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Antibacterial Activity, Phytochemical and Proximate Analysis of *Moringa Oleifera* Seeds Against Clinical Isolates

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This study was carried out to determine the antibacterial activity, phytochemical and proximate analysis of *Moringa oleifera* seeds extracts against some clinical isolates. The antibacterial activity for Aqueous and Ethanol extracts was determined using the agar diffusion method. The Phytochemical Screening of the seed was carried out using Qualitative Analysis (QA) and proximate analysis was carried out according to AOAC methods. The aqueous seed extract showed varying degrees of antimicrobial activity over that of ethanol extract on the microorganisms tested. Two species (*Staphylococcus aureus* and *Salmonella typhi*) presented the lowest MIC of 31.25 and 125mg/ml compared to MBC of 250mg/ml respectively. The phytochemical revealed the presence of Flavonoids, Tannins, Alkaloid, and Saponins. The Proximate Analysis revealed high concentration of Crude lipid ($35.58 \pm 0.42\%$) and lowest concentration of Moisture content ($2.03 \pm 0.01\%$). This study shows that *moringa oleifera* seeds can be as effective as synthetic medicine to combat pathogenic microorganisms if properly utilized.

Keywords: *Moringa oleifera* seeds, Proximate analysis, Phytochemicals; *Staphylococcus aureus*,

1. Introduction

Moringa oleifera is known in Nigeria, it is consumed as food, the Hausas called it *zogale* or *tamakka*, Yorubas called it *Ewe Igbale* or *idagbomonoye* and Igbo called it *Ikwaoyiba* (Thilza *et al.*, 2010). It has been reported to have medicinal value and is highly nutritious which benefits people in terms of providing daily nutritional supplements and boosting their immune systems (Popoola and Obembe, 2013).

Since the introduction of penicillin, scientists began to notice the emergence of a penicillin-resistant strain of *Staphylococcus aureus*, dysentery causing *Shigella* and *Salmonella*, and strains of *gonorrhoea* (WHO, 2003). *Moringa oleifera* has been used for treating microbial infection, inflammation, sexually transmitted disease, and diarrhea (Farooq *et al.*, 2012) for several decades due to its versatile and valuable properties. It has been reported that polypeptides in seeds are one of the best natural coagulants and can bind to many moieties and processes antimicrobial properties that can be used for treatment (Anwara and Rashid, 2007). The seeds can be used as an inexpensive adsorbent for the removal of heavy metal and water purifying powers by flocculating gram-negative

and positive bacterial cells (Sharma *et al.*, 2006; Kawo, 2007). *Moringa oleifera* seeds possess potent antifungal, antibacterial, and flocculating activities (Friere *et al.*, 2015; Ullah *et al.*, 2015).

The medicinal plant is any plant or contains bioactive substances (phytochemicals) and is regarded as secondary metabolites. Antimicrobial agents are important in reducing the global burden of infectious diseases. However, as resistance pathogens develop and spread, the effectiveness of the antibiotics is diminished and causes sectors to treat public health, and for all kinds of antibiotics is diminished and cause serious threat to public health, and all kinds of antibiotics (Abdulrasheed *et al.*, 2015). The World Health Organization (WHO) is coordinating a global campaign to raise awareness of antibiotic resistance (WHO, 2015). Antibiotic resistance is aggravated by the misuse and overuse of antibiotics, whereby it causes an estimated 700,000 death. Each year, Resistance to specific antibiotics has been reported in different parts of Nigeria.

The problem of the emergence of strains that are resistant to most synthesis antibiotics has arisen

due to the extensive use of antibiotics which renders the most of current antimicrobial agents inefficient in controlling some bacterial diseases. This, therefore, makes it an effective antimicrobial agent, and its use will help in solving problems related to the emergence of strains that are resistant to most synthetic antibiotics. The study aims to determine antibacterial activity, phytochemical and proximate analysis of *Moringa oleifera* seeds extract on some clinical isolates.

