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ANTIBACTERIAL ACTIVITY OF *MORINGA OLEIFERA* SEED EXTRACTS ON SOME GRAM NEGATIVE BACTERIAL ISOLATES

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ABSTRACT

The antibacterial activity of aqueous and ethanolic extracts of *Moringa oleifera* (Zogale) seed was examined against some Gram negative bacteria (*Escherichia coli*, *Shigella flexneri* and *Salmonella typhi*). The concentration of the extracts used was between 50mg/ml and 400mg/ml. The results evaluated by zones of inhibition showed that the ethanolic extract at 400mg/ml, 200mg/ml and 100mg/ml were inhibitory to both *Shigella flexneri* and *Escherichia coli* while *Salmonella typhi* was not susceptible to the extract. The aqueous extract of *Moringa oleifera* seeds at the various concentrations tested was inactive against the tested organisms. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were 100mg/ml for the two organisms. The results of this study confirms the efficacy of Moringa seeds against diarrheal agents, *E.coli and Sh. flexneri*, and extends the scope of use of Moringa seeds as a water purifier and water treatment agent. This also indicates that Moringa seeds could be useful in treatment of some gastro intestinal and wound infections caused by gram negative bacteria.

INTRODUCTION

The need for new antimicrobial agents is closely linked with the problem of emergence of strains that are resistant to most synthetic antibiotics. This has arisen due to extensive use of antibiotics, which renders most of the current antimicrobial agents inefficient in controlling some bacterial diseases (Gustavo, *et al.*, 2010). There is increased evidence to proof that medicinal plants may represent an alternative treatment for non severe cases of infectious diseases. They could also serve as possible source of new and cheap antibiotics to which pathogenic strains are not resistant and several works provide scientific bases for the popular use of plants against infectious diseases (Kitula, 2007; Ajibesin *et al.*, 2008; Wu *et al.*, 2008).

Moringa Oleifera has been used extensively in traditional medicine for the treatment of several ailments, promotes digestion, skin diseases, diarrhea, as stimulant in paralytic afflictions, epilepsy and hysteria (Farooq *et al.*, 2012; Mishra *et al*, 2011). Various parts of the plant have been shown to be useful, such as the roots have been experimentally shown to have anti-inflammatory action (Odebiyi and Sofowora, 1999; Ndiaye *et al.*, 2002; Jamil *et al.*, 2007), the leaves, stem bark and seeds have been reported to have therapeutic properties (Anwar and Rashid, 2007).

The seed powder of *Moringa oleifera* works as a natural coagulant which clarifies very turbid water (Broin *et al.*, 2005). Studies on crude ethanol extract of dried *Moringa oleifera* seeds have been suggested to have anti-tumor-promoting activity and also wound healing property, while invitro anti-fungal activities against dermatophytes such as *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum*, and *Microsporum canis* has been reported, indicating that extracts could be used for future development of anti-skin disease agent (Guevara *et al.*, 2007). In Nigeria, *Moringa oleifera* is an edible plant, mostly found in the Northern part of the country. Despite much of the information on the excellent medicinal value of the plant, a lot more work is required to further determine the scope of its value as an alternative, cheap and effective antimicrobial agent in addition to its other uses.

This study is' set out to establish the antibacterial activity of ethanolic and aqueous extracts of *Moringa oleifera* seeds on some clinical bacterial isolates and determine the therapeutic value of the plant.

MATERIALS AND METHODS

Plant Samples and Test Organisms

Seeds of *Moringa oleifera* (Zogale in Hausa) were obtained from Jos South in Plateau state of Nigeria. Plant species were identified at the Plant Science Department of the University of Jos. Bacterial isolates biochemically characterized were obtained from the National Veterinary Research Institute (NVRI) Vom, Plateau State. The organisms include; *Escherichia coli*, *Salmonella typhi* and *Shigella flexneri*.

Preparation of Plant sample for extraction

The seeds were dried under shade and ground to powdered form using a clean sterile mortar and pestle and packaged in an air tight plastic container until used.

