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Research Article

Phytochemical Composition & Bacterial Isolates Associated with Moringa Oleifera Leaves/Seeds

Ogbonna U. S. A.[1]*, Okoh F. N.[2], Obianom, O. A[1]., Agu K.C.[1], Anazodo C. A.[1], Chidubem-Nwachinemere N. O.[1], Abana C. C.[1], Okoli F. A.[1], Egurefa S. O.[2], Nwosu J. C.[3]

[1]Applied Microbiology and Brewing Department, Nnamdi Azikiwe University, PMB 5025, Awka, Nigeria

[2]Department of Science Laboratory Technology, Delta State University, Ozoro.

[3]Department of Biological Sciences, University of Africa, Toru-Orua, Bayelsa, Nigeria

Abstract:

Medicinal plants have posed as natural resources of compounds with pharmacological and nutritional properties aiding humans to prevent and treat diseases. Among several plants evaluated in bio-prospective studies, *Moringa oleifera* is known to have many names based on its many uses: clarifier tree, horseradish tree, and drumstick tree (referring to the large drumstick-shaped pods) and in East Africa, Moringa is known as mother's best friend. This study aimed to determine the bacterial isolates and phytochemical components in *Moringa oleifera* seeds and leaves. The results showed three probable bacterial isolates, which include, *Escherichia coli, Proteus mirabilis,* and *Klebsiella pneumoniae* respectively. The bacteria metabolizes some sugars such as glucose, sucrose, lactose, and maltose which could impact the nutritional value of the leaves and seeds when consumed. Through gram staining, it was confirmed that the three isolates are gram-negative organisms as they appear rod-shaped and turned pink. The phytochemical analysis showed that saponins, tannins, flavonoids, glycosides, alkaloids, phenolics, terpenoids, etc are present in both the leaves and seeds. Moringa leaves and seeds contain high amounts of these phytochemical components which are medicinally important because of their antimicrobial, anti-inflammatory, anti-hypertensive, hypoglycemic, and hyperglycemic activity. The seeds and leaves can be used as a food supplement to increase the nutritional composition of food lacking protein, carbohydrates, and lipids by including them in diets to add to human's daily nutritional needs.

Keywords: Phytochemicals, Bacteria, Moringa oleifera, leaf, Seed

Introduction

Medicinal plants have posed as natural resources of compounds with pharmacological and nutritional properties aiding humans to prevent and treat diseases (El-Sohaimy *et al*., 2015). Among several plants evaluated in bio-prospective studies, *Moringa oleifera* is known to have many names based on its many uses: clarifier tree, horseradish tree, and drumstick tree (referring to the large drumstick-shaped pods), and in East Africa moringa is known as "mother's best friend". Here in Nigeria, its names include Ewe Igbale in Yoruba, Zogelle in Hasusa and Idagbo monoye in Igbo (Chioma, 2013) have stood out in alternative medical therapies, showing benefits for the control of several diseases. Its medicinal potential is derived from secondary metabolites, such as alkaloids, tannins, flavonoids, steroids, saponins, coumarins, quinones, and resins. However, According to Agu *et al.* [43-46] and Okigbo *et al*. [47], between a quarter and two-fifths of agricultural produce is wasted yearly in the tropics due to poor storage conditions at the farm and village level.

M. oleifera is native to Northern India, but currently it is widely distributed in America, Africa, Europe, Oceania, and Asia. Leaves, flowers, pods, and seeds of this tree are considered a food source of high nutritional value in the African continent and other countries, particularly in India, the Philippines, and Pakistan. Three nongovernmental organizations, Trees for Life, Church World Service, and the Educational Concerns for Hunger Organization, have advocated the motto "Natural nutrition for the tropics" to stimulate the use of several plant species as food sources, including *M. oleifera*. Leaves can be consumed cooked or fresh and they can be stored as dried powder unrefrigerated with no nutritional losses, for several months. Undoubtedly, *M. oleifera* adds substantial health benefits to countries where hunger is a problem.