2. Materials and Methods

2.1 Collection and Preparation of *Moringa oleifera* Seeds

Moringa oleifera seeds sample was obtained from Osokoro, behind Government House Dutse, Jigawa state. The seeds were dried under shade and grounded to powdered form using a clean sterile mortar and pestle and packaged in an air-tight plastic container until used.

2.2 Isolation and Identification of the Bacterial Isolate

Clinical isolates of *Staphylococcus aureus* and *Salmonella typhi* was obtained from Sokoto state university, Microbiology laboratory. Gram's staining and biochemical test such as Triple sugar iron, methyl red Voges Proskauer test, indole test, oxidase, coagulase, urease and catalase were carried out as described by Oyeleke and Manga (2008) for identification of microorganisms.

2.3 Preparation of Aqueous and Ethanolic Extract

Forty grams (40g) of the powdered seed was weighed and dissolved in four hundred millilitres (400ml) of distilled water in a beaker and allowed to stand for 48 hrs. This was then heated on a water bath (60°C) and filtered. Hot water was continuously added to a residue and subsequently filtered. The procedure was repeated three times and filtrate then evaporated to dryness on a water bath (60°C) (Lar *et al.*, 2011). The same procedure was repeated for the ethanol extract.

2.4 Preparation of Concentrations of Seed Extract

Using sterile dilution technique, 1g of the ethanol and aqueous extracts was dissolved separately in 4mls of water to give a concentration of 250mg/ml (highest stock culture) followed by serial dilution with distilled water to give various concentrations of 125mg/ml, 62.5mg/ml and 31.25mg/ml. The tubes containing the various

concentrations were labeled and used immediately. Amoxicillin was used as the standard drug (500mg/ml) (Lar *et al.*, 2011).

2.5 Antibacterial Activity by Zone Of Inhibition

The agar-well diffusion method prescribed by NCCLS (2007) was employed in the susceptibility testing. Suspensions of the bacterial isolates were made in sterile normal saline and adjusted to 0.5 McFarland's standard. Each Mueller Hinton (MH) agar plate was uniformly seeded using a sterile swab dipped in the suspension and streaked on the agar plate surface, and the plates were left on the bench for excess fluid to be absorbed. Wells of 6 mm in diameter, 4 mm deep, and about 2 cm apart were punched in the MH agar with a sterile cork-borer. Approximately 100µl of the extracts was dropped into each well which filled them respectively to fullness. The setup was allowed to stabilize for 3 hours before being incubated at 37°C for 24h (Aibinu *et al.*, 2007). The mean zones of inhibition were measured in mm, for all the individual isolates. Positive control was equally filled with extracts.

2.6 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of aqueous and ethanol extracts of the seeds was 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 contain 5ml of single strength of nutrient broth. Five milliliters (5ml) of the crude extract in the desired concentration were introduced into tube one and mixed thoroughly. Five milliliters (5ml) of the content of tube two, 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 (the equivalent of 10^{10} CFU/ml) of the test organisms were added. All the tubes were incubated at $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 24 hours, 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 (Lar *et al.*, 2011).

2.7 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 loop full from each of these tubes was sub-

cultured onto the surface of extract-free nutrient agar and incubated for 24 hours at 35°C±2°C. The lowest concentration at which no growth was observed on the agar was noted as the MBC (Lar *et al.*, 2011).

2.8 Phytochemical Analysis

The phytochemical screening of the seed extracts was carried out to detect the presence or absence of phytochemicals such as Flavonoids, Tannins, Alkaloids, Saponins, and phenols as described by Sofowora (1994).

