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IN VITRO EVALUATION OF ANTIBACTERIAL ACTIVITIES OF SEED AND SHELL EXTRACTS OF *Moringa oleifera* AGAINST SOME HUMAN PATHOGENIC BACTERIA

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Abstract
Phytochemical analyses of aqueous and methanolic extracts of Moringaoleiferaseed powder demonstrated only the presence of saponins, reduced sugar, and carbohydrates.The antibacterial properties of aqueous and methanolic extracts ofMoringaolifera seed powder were determined in vitro against Pseudomonas aeruginosa, Salmonnellatyphi, Staphylococcus aureus and Bacillus subtilis usingdisc diffusion and minimum inhibitory concentration method (MIC). The aqueous extract of the seed powder displayed a potential antibacterial activity against the two tested gram-negative bacteria: Pseudomonas aeruginosa and Salmonnellatyphi, and the two tested gram-positive bacteria: Staphylococcus aureus and Bacillus subtilis. The methanolic extract of the seed powder however displayed antibacterial activity for onlyPseudomonas aeruginosa and Bacillus subtilis. The zones of inhibition for seed powder of aqueous and methanolic extracts were 4.33 - 10 mm and 0.83 - 5 mm respectively. The results suggest that the seed powder extracts of M. oleifera can be used as antibacterial agents that could be developed into chemotherapeutic products.

Keywords: *Moringaoleifera, gram-negative, gram-positive, minimum inhibitory concentration, extracts.*

INTRODUCTION

The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases. The frequency of these life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immuno-compromised patients in developing countries (Al-Bari *et al.*, 2006). The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raised the spectre of 'untreatable' bacterial infections and adds urgency to the search for new infection-fighting strategies (Zy *et al.*, 2005; Rojas *et al.*, 2006). For a long time, plants have been important sources of natural products for human health. World Health Organization (WHO), 2002 noted that majority of the world's population depend on traditional medicine for primary healthcare services because they constitute a major source of natural organic compounds. Plants have their antimicrobial properties as secondary metabolites such as alkaloid and flavonoid compounds. The practice of complementary and alternative medicine is now on the increase in developing countries in response to World Health Organization directives culminating in several pre-clinical and clinical studies that have provided the scientific basis for the efficacy of many plants used in folk medicine to treat infections (Vijaya and Ananthan, 1997; Dilhuydy, 2003). Despite the existence of potent antibiotic and antifungal agents, resistant or multi-resistant strains are continuously appearing, imposing the need for a continuous search and development of new drugs. Some strains of *S. aureus* are capable of producing staphyloxanthin (a carotenoid pigment that acts as virulent factor). It has an antioxidantaction that helps the microbe evade death by reactive oxygen species used by the hostimmune system. It is therefore very necessary that the search for newer antibiotic sources be a continuous process. Plants are the cheapest and safer alternative sources of antimicrobials (Pretorius and Watt, 2003; Sharif and Banik, 2006; Doughari *et al.*, 2007). *M. oringaoleifera* is the most widely cultivated species of a monogeneric family, the Moringaceae that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan (Fahey, 2005). It is widely used for treating bacterial infection, fungal infection, anti-inflammation, sexually transmitted diseases, malnutrition and diarrhoea. *Moringa*species have long been recognized by folk medicine practitioners as having value in the treatment of tumors (Ramachandran *et al.*, 1980). Hence, the present study was undertaken specifically to investigate the role of aqueous and methanolic extracts of *M. oleifera* seed powder as potential antimicrobial agent against some human pathogenic bacteria.

MATERIALS AND METHODS

All practical analysis and evaluations were carried out in Biochemistry and Microbiology laboratories, Federal University of Technology, Bosso campus, Minna-Niger state, Nigeria.

Sample Collection

The pods of *M. oringaoleifera* Lam were collected from Bosso area in Minna, Niger State in the month of August, 2010 and identified at the herbarium unit of Biological Science Department, Federal University of Technology, Minna, Niger State. The pods were air dried, and cracked. The seed powder was further air dried, made into powder, and sieved through 2 mm brass.