Several studies have demonstrated the beneficial effects in humans. *M. oleifera* has been recognized as containing a great number of bioactive compounds (Saini *et al*., 2016). The most used parts of the plant are the leaves, which are rich in vitamins, carotenoids, polyphenols, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins, and saponins. The high number of bioactive compounds might explain the pharmacological properties of *M. oleifera* leaves. Many studies, in vitro and in vivo, have confirmed these pharmacological properties (Leone *et al*., 2015).

The leaves of *M. oleifera* are mostly used for medicinal purposes as well as for human nutrition since they are rich in antioxidants

and other nutrients, which are commonly deficient in people living in undeveloped countries (Popoola and Obembe, 2013). *M. oleifera* leaves have been used for the treatment of various diseases from malaria and typhoid fever to hypertension and diabetes (Sivansankari, 2014). The roots, bark, gum, leaf, fruit (pods), flowers, seed, and seed oil of *M. oleifera* are reported to have various biological activities, including protection against gastric ulcers, antidiabetic (Oyedepo, 2013), hypotensive and anti-inflammatory effects.

In addition to medicinal and nutritional applications, one of the most applied properties of *M. oleifera* is the highly efficient coagulating effect of its seeds, which are used in water treatment. These seeds act as coagulants of organic matter suspended in water and are used in water treatment stations for natural cleaning before performing other cleansing processes.

Furthermore, seeds have more stable activity in different pH ranges, when compared to aluminum sulfate, the most frequently used coagulating substance in water treatment stations. *M. oleifera* has also been assessed for its potential to treat aquaculture wastewater. The results have shown the simultaneous elimination of water turbidity, suspended particles, and microorganisms, making it suitable to completely or partly replace the usual coagulating agents, leading to economic, health, and environmental gains (Sanchez-Martin *et al*., 2010).

Materials and Methods

Sample collection

Fresh and matured leaves and seeds of *Moringa oleifera* were plucked from different Moringa trees in Nnamdi Azikiwe University, Department of Biosciences, Anambra state, Nigeria.

Production of Moringa oleifera

- Fresh leaves of Moringa oleifera were plucked and washed severally with clean water to remove soil particles and other undesirable materials and sun-dried.
- Matured seeds were cracked and separated from the chaff and other impurities and sundried.
- The dried leaves/seeds were ground into powdery form for extraction of oil from the seeds and the phytochemical properties of the leaves.

The powdered leaves and seeds will be packaged in sterile plastic containers.

Microbial Isolation of *Moringa oleifera*

9ml of water will be put into 4 test tubes, then cotton wool to cover the mouth of the test tubes and conical flask, then use foil to seal up the conical flask. Autoclave at 121℃ for 15 minutes at 15 lb. Then 5g of the sample will be measured and put into the beaker. A plastic pipette will be used to get 1 ml of the sample into each test tube. The Moringa sample will be diluted in a ten-fold serial dilution. 1 ml from the 10-1 dilution tube will be dispensed into several sterile petri dishes with the aid of a sterile pipette. Nutrient agar will be poured into the plates and the plates will be rotated for easy mix-up of the sample and the media. All plates will be allowed to solidify, then the incubated plates will be examined for microbial growth.

Isolation of total bacteria & coliform counts of *Moringa* oleifera

After incubation, discrete colonies from bacteria plates will be picked with a sterile wire loop and sun-cultured onto a newly prepared nutrient agar and Sabouraud dextrose agar plates. All plates will be stored appropriately at room temperature for 18-48hours.

Isolation of coliform and other fastidious organisms, the homogenates Moringa leaves will be streaked on general purpose enriched medium (blood agar) and selective and differential medium for members of the family Enterobacteriaceae (MacConkey agar) and incubated aerobically at 37°C for 24 hours. The isolated bacteria will be identified based on colony morphology, Gram staining reaction, and biochemical characteristics using established standardized methods according to Bergey's Manual of Determinative Bacteriology.