2.9 Proximate Analysis

The proximate composition of the *Moringa oleifera* seeds was determined as described by Association of official analytical chemists (AOAC, 2010). All determination were carried out in triplicate

2.9.1 Determination of Moisture Content

Two gram (2g) of the powdered sample were weighed in a beaker of known weight. The sample was then placed in a hot air oven at 105°C for 3 h. The sample was then cooled and weighed again to determine water loss in the powdered sample which was calculated using equation 1.

$$\text{Moisture \%} = \frac{(W_1 - W_2)}{W_0} \times 100 \quad \text{-----1}$$

Where: W_0 =Weight of empty crucible
 W_1 =weight of sample and crucible before heating
 W_2 =weight of sample and crucible after heating

2.9.2 Determination of Crude Lipid

The apparatus used for the estimation of fat is the Soxhlet extractor. To determine the percentage of fat the dried sample of the plant was extracted with petroleum ether. It was then distilled off completely and dried. The oil weight and percentage of oil were calculated.

$$\text{Crude Lipid \%} = \frac{W_2 - W_3}{W_2 - W_1} \times 100 \quad \text{-----2}$$

Where: W_1 =Initial weight of the empty crucible
 W_2 =weight of sample and crucible before heating
 W_3 =weight of sample and crucible after heating

2.9.3 Determination of Crude Fiber

During the acid and subsequent alkali treatment, oxidative hydrolytic degradation of native cellulose and considerable degradation of lignin occurs. The residue obtained after final filtration was weighed, incinerated, cooled, and weighed again. The loss in weight is the crude fiber content.

$$\text{Crude fiber \%} = \frac{(W_1 - W_2)}{W_0} \times 100 \quad \text{-----3}$$

W_0 =Initial weight of the empty crucible
 W_1 =weight of the mixture before heating
 W_2 =weight of the mixed sample after heating

2.9.4 Determination of Ash Content

For estimation of ash, the sample was incinerated at a higher temperature. Briefly, 2g of sample in a crucible were incinerated into the Muffle furnace at 600°C for 5 hours. The crucible was then cooled, the sample was reweighed and the percentage of ash was calculated.

$$\text{Ash \%} = \frac{(W_3 - W_1)}{(W_2 - W_1)} \times 100 \quad \text{-----4}$$

Where: W_1 = weight of the empty crucible
 W_2 = weight of crucible + sample
 W_3 = weight of crucible + ash

2.9.5 Determination of Carbohydrate

The carbohydrate content was determined as Nitrogen Free Extract (NFE) using this formula.

$$\text{CHO} = 100 - (\% \text{ CP} + \% \text{ CF} + \% \text{ CFa} + \% \text{ Ash} + \% \text{ moisture}) \quad \text{-----5}$$

Where CP- Crude Protein, CFa-Crude Fat, CF- Crude Fibre

3. Results and Discussion

3.1 Results

The antibacterial activity of aqueous and ethanol extracts of *Moringa oleifera* seeds, the results shown in table 1 at a concentration of 250mg/ml, 125mg/ml, 62.5mg/ml, and 31.25mg/ml, both *Salmonella typhi* and *Staphylococcus aureus* was susceptible with the zone of inhibition of 14mm, 12mm, 9mm, and 8mm for *Salmonella typhi* while 14mm, 12mm, 10mm, and 7mm for *Staphylococcus aureus* respectively. It's only that, the ethanol extract has lower zones of inhibition of 10mm, 9mm, 7mm, and 5mm for *Salmonella typhi* while 4mm, 3mm, 2mm, and 1mm for *Staphylococcus aureus*. The result of the Minimum Inhibitory Concentration (MIC) of the aqueous and ethanol extract shown in table 2 indicates that *Salmonella typhi* and *Staphylococcus aureus* are susceptible at a concentration of 125 and 31.25mg/ml respectively.

The Minimum Bactericidal Concentration (MBC) *Salmonella typhi* and *Staphylococcus aureus* shown in table 3 indicate that there was a similar minimum bactericidal concentration of 250mg/ml for both ethanol and aqueous solutions.

The results of Phytochemical Screening of *Moringa oleifera* seeds presented in table 4 revealed the presence of Flavonoid, Tannin, and

Saponin in the ethanol extracts, while for aqueous extracts it shows the presence of Flavonoid, Alkaloid, Saponin, and Phenolic while Tannin is absent. The results of Proximate Analysis of *Moringa oleifera* seeds presented in table 5 shows the presence of Crude lipid (35.48 ± 0.42), Crude protein (30.69 ± 0.39), and

Carbohydrate (16.37 ± 0.78) at high concentration while the Crude fiber (9.66 ± 0.14), Ash content (5.76 ± 0.12) and Moisture content (2.04 ± 0.01) presence at a lower concentration.

