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Proximate Analysis and the Antimicrobial Activities of *Moringa Oleifera* Leaves, Seeds and Roots on Some Microorganisms

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Abstract - The antimicrobial activity of different extracts of Moringa oleifera leaves, roots and seeds was investigated against five test bacteria namely Esherichia coli, Staphylococcus aureus,Salmonella typhi, Klebsiella pneumonia and Pseudomonas aeruginosa and the six test fungi namely Fusarium oxysporum, Aspergillus niger, Aspergillus terreus, Aspergillus ochraceus, Penicillium corylophilum and Rhizopus stolonifera. Two solvent 99% Ethanol and hot water were used for extraction of the active ingredients present in the plant. The antibacterial activities were performed using Agar well diffusion method. The result showed that Hot water root extract had maximum zone of inhibition of 12.0mm. The antifungal activity was performed using Micro broth dilution test method. Ethanol root and ethanol leaves extract showed 100% inhibition against Rhizopus stolonifera at the maximum concentration used. Hot water root extract showed 100% inhibition against A. terreus, A. ochraceus and R. stolonifera at the maximum concentration used. In the proximate analysis, The seeds contained moisture content (9.56% 0.01), Ash content (8.25% 0.01), crude lipid (11.52% 0.02),. This antimicrobial study indicate that the root of Moringa oleifera had the broadest spectrum of activity on the test bacteria and test fungi and consequently making it more effective than the leaves and seeds. This study also revealed that the leaves, seeds and roots of Moringa oleifera are nutritionally valuable and are highly recommended for their higher nutritional values based on the proximate analysis.

Keywords: Moringa oleifera, Antimicrobial activities, proximate analysis, Bacteria, Fungi,

1. Introduction

1.1 Moringa Oleifera

The fact that Ancient medicinal system relies on several plant products used by traditionally human communities in many parts of the world for different therapy and prevention of diseases cannot be over emphasized (Okorondu et al., 2015). Moringa oleifera is simply one of the numerous plants with outstanding therapeutic and preventive characteristics and therefore, has been of great contribution from ancient times (Fozia et al., 2012).

Indians have been using it as a regular component of conventional eatables for nearly 5000 years and also people in the northern Nigeria consume it as a local food and tea (Anwar et al., 2005).

1.2 Uses of Moringa Oleifera

Moringa oleifera have found a wide range of application. It is applied industrially and domestically. Every component of the plant is useful. No wonder it is refered to as the wonder plant. Human consumption Moringa leaf powder is used as a 100% natural food supplement and can be consumed in different ways. The leaf powder can be mixed with juices or beverages using a teaspoon.

One teaspoon of Moringa will provide a full range of nutrients required by the body. Moringa leaf powder can also be mixed with vegetables or soup that is prepared for consumption. In Africa, 25 g of Moringa powder is administered to pregnant women daily to
improve prenatal nutrition (Diatta, 2001). Apart from plain leaf powder,
Moringa powder is also sold in capsules. The daily intake is about two capsules a day (one capsule in the morning and one at lunch time). According to Marcu (2005), no negative effects from daily consumption of 11 Moringa leaves and seeds have ever been reported.
Marcu (2005) further indicated that Moringa has the following health benefits:
1. Reduces cholesterol levels and triglycerides (" bad" fats);
2. Controls blood sugar and helps normal sugar and energy balance;
3. Offers vitamins and minerals vital for maintaining normal physiology and
4. Offers powerful anti-aging and anti-inflammatory natural substances, many with anti-cancer properties.

2. Methodology

All reagents and glass wares that were used in the course of this research work was sterilized in an autoclave at a pressure of 121^0C for a period of 15 minute.

2.1 Chemicals and reagents

99% Ethanol, Nutrient agar, Salmonella Shigella Agar (SSA), Eosin Methlylene Blue Agar (EMB), Centrimide Agar, MacConkey Agar, Distilled water.

2.2 Test Organisms

The bacteria used were *Esherichia coli, Staphylococcus aureus, Salmonella typhi, Klebsiella pneumonia* and *Pseudomonas aeruginosa* . *Escherichia coli* was grown on EMB agar, *Staphylococcus aureus* was grown on nutrient agar, *Salmonella typhi* was grown on SSA, *Klebsiella pneumonia* was grown on MacConkey agar and *Pseudomonas aeruginosa* was grown on Centrimide agar.
The fungi used were *Fusarium oxysporum, Aspergillus niger, Aspergillus terreus, Aspergillus ochraceus, Penicillium corylophilum and Rhizopus stolonifera.*

2.3 Extraction of Plant Materials

The method of Aishmma and Mitscher (1979) was used to obtain the plant extract. Hundred grams of each of the grounded samples were added to 350 ml distilled water and 350 ml of 99% ethanol in separate conical flasks and left to stand for 72 hours. The extracts were then filtered separately using whatman no 1 filter paper and the filtrates were evaporated and stored in a deep freezer for further use.