Characteristics of microbial isolates

The characteristics of the microbial isolates will be done by the observation of colonial characteristics, Gram reaction, and biochemical tests. The characteristics of the isolates will be performed by using a gram staining reaction, catalase test, citrate test, motility test, and sugar fermentation test.

Gram staining

The smear of the isolate will be made on clean, non-greasy, dust-free, and air-dried slides. The smear will be flooded with crystal violet and allowed to remain on the slide for 60 seconds. Thereafter, the crystal violet will be washed off with gentle running water. Again, the slide will be flooded with Gram's iodine, allowed to remain for 60 seconds, and washed off. The slide will be decolorized with acetone- alcohol mixture. The slide will be counter-stained with safranin for 60 seconds rinsed with water and allowed to air-dry. The slide will then be viewed under an oil immersion lens microscope (x100). Purple color indicates Gran-positive organisms while red or pink color indicates Gram-negative organisms.

Biochemical characteristics of bacterial isolates

Catalase Test

3ml of 3% solution of hydrogen peroxide (H2O2) will be transferred into a sterile test tube. Then 3 loopfuls of a 24-hour pure culture of the test will be inoculated into the test tube. Through observation, if there is immediate bubbling, it indicates a positive reaction, if there is no bubbling, it indicates a negative reaction.

Motility Test (Handling drop method)

A loopful of 18-24 hours broth culture of the test for bacteria will be placed at the center of a clean grease-five cover-slip. Carefully, the cover slip will be inverted and placed over the concave portion of a hanging drop slide. The cover slide arrangement will be observed for motility x100 Magnification on a compound microscope. Care will be taken to not misinterpret the motion. Results should be recorded as motile or non-motile.

Citrate Test

A 24-hour culture will be inoculated into test tubes containing sterile Simmons citrate agar and then incubated for 24 hours. A positive test is indicated by a change from green to blue color on the surface of the Simmons citrate agar slant.

Sugar Fermentation Test

Each of the isolates will be tested for its ability to ferment a specific sugar. 1g of sugar and 1g of peptide water will be dissolved in 11 ml of water. 5 ml of the solution will be transferred into clean test tubes using sterile pipettes. The test tubes containing peptones water and sugar will be added. Dirham's tube which will be placed inversely and bromothymol blue as an indicator. These will be sterilized for 10 minutes and allowed to cook before inoculating the inocula. The test tubes will be incubated for 3days. The production of acid and gas or acid only indicates the utilization of sugars.

Qualitative phytochemical screening

Phytochemical analysis extract was carried out using the method described by Odebiyi and Sofowora (1978) for the detection of saponins, tannins, phenolics, alkaloids, steroids, triterpenes, phlorotannins, glycosides, and flavonoids.