Table 1: Antibacterial Activity of Aqueous and Ethanol Extracts of *Moringa Oleifera* Seeds on Test Organisms

Test organisms	Extract concentrations (Zone diameter of inhibition in mm)					
	250	125	62.5	31.25	Control	Extract
<i>Salmonella typhi</i>	14	12	9	8	13	AE
	10	9	7	5	12	EE
<i>Staphylococcus aureus</i>	14	12	10	7	15	AE
	4	3	2	1	8	EE

Key: mm= Millimeter, Control= Amoxicillin, AE=Aqueous Extract, EE=Ethanol Extract

Table 2: Minimum Inhibitory Concentration (MIC) of *Moringa Oleifera* Seeds

Test organisms	Aqueous(mg/ml)	Ethanol (mg/ml)
<i>Salmonella typhi</i>	125	125
<i>Staphylococcus aureus</i>	31.25	62.5

Key: mg= Milligram, and ml= Milliliter.

Table 3: Minimum Bactericidal Concentration (MBC) of *Moringa Oleifera* Seeds

Test organisms	Aqueous (mg/ml)	Ethanol (mg/ml)
<i>Salmonella typhi</i>	250	250
<i>Staphylococcus aureus</i>	250	250

Keys: mg= Milligram, and ml= Milliliter

Table 4: Phytochemical Analysis of Aqueous and Ethanol Extract of *Moringa Oleifera* Seeds

Constituents	Ethanol Extracts	Aqueous Extracts
Flavonoids	+	+
Tannins	+	-
Alkaloids	-	+
Saponins	+	+
Phenolic	-	+

Key: + Means present, and - Means absent

Table 5: Proximate Analysis of *Moringa Oleifera* Seeds

Parameters	Concentration (%)
Moisture content	2.03 ± 0.01
Crude lipid	35.58 ± 0.42
Crude fiber	9.77 ± 0.14
Ash content	5.69 ± 0.12
Carbohydrate	16.40 ± 0.78
Crude protein	30.53 ± 0.39

Key: All values are mean \pm standard deviation (S.D) of triplicate measurement.

3.2 Discussion

The antibacterial activity of aqueous and ethanol extracts of dried *moringa oleifera* seeds was determined using two organisms, which were gram-positive and gram-negative bacteria, *Staphylococcus aureus* and *Salmonella typhi* respectively. The aqueous extract for both organisms revealed high susceptibility of action against that of ethanol extract which shows lower susceptibility to antibacterial activity. It might be due to the Phytochemical reaction is more

reactive in aqueous extract than in ethanol extract. Our findings confirm other reports on the antibacterial activity of *moringa oleifera* seed extracts (Jamil *et al.*, 2017).

The MIC of the aqueous and ethanol was obtained between 62.5-31.25mg/ml for *Staphylococcus aureus* while MBC was obtained at 250mg/ml which indicates that the seed extracts were bacteriostatic at lower concentrations and bactericidal at higher

concentrations. This could be attributed to the differences in the composition of the extract. Our findings differ from that of the report of Nepolean *et al.*, (2009) who reported that the ethanol 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. The antibacterial activity exhibited on some bacterial isolates by ethanol *moringa oleifera* seed extract could be a result of the presence of flavonoids since these phytochemicals are reported to confer antibacterial activity (Hausteen *et al.*, 2015). The presence of some phytochemical substances especially a short polypeptide found in *moringa oleifera* seed extracts was reported to act directly on microorganisms and result in growth inhibition by disrupting cell membrane synthesis or synthesis of essential enzymes (Bukar *et al.*, 2010).