2.4 Preparation of concentrations of extract

Using serial dilution technique, 5g of the evaporated ethanol and aqueous extracts were dissolved separately in 10ml of water to give concentration of 0.50g/ml(highest stock culture) followed by serial dilution with distilled water to give various concentrations of 0.4g/ml,0.30g/ml and 0.02g/ml. The tubes containing the various concentrations were labeled and used immediately. Hot water and 99% ethanol without the plant samples was used as negative control. The antibacterial screening was carried out using agar well diffusion method as described by (chessbrough, 2006). One milliliter of sterile Antibacterial susceptibility screening (Agar well diffusion method). Nutrient agar was poured into each of the petri dishes and allowed to set. The pure culture of *Staphylococcus aureus, Salmonella shigella, Escherichia coli, Bacillus subtilis and Pseudomonas aeruginosa* were streaked unto the surface of the sterile plates and four wells were made aseptically on each of them with a sterile cork borer. The different concentration of the extracts were introduced into the separate wells and allowed to diffuse into the medium for about 1 hour and then incubated at 37oc for 24 hours. After incubation, the diameters of zone of inhibition were taken and the negative controls were also checked for antimicrobial activity.

2.5 Minimum inhibitory Concentration (MIC)

The MIC of the potent extracts was determined by macro broth dilution techniques (Boron and Feingold, 1990). Multiple fold dilution was made to get ten different concentrations of the extracts and standardized suspensions of the test organisms were inoculated into a series of sterile tubes of peptone water containing dilutions (0.05, 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, 0.80, 0.90g/ml) of seeds, leaves, and root extracts and incubated at 37^{o} C for 24hours. The MICs were read as least concentration that inhibited any visible growth (absence of turbidity) of the test organisms. Antifungal susceptibility screening (Micro broth dilution test): Two milliliters each of equal volume of the different concentrations of extracts and yeast extract broth were dispensed into sterilized test tubes. Specifically, 0.1ml of standardized fungi inoculums from $5.0x10^4$ CFU/ml was added to each of the test tubes above and the tubes incubated at 28±2 ^{o} C for 72 hours (khosravi and Behzadi A.,2006). Inoculated tubes containing broth and solvent without plant extracts were incubated alongside to serve as negative control.
After incubation, turbidity was observed and the sensitivity of the fungi to the test extracts was recorded as described by Murugan *et al*. (2007).

2.6 Proximate analysis

The moisture, crude protein, crude fat, total ash and crude fibre contents of the samples were determined using standard methods of the Association of Official Analytical Chemists (AOAC, 2006). Moisture content was determined by heating 2.0g of each fresh sample to a constant weight in a crucible placed in an oven maintained at 105^0 C. The dried samples from the moisture content analysis were used in the determination of the other proximate content. Crude protein (% total nitrogen x 6.25) was determined by the kjeldahl method using 2.0g samples: crude fat was obtained by exhaustively extracting 5.0g of each sample in a soxhlet apparatus using petroleum ether (boiling point range 40-60^{0}C) as the extractant. Ash was determined by the incineration of 10.0g samples placed in a muffle furnace maintained at 550^{0}C for 5hours. Crude fiber was obtained by digesting 2.0g of sample with H_2SO4 and NaOH and incinerating the residue in a muffle furnace maintained at 550^0 C for 5hours. Carbohydrate was calculated by adding all the other proximate content and subtracting from 100. Each analysis was carried out in duplicates.

3.0 Results and Discussion

Table 1 present the result of antibacterial activity of *Moringa oleifera* extracts on some bacteria isolates. It can be deduced that *Moringa oleifera* root extracted with ethanol had the broadest spectrum of activity on the test bacteria. The results show that it had activity against all five test bacteria namely *Salmonella typhi* (6.0-8.0mm), *Staph. aureus* (7.0-8.0mm). *E coli* (8.0-12.0mm) and *Klebsiella pneumonia* (6.0-8.0mm) at concentrations ranging from 0.20g/ml to 0.50g/ml while it only showed activity on *Psudomonas aeruginosa* (2.5mm) at concentration of 0.40g/ml and 0.50g/ml. *Moringa oleifera* root extracted with hot water was active on *E.coli* (4.0-6.0mm), *Staph. aureus* (4.0-5.0mm), *Salmonella typhi* (4.50-5.0mm), *Klebsiella pneumonia* (2.0-5.0mm) at concentrations ranging from 0.20g/ml to 0.50g/ml while it only showed little activity on *Psudomonas aeruginosa* (2.0mm) at concentrations of 0.4g/ml and 0.5g/ml.