Aqueous extraction

Forty gram (40g) of the powdered seed was weighed using a weighing balance and transferred into a 1 liter beaker. Three hundred milliliter (300ml) of distilled water was added to the powder and allowed to stand for 48 hrs. This was then heated on a water bath (60°C) and filtered while hot. Hot water was continuously added to the residue and subsequently filtered. The procedure was repeated three times and the filtrate was then evaporated to dryness on a water bath (60°C).

Ethanolic extraction

Forty grams (40g) of the seed powder was weighed using a weighing balance and transferred to a one liter beaker. Three hundred (300ml) of methanol was added to the powder and allowed to stand for 48 hrs. The residue was then transferred to a soxhlet apparatus with ethanol for 48hrs and then evaporated to dryness on a water bath.

Preparation of media

Nutrient agar was prepared according to the manufacturer's instruction and dispensed into universal bottles in 20mls per aliquots. The bottles were then sterilized at 121°C for 15minutes and allowed to cool to about 45°C before dispensing into petridishes.

Preparation of concentrations of seed extract

Using sterile dilution technique, 4g of the ethanolic and aqueous extracts were dissolved separately in 10mls of water to give concentration of 400mg/ml (highest stock culture) followed by serial dilution with distilled water to give various concentrations of 200mg/ml, 100mg/ml and 50mg/ml. The tubes containing the various concentrations were labeled and used immediately. Gentamycin was used as the standard drug (125mg/ml).

Determination of antibacterial activity of aqueous and ethanolic extracts of *Moringa oleifera* seed

The agar well diffusion method was used in assessing the antibacterial activity of the plant extracts. To the various test tubes containing 10 ml nutrient broth, 0.1ml of the bacterial inoculum was added and mixed for homogeneity and was incubated for 3hrs. To all the test tubes (1-9) 0.1ml of broth cultures with estimated concentration of 10^8cfu/ml of bacterial inoculum. Standardization of the broth cultures was done as described by Cheesbrough, (2000) by diluting 1ml of broth culture to 5mls of nutrient broth and visually comparing the turbidity to that of 0.5 Macfarland turbidity standards after incubating at 37°C for 3-5 hours. The nutrient agar was poured into sterilized Petri-dishes, allowed to solidify for 30 minutes and dried. The test organisms were inoculated onto the sterile plates. Five wells of 9 mm in diameter each were aseptically bored using a sterile cork borer on each agar plate. On each agar plate, about 0.3mls of the seed extract of varying concentration was added to 4 of the wells and to the 5th well, gentamycin was added as a positive control. The same procedure was applied for the aqueous extract. The plates were then incubated at 35°C ± 2°C for 18-24hrs. Effect of the extract was assessed by measuring the diameters of zones of inhibition to the nearest milliliter, and then compared with the standard gentamycin.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations of the aqueous and ethanolic extracts of the seeds were determined by the broth dilution method. Eighteen (18) tubes labeled 1-9 were used for each extract. The first contained 5mls of double strength of nutrient broth, while the remaining contained 5ml of single strength of nutrient broth. Five milliliter (5ml) of the crude extract in the desired concentration was introduced into tube one and mixed thoroughly. Five milliliters (5ml) of the content of tube one was transferred into tube two, it was also mixed thoroughly and 5mls of the content of tube two was also transferred into test tube three. The procedure was

repeated for the remaining test tubes to tube 8 while tube 9 contained no drug. To each of the test tubes (1-9) 0.1ml of broth cultures (equivalent of 10^8cfu/ml) of the test organisms was added. All the tubes were incubated at 35°C ± 2°C for 18-20 hrs, after which they were examined for bacterial growth. The minimum inhibitory concentration (MIC) of the crude extract is the lowest concentration of the extract that is capable of inhibiting the growth of specified inoculum of a test organism.

Determination of Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) was determined by first selecting the tubes that showed no growth during the MIC determination. One loopful from each of these tubes was sub-cultured onto the surface of extract free nutrient agar and incubated for 24hrs at 35°C ± 2°C. The lowest concentration at which no growth was observed on the agar was noted as the MBC.