Test Microorganisms

The four bacterial strains used in this study are two gram-negative: *Pseudomonas aeruginosa*and *Salmonella typhi*, and two gram-positve: *Bacillus subtilis and Staphylococcus aureus*. All the tested strains were collected from the Department of Microbiology, Federal University of Technology, Bosso Campus Minna, Niger State. The bacteria were grown at 37°C (in incubator) and maintained on nutrient agar slants at 4°C.

Plant Sample Extraction

Aqueous Extracts of Seed Powder

Fifty gramsseed powderof *M. oringaoleifera* was placed in 400 ml of distilled water in a round bottom flask and fixed to a reflux extractor via glass adaptor for four hours at 100°C. This was filtered off into a clean beaker using muslin cloth and subjected to steam bath evaporation at 40°C. The extract obtained was stored in the refrigerator at 4°C till ready for antibacterial activity test (Akueshi *et al.*, 2002).

Methanol (100%) Extracts of Seed Powder

Fifty grams seed powder of *M. oringaoleifera* was placed in 200 ml of methanol (100%) in a round bottom flask and fixed to a reflux extractor via glass adaptor for four hours at 60°C. This was filtered off using muslin cloth into a clean beaker and subjected to steam bath evaporation at 40°C. The extract obtained was stored in a refrigerator at 4°C for antibacterial activity test (Akueshi *et al.*, 2002).

Phytochemical Screening of Extracts

Phytochemical tests for various constituents of extracts were carried out by the methods of Trease and Evans (1983). The extracts were screened for the presence of alkaloids, flavonoids, saponins, glycosides, tannins, reduced sugar, carbohydrates, amino acids, steroids, phenols, volatile oils and proteins.

Experimental Design

The antibacterial assay involves the antibacterial activity of samples of *M. oleifera* extracts;

I. Aqueous extract of seed powder;
II. Methanolic extract of seed powder.

***In vitro* Antibacterial Test**

The *In vitro* antibacterial test was carried out by disc diffusion method (Bauer *et al.*, 1996; Barry, 1980) using 25 ìl of standardized suspension of tested bacteria spread on nutrient agar plates. The discs (5 mm in diameter) were impregnated with 10 ìl of 20 mg ml^{-1} (200 ìg $disc^{-1}$), air-dried and placed on seeded agar plates. Negative controls were prepared using the same solvents to dissolve the plant extracts. Tetracycline (30 ìg disc-1).was used as positive control to determine the sensitivity of bacterial strain. The plates were incubated at 37°C for 24hrs. Antimicrobial activity was evaluated by measuring the zones of inhibition against the tested bacteria. Each assay was carried out in triplicate.

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) of two samples of *M. oleifera* was determined by two-fold serial dilution method (Chandrasekaran and Venkatesalu, 2004). The dose levels of seed powder of 20 mg ml^{-1} was serially diluted in a nutrient broth of 5 ml with varying concentrations; 10, 5, 2.5, 1.25 and 0.625 mg ml^{-1}. The test tubes were incubated at 37°C for 24 hrs. Controls were used with the test organisms, but with distilled water instead of the plant extract. The least concentration of the samples with no visible growth was taken as the MIC (Adesokan, 2007).

RESULTS AND DISCUSSION

Table 1 shows the phytochemical properties of the extracts. The two extracts demonstrated trace presence of saponins, reduced sugar and carbohydrate.

Table 2 shows diameter of zones of inhibition of bacterial growth at varying concentrations of seed powder of *M. oringaoleifera*. The aqueous extract of seed powder showed stronger antibacterial activity against the studied gram-negative bacteria (*Psuedomonasaeruginosa* and *Salmonella typhi*) and gram-positive bacteria (*Bacillus subtilis*and *Staphylococcus aureus*) with the respective diameter zones of inhibition as: 11.33±1.52, 12.00±2.64 and 5.00±3.00, 4.33±2.08 mm. Methanolic extract of seed powder also exhibited a relatively potent inhibitory effect against all the tested gram-negative bacteria (*Psuedomonasaeruginosa* and *Salmonella typhi*) and gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) with their respective diameter zones of inhibition was 3.33±2.86, 5.00±1.0 and 1.5±1.32, 1.83±1.25 mm.