1. Alkaloids: 1cm^3 of 1%HCl was added to 3cm^3 of the extracts in a test tube. The mixture was heated for 20 minutes, cooled and filtered. The filtrate was used in the following tests: 2 drops of Wagner's reagent were added to 1cm^3 of the extracts. A reddish-brown precipitate indicates the presence of alkaloids
2. Tannins: 1cm^3 of freshly prepared 10% KOH was added to 1$cm^3$of the extracts. A dirty white precipitate indicates the presence of tannins.
3. Phenolics: 2 drops of 5% $FeCl_3$ was added to 1cm^3 of the extracts in a test tube. A greenish precipitate indicates the presence of phenolics.
4. Glycosides: 10cm^3 of 50% H_2SO_4 was added to 1cm^3 of the extracts, and the mixture was heated in boiling water for 15 minutes. 10cm^3 of Fehling's solution was added and the mixture boiled. A brick red precipitate indicates the presence of glycosides.
5. Saponins: Frothing test: 2cm^3 of the extract in a test tube was vigorously shaken for 2 minutes. Frothing indicates the presence of saponins.
6. Flavonoids: 1cm^3 of 10% NaOH was added to 3cm^3 of the extracts. A yellow colouration indicates the presence of flavonoids.
7. Steroids: salakowsti test: 5 drops of concentrated H_2SO_4 was added to 1cm^3 of the extracts. Red colouration indicates the presence of steroids
8. Phlobatannins: 1cm^3 of the extracts was added to 1% HCl. A red precipitate indicates the presence of phlorotannins.
9. Triterpenes: 5 drops of acetic anhydride were added to 1cm^3 of the extracts. A drop of concentrated H_2SO_4 was then added and the mixture was steamed for 1 hour and neutralized with NaOH followed by the addition of chloroform. A blue-green color indicates the presence of triterpenes.
10. Phytosterols (Finar 1986): liberman-burchard's test: 50mg is dissolved in 2ml acetic anhydride. To this, one or two drops of con.$H_2SO_4$is added slowly along the sides of the test tube. An array of color changes shows the presence of phytosterols.
11. Fixed oils and fats (kokate, 1999) A small quantity is processed between two filter papers, oil stain on the paper indicates the presence of fixed oil.
12. Terpenoids: 5ml of aqueous extract of the sample is mixed with 2ml of $CHCl_3$ in a test tube 3ml of con. H_2SO_4 is carefully added to the mixture to form a layer. An interface with a reddish-brown coloration is formed if a terpenoid constituent is present.
13. Amino acid (Yasuma and Ichikawa 1953): Two drops of ninhydrin solution (10mg of ninhydrin in 200ml of acetone) are added to two ml of aqueous filtrate. A characteristic purple color indicates the presence of amino acids.

Determination of coumarins

Add 0.5 ml of 5N NaOH to the solution for 1 ml of the extract (0.5 g in 1ml of ethanol), heat at 80 ^{0}C for 5 min, cool, add 0.75 ml of 5 N H2SO4, mix thoroughly, add 0.25 g of anhydrous NaHCO3, mix, and transfer to the extractor. Rinse the flask with distilled water and transfer to the extractor and make up to 50 ml. extract for 3 hrs with pet. Ether, remove the inner tube, and transfer the

pet ether in the extractor to the extraction flask. Add 20 ml of water to the pet ether extract and carefully evaporate the pet ether in a water bath at 50-55 ^{0}C.

Transfer the aqueous solution to a volumetric flask, and make up to 50 ml with continuous mixing. Pipette 25 ml into a flask and add 1% Na2CO3 solution, heat in a water bath at 85 ^{0}C for 15 min and cool. Add 5 ml of the diazonium solution, and let stand for 2 hours. Read the absorbance at 540 nm against the reagent blank. Calculate the cumarin content from the standard curve.

Estimation of total phenolic content

The total phenolic content of the sample was estimated according to the method of Makkar et.al (1997). The aliquots of the extract were taken in a test tube and made up to the volume of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially to the test tube. Soon after vortexing the reaction mixture, the tubes were placed in the dark for 40 min. and the absorbance was recorded at 725 nm against the reagent blank. Using Gallic acid monohydrate, a standard curve was prepared. The linearity obtained was in the range of 1-10 µg/ml. using the standard curve, the total phenolic content was calculated and expressed as Gallic acid equivalent in mg/g of extract.

Total flavonoid assay

Total flavonoid content was measured by aluminum chloride colorimetric assay. 1 ml of extracts or a standard solution of Quercetin (500µg/ml) was added to a 10 ml volumetric flask containing 4 ml of distilled water. To the above mixture, 0.3 ml of 5% NaNO2 was added. After 5 minutes, 0.3 ml of 10% AlCl3 was added. At 6th min, 2 ml of 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against the prepared reagent blank at 510 nm.

The total flavonoid content of the flower was expressed as a percentage of Quercetin equivalent per 100 g of fresh mass.