The result of phytochemical analysis of ethanol and aqueous extracts of *moringa oleifera* seed shows the presence of Flavonoid, Tannin, and Saponin whereas Alkaloid and Phenolic were not detected for ethanol extracts. While it revealed the presence of Flavonoids, Alkaloids, Saponins, and Phenolic. While Tannin were detected for aqueous extracts. This finding correlated with that of Josephine *et al.*, (2015) who reported that the ethanol seed extract of *moringa oleifera* revealed the presence of phytochemicals such as saponins, tannins, cardiac glycosides, flavonoids, and anthraquinones. Bukar *et al.*, (2010) stated the presence of flavonoids, Tannin, and Alkaloid in ethanolic extract of *moringa oleifera*.

The Proximate Analysis of the *moringa oleifera* seeds in table 5 revealed the highest concentration of Crude lipid at $35.68 \pm 0.42\%$ while the lowest concentration was recorded in Moisture content with $2.03 \pm 0.01\%$. The high Crude fat and protein of *moringa oleifera* seeds may be used as a good source of balanced diets, while the presence of low moisture is an indication that the seed can be preserved for a long period. This finding is in agreement with Peter and Mumini (2017) who study the nutritional composition of *Cortinarius* species and *moringa oleifera* seed with slightly above crude fat and protein. Similar finding with Bonomi *et al.*, (2018) which also reported low moisture content of $1.26 \pm 0.04\text{g}$ from *moringa oleifera* seed oil.

4. Conclusion

The aqueous seed extract showed high zone of inhibition activity than ethanol extract on the microorganisms tested. Two species (*Staphylococcus aureus* and *Salmonella typhi*) presented the lowest MIC of 31.25mg/ml and MBC of 250mg/ml which is similar to that of the antibiotic standard, indicating a potent source of new antibiotic alternative. It revealed the presence of Flavonoids, Tannin, Saponins while Alkaloid and Phenolic were absent for ethanol extracts whereas Flavonoids, Alkaloid, Saponin, and Phenolic were present but only Tannin is absent for the aqueous extracts. The Proximate analysis revealed the high Concentration of Crude lipid and the lowest Concentration of Moisture content. This study demonstrated that *moringa oleifera* seeds can be as effective as antibacterial drugs and also as a source of a balanced diet.

Conflict of interest

The authors declare no conflict of interest.

References

- Abdulrasheed, M., Ibrahim, I. H., Mubarak, M. A. and Umar, F.A.(2015). Comparison of Antimicrobial activity of seed oil of Garlic and *Moringa oleifera* against some food-borne microorganisms. *Bayero Journal of pure and Applied sciences*, 8(2):196-201
- AOAC (2010). Official methods of analysis. 20th ed., *Association Of Official Analytical Chemist*. Washington DC, USA. pp 24 - 40
- Aibinu, S.L, and Nahar, N.M., (2007) Microscopic Evaluation of the Antimicrobial Activity of Seed Extracts of *Moringa Oleifera*. *Pakistan Journal of Botany*, 20: (3): 939-945.
- Anwar, F, and Rashid, U. (2007). Physico-chemical characteristics of *Moringa oleifera* seeds and seed oil from a wild provenance of Pakistan. *Pakistan Journal of Botany*, 39: (5): 1443-1453.
- Bonomi, Z.M., Al Amin, A. S., Gadanya, M.A. and Babandi, A.(2018). Characterization and proximate analysis of *Moringa Oleifera* seed oil cultivated in Kano Nigeria. *Nigerian Journal Of Nutritional Sciences*, 39:2
- Bukar A., Uba A., and Oyeyi T. (2010). Antimicrobial profile of *Moringa oleifera* lam extracts against some food-borne