Table 1: Phytochemical Constituents of *Moringaolifera* Seed Powder

Phytochemical Components	Aqueous Extract	Methanolic Extract
Saponins	+	+
Tannins	-	-
Flavonoids	-	-
Alkaloids	-	-

Table 3: Minimum Inhibitory Concentration (MIC) of *M. oringaoleifera*seed powder and shell extracts against the tested human pathogenic bacteria.

Bacteria		Aqueuosextracts[a]		
		Seed powder	Seed powder	Distilled Water
Gram-Negtive	*Pseudomonas aeruginosa*	2.5	10	-
	Salmonella typhi	2.5	ND	-
Gram-positive	*Bacillus subtilis*	5	10	-
	Staphylococcus aureus	5	ND	-

KEY:

[a]Minimum inhibitory concentration (values in mg ml^{-1}).

ND: Not detected

Distilled water is the control.

Table 3: Minimum Inhibitory Concentration (MIC) of *M. oringaoleifera* seed powder and shell extracts against the tested human pathogenic bacteria.

Bacteria		Aqueous extracts*		
		Seed powder	Seed powder	Distilled Water
Gram-Negtive	*Pseudomonas aeruginosa*	2.5	10	-
	Salmonella typhi	2.5	ND	-
Gram-positive	*Bacillus subtilis*	5	10	-
	Staphylococcus aureus	5	ND	-

KEY:

*Minimum inhibitory concentration (values in mg ml^{-1}).

ND: Not detected

Distilled water is the control.

Displays of strong inhibition of aqueous extract of seed powder against all the tested bacteria were notice within the concentrations of 2.5 mg ml^{-1} and 5.0 mg ml^{-1}. The methanolic extract of the seed powder however showed inhibition only for *Pseudomonas aeuginosa* and *Bacillus subtilis* at 10 mg ml^{-1}. The highest zones of inhibition against all the tested bacteria were found in the aqueous extract of the seed powder and this showed relativity with the positive control drug, tetracycline, (with respect to *Salmonella typhi* and *Bacillus subtilis)*. The methanolic extract of the seed powder also showed inhibitory potency but was less by half the activity of the aqueous extracts on all tested bacteria. The inability of the methanolic extract to effectively inhibit some of the tested bacteria could be due to incomplete extraction of its active components. The secondary metabolites like alkaloids and flavonoids may therefore be too low to demonstrate antibacterial activities against the tested bacteria. The alkaloids are nitrogenous heterocyclic organic compounds produced by plants to protect it self against predators. Saponins on the other hand have anti-inflammatory, anticholinergic, and hepatoprotective effects. The lowest MIC wasrecorded in respect of the two gram-negative bacteria (*P. aeruginosa* and *Salmonella typhi*). These suggest that *M. oleifera* seed powder used contain bio-active components whose antibacterial activities against the tested gram-negative, and gram-positive bacteria are closely related to that of the antibiotic, tetracycline. The *in vivo* activity of the aqueous extract of the seed powder of *M. oringaoleifera* showed a better antibacterial activity over the methanolic extract. This is observed by the stronger inhibitory potentials exhibited by the aqueous extract. The claim by the traditional medical practitioners that *M. oleifera* seed powder is used to treat some infections has not been authenticated by our research. It may however have some potential applications in pharmaceutical industry for treating some pathogenic bacteria. Other solvents can be used for extraction (e.g. ethanol) to see whether it will show greater efficacy.

CONCLUSION

The inability of the two extracts to effectively inhibit the tested bacteria could be due to incomplete extraction of the active components especially alkaloids, tannins, and saponins. If seed extracts are to be used for medicinal purposes, issues of safety and toxicity should also be considered.

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