RESULTS

Antibacterial activity of aqueous and ethanolic extracts of *Moringa oleifera* seeds

For the ethanolic extract of the seed of *Moringa oleifera,* the results showed that at concentrations of 400mg/ml, 200mg/ml and 100mg/ml, Escherichia *coli* and *Shigella flexneri* were susceptible with zones of inhibition of 16mm, 12mm and 9 mm for *E. coli.* Similarly, *Sh. flexneri* had inhibition zones of 15mm, 12mm and 9 mm. *Salmonella typhi* was resistant to the ethanolic extract. The three organisms were not affected by the 50mg/ml concentration.

All the organisms tested were unaffected by the aqueous extract with no zones of inhibition observed compared with the control antibiotic which was effective on the 3 organisms, with zones of inhibition of 18mm, 20mm and 13mm for *E. coli, Sh. flexneri and S. typhi* respectively (Tables 1 and 2).

Minimum Inhibitory Concentration (MIC)

The result of the Minimum Inhibitory Concentration (MIC) of the ethanolic extract showed that *Escherichia coli and Shigella flexneri* were susceptible or sensitive at a concentration of 100mg/ml which is the lowest concentration of the extract which inhibited bacterial growth resulting in visually clear tubes after 24 hours incubation. But *Salmonella typhi* was resistant (Table 3).

Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) of Escherichia *coli* and *Shigella flexneri* for the ethanolic extract was 100mg/ml. This was the lowest concentration, from which there was no bacterial growth during MIC determination that was sub-cultured onto extract free nutrient agar. The plates were examined after 24 hours incubation of the test organisms (Table 4). This was not determined for the aqueous extract.

DISCUSSION

The antibacterial activity ethanolic and aqueous extracts of dried *Moringa oleifera* seeds was determined using three gram negative organisms, *Escherichia coli, Shigella flexneri and Salmonella typhi.* The aqueous extract had no effect on the test organisms at the various given concentrations, but there was appreciable antimicrobial activity demonstrated by the ethanolic seed extract with *Escherichia coli* and *Shigella flexneri* being susceptible. *Salmonella typhi* showed no susceptibility to both extracts. Our findings confirm other reports on the antibacterial activity of *Moringa oleifera* seed extracts (Jamil *et al*, 2007, Caceres *et al*, 1991; Mishra *et al*, 2011; Saadabi and Abu, 2011; Farooq *et al*, 2012).

Oluduro *et al*, (2011) used methanolic and aqueous extracts of the Moringa seeds and found appreciable inhibitory effect against bacterial isolates from wound infections, including *E.coli.* They reported that the extracts had broad spectrum of activity. Khesorn (2006) had also found that methanolic and purified dichloromethane extracts of the seeds had antibacterial action against both Gram positive and Gram negative organisms. Jabeen *et al*, (2008) reported that the seed extracts of Moringa were more effective against *Pasteurella multocida* and *Bacillus subtillis* than against *E. coli.* Our findings differ with that of report of Nepolean *et al*, (2009) who reported that the ethanolic extracts of Moringa seeds have high antibacterial activity against *Salmonella typhi*, while the aqueous extract had low activity against the same organism. Another study by Walter *et al*, (2011) showed that both methanol and n-hexane extracts of *Moringa oleifera* and *Moringa stenopetala* displayed antimicrobial activity against *S. typhi*, even though it was resistant to the ethanol extract in our study. Findings from this work reveal that Moringa seed extract had both bactericidal and bacteriostatic activity on *Escherichia coli* and *Shigella flexneri,* which are both gram negative organisms. This indicates that the seed extracts could also be used in the treatment of

some gastro intestinal or wound infections caused by gram negative bacteria.