Saponins

The spectrophotometric method of Brunner (1984) was used for the analysis of saponins. Briefly, 1g of the finely ground dried sample was weighed into a 250 ml beaker, and 100 ml of isobutyl alcohol was added. The mixture was shaken on a UDY shaker for 5 hours to ensure uniform mixing. Thereafter, the mixture was filtered through a Whatman No. 1 filter paper into a 100ml beaker containing 20ml of 40% saturated solution of $MgCO_3$. The resulting mixture was again filtered to obtain a clear colorless solution. One milliliter of the colorless filtrate was pipette into a 50ml volumetric flask and 2ml of 5% $FeCl_3$ solution was added and made up to the marked level with distilled water. This was then allowed to stand for 30 minutes for a blood-red color to develop. 0-10ppm saponins standard was prepared from saponins stock solution. The standard solutions were treated similarly with 2ml of 5% $FeCl_3$ solution as earlier described. The absorbance of the samples as well as standard saponin solutions was read after color development using a Jenway V6300 spectrophotometer at a wavelength of 380nm.

Percentage saponin was calculated using the formula:

$$\% \text{ saponin} = \frac{\text{Absorbance of sample} \times \text{Average gradient} \times \text{Dilution factor}}{\text{Weight of sample} \times 10{,}000}$$

Alkaloids

The quantitative determination of alkaloids was done by distillation and titrimetric methods as described by Henry (1973). Briefly, 2g of finely ground sample was weighed into a 100ml beaker, and 20 ml of 80% absolute alcohol was added to give a smooth paste. The mixture was transferred to a 250ml flask and more alcohol added to make up to 1g of magnesium oxide was then added. The mixture was digested in a boiling water bath for an hour and a half under a reflux air condenser with occasional shaking. The mixture was filtered while hot through a Buchner funnel. The residue was poured back into the flask and re-digested for another thirty minutes with 50ml alcohol after which the alcohol was evaporated. Distilled water was added to replace the lost alcohol. When all alcohol has evaporated, 3 drops of 10% HCl was added. The whole solution was later transferred into a 250ml volumetric flask; 5ml of Zinc acetate solution and 5ml of potassium ferricyanide solution were thoroughly mixed to give a homogenous mixture. The flask was allowed to stand for a few minutes, filtered through a dry filter paper, and 10ml of the filtrate was transferred into a separating funnel and the alkaloids present were extracted vigorously by shaking with five successive portions of chloroform. The residue obtained was dissolved in 10ml of hot distilled water and transferred into a Kjeldahl tube with the addition of 0.2g of selenium for digestion to a clear colorless solution. The clear colorless solution was used to determine Nitrogen using the Kjeldahl distillation apparatus the distillate was back titrated with 0.01N HCl and the titre value obtained was used to calculate the % Nitrogen using the formulae:

$$\%N = \frac{\text{Titre value} \times \text{Atomic mass of Nitrogen} \times \text{Normality of HCl} \times 100}{\text{Weight of sample (mg)}}$$

$$\% \text{ Alkaloid} = \% \text{ Nitrogen} \times 3.26$$

Where 3.26 is a constant

Tannins

The method of Swain (1979) was used to determine the quantity of tannins. 0.20g of sample was measured into a 50ml beaker 20ml of 50% methanol was added and covered with paraflim and placed in a water bath at 77-80˚C for 1 hour. It was shaken thoroughly to ensure uniform mixing. The extract was filtered using a double-layered Whatman No. 41filter paper into a 100ml volumetric flask.20ml water was added and 2.5ml Folin-Denis reagent and 10ml of 17% Na_2CO_3 were added and mixed properly. The mixture was made up to mark with water mixed well and allowed to stand for 20 minutes. A bluish-green color will develop at the end of the range. 0-10ppm was treated similarly as 1ml sample above.

The absorbances of the Tannic acid standard solutions as well as samples were read after colour development on a spectronic 21D spectrophotometer at a wavelength of 760nm. % Tannin was calculated using the formula:

$$\% \text{ Tannin} = \frac{\text{Absorbance of sample} \times \text{Average gradient} \times \text{Dilution factor}}{\text{Weight of sample} \times 10{,}000}$$

Glycosides

10ml of the extract was pipette into a 250ml conical flask. 50ml Chloroform was added and shaken on a Vortex Mixer for 1 hour. The mixture was filtered into a conical flask. 10 pyridine and 2 ml of 2% sodium nitroprusside were added and shaken thoroughly for 10 minutes. 3ml of 20% NaOH was later added to develop a brownish-yellow color.