Moringa oleifera leaf extracted with ethanol was active on *E.coli* (6.5-10.0mm), *Staph. aureus* (6.0-9.0mm), *Salmonella typhi* (6.0-9.0mm), *Klebsiella pneumonia* (4.0-7.0mm) at concentrations ranging from 0.20g/ml to 0.50g/ml, *Pseudomonas aeruginosa* showed only little sensitivity (2.0mm) at concentration of 0.4g/ml and 0.5g/ml while *Moringa oleifera* leaf extracted with hot water showed activity on *E.coli* (4.5-5.0mm), *Staph. aureus* (3.5-4.0mm), *Salmonella. typhi* (4.0-4.5mm), *Klebsiella. pneumonia* (2.0-4.0mm) at concentrations ranging from 0.20g/ml to 0.50g/ml. *Pseudomonas aeruginosa* showed only little sensitivity on 0.50g/ml with 2.00mm. *Moringa oleifera* seed extracted with ethanol was active on *E.coli* (4.5-8.0mm), *Staph. aureus* (4.0-6.0mm), *Salmonella typhi* (4.0-6.5mm), *Klebsiella pneumonia* (3.0-4.0mm) at concentrations ranging from 0.20g/ml to 0.50g/ml but did not show any activity on *Pseudomonas aeruginosa* while *Moringa oleifera* seed extracted with hot water was active on *E.coli* (3.0-5.0mm), *Staph. aureus* (2.0-3.0mm), *Salmonella typhi* (3.0-4.0mm) at concentrations ranging from 0.20g/ml to 0.50g/ml, *Klebsiella pneumonia* showed zone of inhibition of 2mm at all the concentrations used. This results are in line with report of Oluduro A.O,(2012) which stated that Moringa

leaves possesses inhibitory properties, which serve as an alternative therapy for wounds, a good source of nutrient supplement and prevention of fungal infections.

Table 2 shows the result of antifungal activities of *Moringa oleifera* extracts on the test fungal. Hot water root extract and ethanol root extract inhibited the growth of *Aspergillus terrus, Aspergillus ochraceusk, Rhizopus stolonifera* by 100% at 0.50g/ml. Ethanol leaf extract showed no sensitivity at all concentration used but inhibited the growth of *Rhizopus stolonifera* by 100% at 0.40 and 0.50g/ml. Hot water leaf extract inhibited the growth of *Aspergillus ochraceus, Aspergillus niger and Aspergillus terrus* by 50% at 0.50g/ml and *Rhizopus stolonifera* by 75% at 0.50g/ml. Ethanol seed extract inhibited the growth of *Fusarium oxysporum* by 25% at 0.50g/ml and *Aspergillus niger, Aspergillus terrus, Aspergillus ochraceus, penicillum corylophilum, Rhizopus stolonifer* by 50% at 0,50g/ml. Hot water seed extract inhibited the growth of *Rhizopus stolonifer* by 75% at 0.50g/ml; it also inhibited the growth of *Aspergillus niger and Aspergillus ochraceus* by 50% at 0.50g/ml and *Aspergillus terrus, Fusarium oxysporum and penicillum corylophilum* by 25% at 0.50g/ml. All the extracts showed little or no activity on the test fungi at low concentrations of 0.20 and 0.30g/ml. Comparatively, it was observed that the root extract of *Moringa oleifera* exhibited more antifungal activity on the test fungal than the leaf and seed as it inhibited the growth of *Aspergillus terrus, Aspergillus ochraceus and Rhizopus stolonifera* completely at concentration of 0.50g/ml. the root extract showed more antifungal activity on *Rhizopus stolonifera* than the leaf and seed extracts. Ibegbulem C.R (2012) affirm the fact that the root extract of *Moringa oleifera* exhibited more antifungal activity on the test fungal than the leaf and seed**.**

Chart 1: The proximate screening result indicates that *Moringa oleifera* contains high amount of carbohydrate and crude protein in the leaves. The carbohydrates content in the leaves are 53.48% while roots has 51.90% and the seeds has 39.18% respectively. The protein content in leaves are 24.98% while 12.29% protein in roots and seeds has 17.99% respectively. Crude lipid in seeds is 11.52%, leaves 2.66% and roots is 2.59%.