Table 1: Antibacterial activity of Ethanolic extracts of seeds of *Moringa oleifera* on test organisms

	Zones of inhibition (Diameter in mm)				
Test organisms	400	200	100	50	Gentamycin (125mg/ml)
Escherichia coli	16	12	09	-	18
Shigella flexineri	15	12	09	-	20
Salmonella typhi	-	-	-	-	13

Key: - = No zone of inhibition (growth)

Table 2: Antibacterial activity of Aqueous extracts of seeds of *Moringa oleifera* on test organisms

	Zones of inhibition (Diameter in mm)				
Test organisms	400	200	100	50	Gentamycin (125mg/ml)
Escherichia coli	+	+	+	+	18
Shigella flexineri	+	+	+	+	20
Salmonella typhi	+	+	+	+	13

Key : + = Positive bacterial growth, due to inactivity of the aqueous extract, after 24hours incubation. Zones of inhibition seen only for Gentamycin control

Table 3: The Minimum Inhibition Concentration of Ethanolic extract of *Moringa oliefera* on test organisms

	Concentration of extracts (mg/ml)				
Test organisms	150	100	50	25	MIC
Escherichia coli	-	-	+	+	100
Shigella flexineri	-	-	+	+	+
Salmonella typhi	+	+	+	+	100

Key: - = No bacterial growth; + = Bacterial growth

Table 4: Minimum Bactericidal Concentration of Ethanolic Extract of seeds of *Moringa oleifera* on test organisms

	Concentration of extracts (mg/ml)				
Test organisms	150	100	50	25	MBC
Escherichia coli	-	-	+	+	100
Shigella flexineri	+	+	+	+	Nil
Salmonella typhi	-	-	+	+	100

Key: MBC = Minimum bactericidal concentration; - = Negative bacterial growth; + = Positive bacterial growth; Nil = No MBC value

The antibiotic nature of moringa seeds is attributed to the oil contained in it, which on consumption forms a thin film over the intestinal wall, thus reducing or preventing pathogens from penetrating the intestinal walls (Caceres and Lopez, 1991; Caceres *et al.*, 1991; Nwosu and Okafor, 1995). Other studies have also shown that the antibacterial activity of *Moringa oleifera* seeds is linked with a gum produced in the seed (Madsen *et al.*, 1987; Fuglie, 1999; Harristoy *et al.*, 2005). Although we did not evaluate the phytochemical constituents in the seed, other researchers had reported that tannins, saponins, flavanoids and terpenoids occur in the Moringa seed extracts (Nepolean *et al.*, 2009).

There are also reports that the moringa seed oil contains antiseptic and anti-inflammatory properties and can heal minor skin complaints such as burns, insect bites and rashes. It has been reported that crushed moringa seeds when added to dirty and bacteria laden water have the capacity to remove the impurities. It is believed that, moringa seeds will work better than most commonly used

water purifiers such as aluminium sulfate which could be toxic (Anwar and Rashid, 2007). Our findings show that the ethanolic extract of moringa seed has antimicrobial properties against pathogenic microorganisms in drinking water which also agrees with the works of Shekhar, *et al.* (2000), where crude ethanol extract of *M. oleifera* tested against *E. coli*, *S. typhi*, *V. cholera*, *Shigella dysentriae* and *Pseudomonas pyocyaneus*, showed activity against *E. coli* at reduced extract concentrations. On the contrary, a research conducted by Vaghasiya and Chanda, (2007) showed that *M. oleifera* crude extracts had no activity against *E. coli*.

CONCLUSION AND RECOMENDATION

Only the ethanolic seed extract showed varying degrees of antimicrobial activity on the microorganisms tested. Ethanol extracts exhibited a higher degree of antimicrobial activity as compared with aqueous extracts. Two species (*Escherichia coli, and Shigella flexneri*) presented the lowest MIC compared to the antibiotic standard, indicating a potent source of new antibiotic alternative. However, further work is needed to isolate the secondary metabolites from the extract in order to test for specific antimicrobial activity. There is also the need for more work to be carried out to establish the conflicting findings from various laboratories on the efficacy of the seed extracts on *S. typhi* the causative agent of typhoid fever.This *in vitro* study demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. According to World Health Organization, microbial resistance to conventional water treatment mechanisms is on the rise and medicinal plants offer a good source of alternative (Walter, *et al.*, 2011). *Moringa oleifera* represents an economic and safe alternative to treat infectious diseases in addition to its many other uses. Its use is thus highly encouraged and more work should be carried out to determine the antibacterial constituents that can be used for drug formulation.

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