. oleifera* has a notable antibacterial activity against the microorganisms tested. The maximum antibacterial activity was observed against *E. coli* (15 mm) in aqueous extract, *Pseudomonas aeruginosa* and *Salmonella typhi* (15mm) in methanol extract. The minimum inhibitory concentration was ranged from 400 to 300 mg/ml. **Conclusion:** The *M. oleifera* leaf extract was found to contain some bioactive constituents, however further phytochemical studies and their characterization will be needed to isolate the active constituents and evaluate the antimicrobial activities against a wider range of bacterial pathogens.

KEYWORDS: *Moringa oleifera*, enteric pathogenic, phytochemical.

INTRODUCTION

The medicinal use of herbs is said to be as old as mankind [1]. Medicinal plants have grown enormously from the use of herbal products as natural cosmetics to self medication by the general public owing to their effective antimicrobial activities [2]. Due to the problem of drug resistance, scientists in Africa and other developing countries are conducting researches into local plants and herbs in view of their usage in traditional medicine [3]. The extracts of many plant species have become popular in recent years and attempts have been on to characterize their bioactive principles which are the hallmark of the various pharmaceutical and medical applications.

The plant *Moringa oleifera* was first described in Northern India around 2000BC as a medicinal herb [4]. It belongs to the family of shrubs and trees called Moringaceae. The seeds of *Moringa oleifera* contain edible oil which is used for medicinal purposes, perfumes and skin lotion [4]. The Moringa tree spreads East-Wards from India to the lower parts of China, South East Asia, Philippines, Egypt, and in America. Also, it is cultivated throughout the Middle East, Africa and Nigeria [5].

However, it has been known for its healing properties and ability to reverse malnutrition [4]. Moringa plant is claimed to have enormous medicinal properties which include; anti-inflammatory, anti-ulcerative, anti-epileptic, anti-hypertensive, antioxidant, anti-diabetic, hepatoprotective and cholesterol lowering activity etc [6].

Enteric bacteria are gram negative rods with facultative anaerobic metabolism. They inhabit the intestinal tract of animals and humans in health and disease [7]. They belong

to the family Enterobacteriaceae, members of this family include *Salmonella spps* which are mostly human intestinal pathogen and *Escherichia coli* which form part of the intestinal normal flora [7]. However, Moringa leaf extract is a herb of choice because of its traditional use for the treatment of typhoid, in Jos, Plateau State, Nigeria.

This study was therefore undertaken to investigate if Moringa oleifera leaf extract possess antibacterial effect on enteric pathogens.

MATERIALS AND METHODS

Plant source and identification

The fresh leaves of *Moringa oleifera* were collected from Moringa trees grown by residents of mission layout at Bukuru, Plateau State. The leaves were identified and authenticated in the herbarium, Department of Plant science University of Jos, Nigeria. The collected leaves were shade dried and then ground to coarse powder.

Preparation of crude extracts

Fifty (50 g) of the powdered dried leaves were transferred into a conical flask and the content was soaked in 300 ml of methanol 72 hrs at room temperature. It was then agitated using mechanical shaker. The resulting suspension was filtered using sterile Whatman filter paper No 1. The filtrate obtained was evaporated at controlled temperature of 60^{o}C to dryness in a water bath and weighed on chemical balance [8, 9]. The resulting extract was preserved in a refrigerator. The same procedure was adopted for the aqueous extraction using water as a solvent and the percentage yield was determined.

Phytochemical screening

The phytochemical screening of the crude methanolic and aqueous extract of *Moringa oleifera* was carried out by standard protocols [10] at the Department of pharmacognosy Faculty of Pharmaceutical sciences University of Jos, Nigeria.

Source of test organisms

The test organisms were obtained from the culture collections of the Department of microbiology Federal college of Vetenary and medical laboratory technology Vom, Plateau state, Nigeria. The bacteria isolates obtained were *Escherichia coli, Proteus mirabilis, Salmonella typhi, Pseudomonas aeruginosa, Klebsiella spp. and Shigella spp.*

Culture media

Nutrient agar was used for the antimicrobial sensitivity test. They were prepared according to the manufacturers' instruction.

Standardization of inoculums

The test organisms were inoculated by transferring a loopful of each organism into peptone water, then it was incubated at 37^{o}C for 18hrs. Suspensions of the organisms were made from peptone water, and were shaken to achieve homogenous suspensions. The homogenous suspension of inoculum was adjusted to McFarland's standard 0.5 [11].

Antibacterial activity of leaf extracts

The antibacterial efficacy of methanolic and aqueous leaf extract of *Moringa oleifera* was tested by agar well diffusion method [12]. The cultures from the standardized broth were aseptically swabbed on sterile Nutrient agar plates using sterile cotton swabs. The wells of 6 mm were punched in the inoculated plates using a sterile borer; the base of each hole was filled with molten agar to seal the bottom of the plate. Aliquots of 100 µl volume of aqueous and methanolic leaf extract of (400 mg/ml) concentration were transferred into labeled wells. The wells were also filled with 100 µl positive controls (ciprofloxacin10µg and Ofloxacin 5 µg) and distilled water was used as negative control. The plates were incubated at 37^{0}C for 24 hrs and the zones of inhibition were recorded.