- microorganisms. *Bayero Journal of Pure and Applied Science* **3**: 43-48.
- Cheesebrough, M. (2006). *District Laboratory practice in tropical countries*. Part 1, 2nd edition Pp 12-17
- Farooq, F. Rai, Tiwari, M., Khan, V. and Farooq, S. (2012). Medicinal properties of *Moringa oleifera*, An overview of a promising healer. *Journal of Medicinal Plants Research*, **6**: (27): 4368-4374.
- Freire, J.E.C., Vasconcelos, I.M. and Moreno, F.B.M.B (2015). Mo-CBP3, an antifungal chitin-binding protein from *Moringa oleifera* seeds, is a member of the 2S albumin family. *PLoS ONE*, 0119871 2015;10:e0119871.doi:10.1371/journal.pone.0119871
- Hausteen, M.Z., and Crooseyer B.T., Idioma G.Y. (2015). Isolation and characterization of a flocculating carbohydrate from *Moringa oleifera* Lam. *Biochimica Biophysica Acta* **1143**: 177-281.
- Jamil, A., Shahid, M. M., Khan, and M., Ashraf, (2017). Screening of some medical plants for isolation of fungal proteins and peptides. *Pakistan Journal of Botany*, **39**: (1): 211-221T
- Josephine, N.K., Gabriel, S.B., Lonzy, O.J.O, and Jasper, W.O. (2015). Phytochemicals and uses of *Moringa oleifera* leaves in Uganda rural communities, *Journal of Medical Plants Research*, **4**(9): 753-757
- Kawo, A. H (2007). Water purification potentials and in-vivo toxicity evaluation of the aqueous and petroleum ether extracts of *Calotropis Procera* (Alt. F) Ait.F. latex and moringa oleifera lam seed powder. Ph. D thesis Microbiology Unit, Department of Biological Sciences, Kano. 184pp
- Lar, P.M., Ojile, E., Dashe, E., and Oluoma, J (2011). Antibacterial Activity of *Moringa Oleifera* Seed Extracts on Some Gram-Negative Bacterial Isolates, *African Journal of Natural Sciences* **14**: 57 - 62
- NCCLS (2007). National Clinical Chemical of Laboratory standards: Performance standards for antimicrobial susceptibility testing: 17th informational supplement, M2-A9, and M7-A7 Wayne, P.A, USA.
- Nepolean, P., Anitha, J., and Emilin, R.R. (2009). Isolation, analysis, and identification of phytochemicals of antibacterial activity of *Moringa oleifera*. *Current biotic*, **3**(1) 33-39.
- Oyeleke, S. B. and Manga, S. B. (2008). *Essential laboratory practical in microbiology*. 1st edition. TOBEST publishers, Minna Nigeria. pp 36-37.
- Peter, T.O and Mumini, I.A.(2017). Comparative study of Nutritional composition and phytochemical constituents of *Cortinarius* species and *Moringa oleifera* seed. *Der pharma chemica*, **9**(11):30-34.
- Popoola, J.O and Obembe, O.O. (2013). Local Knowledge, use pattern, and geographical distribution of *Moringa Oleifera* Lam (Moringaceae) in Nigeria. *Journal of Ethanopharmacol*, **150**,162-691.
- Sharma, P., Kumari, P., Srivastava, M.M and Srivastava, S. (2006). Removal of Cadmium from aqueous system by shelled *M. oleifera* lam seed powder. *Bioresource Technology* **97**:299-305
- Sofawora, E. A. (1994). *Medicinal plants and Traditional Medicine in Africa*, University of Ife Press, Nigeria pp1-23
- Thilza, I., Sanni, S., Zakari, A., Muhammed, T. and Musa , B (2010). In vitro antimicrobial activity of water extract of *moringa oleifera* leaf stalk on bacteria normally implicated in eye disease. *Academia Arena* **2**:80-83.
- Ullah, A., Mariutti, R. B. and Masood, R.(2015). Crystal structure of mature 2S albumin from *moringa oleifera* seeds. *Bio and Bio Res Commun*. **4**(6):365-371.doi:10.1016/j.bbrc.2015.10.087
- Walter, A., Samuel, W., Peter, A., and Josph, O. (2011). Antibacterial activity of *Moringa oleifera* and *Moringa stenapetala* methanol and n-hexane seed extracts on bacteria implicated in water-borne diseases. *Africa journal microbiology Res*. **5**,153-157.
- World Health Organization (2003). World Health Report. In World health organization(Ed) Geneva, Switzerland, WHO Publications/office pp1-50
- World Health Organization (2015). Antibiotic resistance: multi-country public awareness survey. WHO Library cataloguing-in-Publication Data. Geneva; ISBN 978 92 4 150981.