Glycoside standard of concentration ranging from 0-5mg/ml were prepared from 100mg/ml stock glycoside standard. The series of standards 0-5mg/ml were treated similarly to the sample above.

The absorbances of the sample as well as standards were read on a spectronic 21D Digital spectrophotometer at a wavelength of 510nm.

% Glycoside was calculated using the formula:

$$\% \text{ Glycoside} = = \frac{\text{Absorbance of sample} \times \text{Average gradient} \times \text{Dilution factor}}{\text{Weight of sample} \times 10{,}000}$$

Steroids:

0.05g of sample extract was weighed into a 100 ml beaker. 20ml of chloroform-methanol (2:1) mixture was added to dissolve the extract upon shaking for 30 minutes on a shaker. The whole mixture until free of steroids. 1ml of the filtrate was pipette into a 30ml test tube and 5ml of alcoholic KOH was added and shaken thoroughly to obtain a homogenous mixture.

The mixture was later placed in a water bath set at 37˚C-40˚C for 90 minutes. It was cooled to room temperature and 10ml of petroleum ether was added followed by the addition of 5ml distilled water. This was evaporated to dryness in the water bath. 6ml of Liebermann Buchard reagent was added to the residue in a dry bottle and absorbance was taken at a wavelength of 620nm on a spectronic 21D digital spectrophotometer. Standard steroids of concentration of 0-4mg/ml were prepared from 100mg/ml stock steroid solution and treated similarly to the sample as above.

% steroid was calculated using the formula:

$$\% \text{ steroid} = = \frac{\text{Absorbance of sample} \times \text{Average gradient} \times \text{Dilution factor}}{\text{Weight of sample} \times 10{,}000}$$

Phlobatannins

0.05g of sample extract was weighed into a 50 ml beaker. 20ml of 50% methanol was added covered with parafilm and placed in a water bath set at 77-80˚C for 1 hour.

The mixture was properly shaken to ensure uniform mixing and later filtered through a Whatman No 1 filter paper into a 50ml volumetric flask using aqueous methanol to rinse, and makeup to mark with distilled water.

1 ml of the sample extract was pipette into a volumetric flask. 20 water, 2.5ml Folin-Dennis reagent, and 10ml of 17% sodium carbonate were added to the solution in the 50 flask. The mixture was homogenized thoroughly for 20 minutes. 0-5mg/ml of phlobatannins standard concentration were prepared from 100mg/ml phlobatannins stock solution and treated like the sample above.

% phlobatannins was calculated using the formula:

$$\frac{\text{Absorbance of sample} \times \text{Average gradient} \times \text{Dilution factor}}{\text{Weight of sample} \times 10{,}000}$$

Anthraquinones

The method of Lewis, (1974) was used. 0.5g of sample was weighed into a 250ml beaker and 60ml benzene was added and stirred with a glass rod to prevent lumping. This was filtered into a 100ml volumetric flask and 0.2% Zinc dust was added followed by the addition of 50ml hot 5% NaOH solution. The mixture was heated just below boiling point for five minutes and then rapidly filtered and washed once in water. The filtrate was again heated with another 50ml of 5% NaOH to develop a red color.