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration of the aqueous and methanolic extract of Moringa leaf was determined using the following concentrations 400mg/ml, 300mg/ml, 200mg/ml, 100mg/ml, 50mg/ml, and 25mg/ml. The cultures from the standardized broth were aseptically swabbed on sterile Nutrient agar plates using sterile cotton swabs. The wells of 6 mm were punched in the inoculated plates using a sterile borer. Aliquots of 100 µl of different concentrations of aqueous and methanolic leaf extract were transferred into labeled wells. The plates were incubated at 37^{0}C for 24hrs after wish it was examined for presence or absence of growth. The MIC was taken as the lowest concentration that prevented bacteria growth [13].

RESULTS

The results of inhibitory effect of methanolic and aqueous leaf extract of *Moringa oleifera* are shown in Table 1. The aqueous extract was found to be inhibitory to all bacterial isolates as seen in the diameter of zone of inhibition. The order of activity against selected bacteria by aqueous extract was *E. coli > S. typhi, Shigella spp.>* Klebsiella *spp, P. aeruginosa > P. mirabilis.* While the methanolic extract showed inhibitory effect to only three out of the six bacterial isolates. The zone inhibition values of the extracts against tested bacteria ranged from 9 to 15mm. Ciprofloxacin and Ofloxacin showed inhibition zones that ranged from 9 to 20mm. Table 2, showed the minimum inhibitory concentrations obtained for aqueous and methanolic extract MIC. MIC values for aqueous and methanolic extract ranged from 300 to 400 mg/ml for all bacterial isolates. There was no MIC in methanolic extract for *E. coli, Shigella spp., Proteus mirabilis.*

The results from MIC indicated that *Pseudomonas aeruginosa* and *Salmonella typhi* was the most sensitive bacteria to the *Moringa oleifera* leaf extract, being negatively affected at lowest concentration of 300 (mg/ml). Therefore aqueous extract had the highest antimicrobial activity compared to methanolic extract. The medicinal value of plants depends on the presence of phytoconstituents. The phytochemical screening revealed the presence of various phytoconstituents such as carbohydrates, cardiac glycosides, steroids, alkaloids, anthraquinones, saponins, flavonoids and tannins. The results of phytoconstituents presence are reported in Table 3.

Table 1: Antibacterial sensitivity of aqueous and methanol leaf extract

Zone of inhibition in mm					
	Extract (400mg/ml)		Control drugs		Negative control
Organisms	**Aqueous**	**Methanol**	**OFX(5 µg)**	**CPX(10 µg)**	**Distilled water**
P. aeruginosa	11	15	17	13	0
E. coli	15	0	18	17	0
S. typhi	12	15	20	17	0
Klebsiella spp.	11	11	19	20	0
Shigella spp.	12	0	9	0	0
P. mirabilis	9	0	18	19	0

Key : OFX=Ofloxacin, CPX=Ciprofloxacin

Table 2: Minimum inhibitory concentrations of aqueous and methanolic extract (mg/ml).

	Aqueous Extract						Methanolic Extract					
Test organisms	**400**	**300**	**200**	**100**	**50**	**25**	**400**	**300**	**200**	**100**	**50**	**25**
P. aeruginosa	+	+	-	-	-	-	+	-	-	-	-	-
E. coli	+	-	-	-	-	-	-	-	-	-	-	-
S. typhi	+	+	-	-	-	-	+	-	-	-	-	-
Klebsiella spp.	+	-	-	—	-	-	+	-	-	-	-	-
Shigella spp.	+	-	-	-	-	-	-	-	-	-	-	-
P. mirabilis	+	-	-	-	-	-	-	-	-	-	-	-

Key: + inhibition, - No inhibition

Table 3: Phytochemical screening of leaves of *Moringa oleifera*

Constituents	Methanol extract	Aqueous extract
Alkaloids	+	+
Saponins	-	+
Tannins	+	+
Flavonoids	+	+
Carbohydrates	+	++
Steroids	+	-
Anthraquinones	-	-
Cardiac glycosides	++	+

DISCUSSION

The present study has revealed that the leaf extracts of *Moringa oleifera* possess appreciable antimicrobial activity against the tested organisms. The aqueous extract showed greater antibacterial effect than the methanol extract, this may be due to the fact that most polar bioactive compounds

of the plant are soluble in water than in methanol. Aqueous extract showed a Minimum Inhibitory Concentration (MIC) to *Salmonella typhi* and *Pseudomonas aeruginosa* at a concentration of 300 mg/ml while methanol extract only maintained MIC at a concentration of 400 mg/ml. This means that only higher concentrations of methanol extract will further inhibit the growth of the pathogens.

The antibacterial activity of *Moringa oleifera* against enteric pathogens as revealed in this study agreed with the report of Devendra in India [14] who revealed that the Moringa *oleifera* leaf had a high antibacterial activity against gram negative bacteria.

From the present study *Moringa oleifera* aqueous extract was observed to be more potent than the methanol extract this finding is contrary to work reported by Singh in India [15].

Flavonoids one of the bioactive constituents is known to inhibit or kill many bacteria strains, it also inhibit viral enzymes such as reverse transcriptase and protease, it destroys some protozoa, yet their toxicity to animal cells is low [16]. With increasing development of resistance by bacteria to antimicrobials Moringa *oleifera* leaves provides evidence based result for the development of alternative drugs against bacteria pathogens.

CONCLUSION

The results from the present study indicate that *Moringa oleifera* leaves contained various types of compounds with potential pharmacological activity against bacterial pathogens associated with gastrointestinal tract. Aqueous extract exhibit a higher antimicrobial activity to bacterial isolates compared to methanolic extract. We recommend further research work involving more detailed in vitro and in vivo investigations to establish which components of the extract are biologically active in terms of antibacterial activity to enteric pathogen.

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