Table 1: Antimicrobial activities of *Moringa oleifera* extracts on bacterial isolates
Zones of Inhibition (MM)

Extract	Conc.(g/ml)	SA	ST	EC	PA	KP
HWS	0.20	2.0	3.0	3.0	0.0	2.0
	0.30	2.0	3.0	3.0	0.0	2.0
	0.40	2.5	3.0	3.0	0.0	2.0
	0.50	3.0	4.0	3.0	0.0	2.0
HWL	0.20	3.5	4.0	4.5	0.0	2.0
	0.30	3.5	4.0	4.5	0.0	2.5
	0.40	4.0	4.5	5.0	0.0	3.0
	0.50	4.0	4.5	5.0	2.0	4.0
HWR	0.20	4.0	4.5	4.0	0.0	2.0
	0.30	4.0	4.5	4.5	0.0	2.0
	0.40	4.0	4.5	5.0	2.0	3.5
	0.50	5.0	5.0	6.0	2.0	5.0
ES	0.20	4.0	4.0	4.5	0.0	3.0
	0.30	5.0	4.0	5.5	0.0	3.0
	0.40	5.5	5.5	7.0	0.0	4.0
	0.50	6.0	6.5	8.0	0.0	4.0
EL	0.20	6.0	6.0	6.5	0.0	4.0
	0.30	7.0	6.0	7.0	0.0	4.0
	0.40	7.0	7.5	8.0	2.0	6.0

	0.50	9.0	9.0	10.0	2.0	7.0
ER	0.20	7.0	6.0	8.0	0.0	6.0
	0.30	7.0	6.5	8.0	0.0	7.0
	0.40	8.0	7.5	10.0	2.5	8.0
	0.50	8.0	8.0	12.0	2.5	8.0
Negative Control	Hot water	0.0	0.0	0.0	0.0	0.0
99% Ethanol	0.0	0.0	0.0	0.0	0.0	0.0

Isolate Keys: HWS= Hot water+ Seed extract, HWL= Hot water + Leaf extract, HWR= Hot water+Root extract, ES= Ethanol +Seed extract, EL= Ethanol + Leaf extract, ER= Ethanol +Root extract, SA= *Staphylococcus aureus*, ST= *Salmonella typhi*, EC= *Escherichia coli*, PA= *Pseudomonas aeruginosa*, KP=*Klebsiella pneumonia*

Table 2: Antfungal activities of *Moringa oleifera* extract on some fungi isolates based on turbidity

Extract	**Conc.(g/ml)**	**FO**	**AN**	**AT**	**AO**	**PC**	**RS**
HWS	0.20	++++	++++	++++	++++	++++	++++
	0.30	++++	++++	++++	++++	++++	+++
	0.40	++++	++++	+++	+++	+++	++
	0.50	+++	++	+++	++	+++	+
HWL	0.20	++++	++++	+++	++++	++++	+++
	0.30	++++	++++	++	++++	+++	+++
	0.40	+++	+++	++	++	+++	+
	0.50	+++	++	++	++	+++	+
HWR	0.20	++++	++++	+++	++	+++	++
	0.30	++++	++++	++	++	++	++
	0.40	+++	++	+	+	++	+
	0.50	+++	+	-	-	++	-
ES	0.20	++++	++++	++++	+++	+++	++++
	0.30	+++	+++	+++	+++	+++	++++
	0.40	+++	+++	++	++	+++	+++
	0.50	+++	++	++	++	++	++
EL	0.20	++++	++++	+++	+++	+++	++
	0.30	+++	++++	+++	++	+++	++
	0.40	+++	+++	++	++	++	-
	0.50	+++	++	++	++	++	-
ER	0.20	++++	++++	+++	++	+++	+
	0.30	+++	+++	+	+	++	+
	0.40	+++	++	+	+	++	-
	0.50	+++	+	-	-	+	-
Negative Control	Hot water	++++	++++	++++	++++	++++	++++
	99%Ethanol	++++	++++	++++	++++	++++	++++

Key: HWS= Hot water+ Seed extract, HWL= Hot water + Leaf extract, HWR= Hot water+Root extract, ES= Ethanol +Seed extract, EL= Ethanol + Leaf extract, ER= Ethanol +Root extract, FO=*Fusarium oxysporum*, AN=*Aspergillus niger*, AT= *Aspergillus terreus*, AO= *Aspergillus ochraceus*, PC=*Penicillum*

corylophilum, RS= *Rhizopus stolonifera*, ++++= *Normal growth*, +++= 25% inhibition, ++= 50% inhibition, += 75 inhibition, -= 100% inhibition.

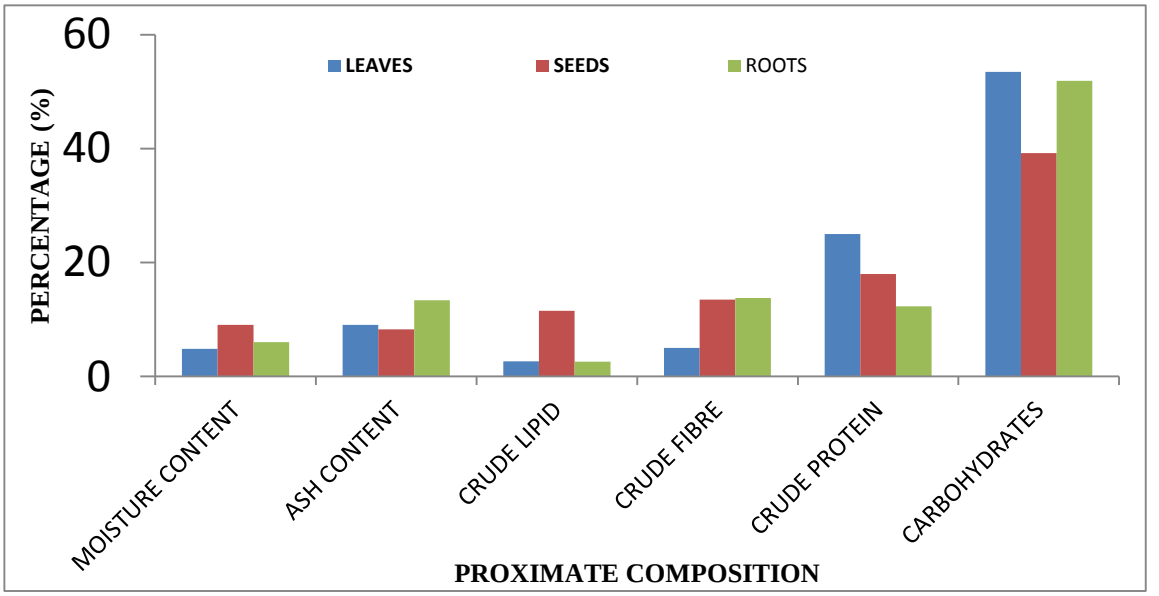

Fig 1: Proximate Composition of Moringa Leaves, Roots And Seeds

4. Conclusion and Recommendation

The result of this study indicated that both the aqueous and ethanol extracts of *Moringa oleifera* leaves, seeds and roots possess antibacterial properties but the roots had higher antibacterial activity that the leaves and seeds. *Pseudomonas aeruginosa* showed resistance to seed extracted with hot water and ethanol and only showed little sensitivity to all the extracts used. In this study, the ethanol extracts were more effective than the aqueous extracts, indicating that ethanol is a better solvent than water.

The result of the antifungal screening showed that both root extract of *Moringa oleifera* exhibited very high antifungal activity on the test fungi as it inhibited the growth of *Aspergillus terrus, Aspergillus ochraceus and Rhizopus stolonifera* by 100% at concentration of 0.5gml, Ethanol leaf extract also exhibited 100% antifungal activity on the growth of *Rhizopus stolonifera* at 0.40 and 0.5gml concentrations. All the extracts exhibited little or no antifungal activity at low concentrations. *Rhizopus stolonifera* was observed to be the most sensitive organism to the extracts. This is in consonance with the report of Ibegbulem C.R (2016) which affirmed that roots of Moringa has the highest activity on test isolates at the concentration of 125mg/ml. The following can therefore be recommended

1. The leaves and seeds of Moringa oleifera can be consumed to reduce antimicrobial activity of pathogenic microorganisms when they infect humans.

2. Furthermore, pharmaceutical companies should pay attention to the roots, leaves and seeds of *Moringa oleifera* as they can become a potent source of new antimicrobial alternative. *Moringa oleifera* represents an economic and safe alternative to treat infectious diseases.

3. The government and the people should also take notice of *Moringa oleifera* as it can be used as a new means of water treatment because of its ability to destroy organisms of fecal origin like *Escherichia coli* and *Salmonella typhi*.

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