Standard anthraquinone solutions of range 0-5mg/l were prepared from 100mg/l stock anthraquinone and treated similarly with

0.2% Zinc dust and NaOH like the sample. The absorbances of the sample as well as that of standard concentrations were read on a Digital Spectrophotometer at a wavelength of 640nm.
The percentage of anthraquinone is calculated using the formula:

$$= \frac{\text{Absorbance of sample} \times \text{Average gradient} \times \text{Dilution factor}}{\text{Weight of sample} \times 10{,}000}$$

Triterpenes

0.50g of sample was weighed into a 50ml conical flask and 20ml 0f 2:1 chloroform-methanol mixture was added, shaken thoroughly, and allowed to stand for 15 minutes. The supernatant obtained was discarded, and the precipitate was re-washed with another 20ml chloroform-methanol mixture for recentrifugation.
The resultant precipitate was dissolved in 40ml of 10% Sodium Dodecyl Sulphate (SDS) solution 1ml of 0.01M ferric chloride solution was added to the above at 30 seconds intervals; shaken well, and allowed to stand for 30 minutes. Standard triterpenes of the concentration range
0-5mg/ml were prepared from 100mg/l stock triterpenes solution from sigma-Aldrich chemicals, U.S.A. The absorbance of the sample as well as that of standard concentrations of triterpenes were read on a digital spectrophotometer at a wavelength of 510nm. The percentage of triterpenes was calculated using the formula:

$$= \frac{\text{Absorbance of sample} \times \text{Average gradient} \times \text{Dilution factor}}{\text{Weight of sample} \times 10{,}000}$$

Determination of total anthocyanins

The total anthocyanin compounds of the samples were estimated using a UV-spectrophotometer by the pH differential method reported by Abu Bakar et al. (2009) with slight modifications. Two buffer systems, potassium chloride buffer, pH 1.0 (0.0025 M) and sodium acetate buffer, pH 4.5 (0.4 M) were used. Briefly, 400 µl of extract (3 mg of ground beans in 10 ml absolute methanol) was mixed in 3.6 ml of corresponding buffer solutions and read against a blank at 510 and 700 nm.
Absorbance (Ad) was calculated as:
Ad = (A510 - A700) pH1.0 - (A510 - A700) pH4.5
Anthocyanin concentration in the extract was calculated and expressed as cyaniding-3 glycoside (mg g-1) equivalent:
Ad × MW × DF × 1000 / (Ma × 1)
Where Ad is the difference in absorbance, MW is the molecular weight for cyaniding-3-glucoside (449.2), DF is the dilution factor of the samples and Ma is the molar absorptivity of cyaniding3-glucoside (26.900). Results were expressed as mg of cyaniding-3-glucoside equivalents in 100 g of dried sample.

Determination of proanthocyanidins

Proanthocyanidin content was determined by vanillin-H2SO4 assay as described by Chang et al. (2007) with minor modifications. A volume, of 1.0 ml aliquots of bean extract (3 mg in 10 ml absolute methanol) was mixed with 2.5 ml of 1.0% (w/v) vanillin in absolute methanol and then with 2.5 ml of 25% (v/v) sulfuric acid in absolute methanol to undergo vanillin reaction with polyphenols in bean species. The blank solution was prepared in the same procedure without vanillin. The vanillin reaction was carried out in a 25°C water bath for 15 min. The absorbance at 500 nm was read and the results were expressed as (+) catechin equivalent by a calibration method.

Results

In this study, two samples were used which consisted of *Moringa oleifera* leaves and seeds which were collected from Nnamdi Azikiwe University Premises.

Microscopic and biochemical tests for microbial identification

For the bacterial isolates, three probable isolates which include *Escherichia coli*, *Proteus mirabilis*, and *Klebsiella pneumoniae* grew on the Eosin Methylene Blue Agar 1 while one isolate which is *Proteus mirabilis* on Eosin Methylene Blue Agar 2. The isolates and their color colony are represented in Table 1. The colony count is represented in Table 2. After 24 hours of sub-culturing using the Nutrient Agar, colonial morphology was checked and the results are shown in Table 3. The gram stain results and the results of the various biochemical tests are represented in Tables 4 & 5.
For the phytochemical analysis, the quantitative and qualitative analysis were analyzed which consisted of some antioxidants as listed in Tables 6 &

TABLE 2.1: PROBABLE ISOLATES

For EMB Agar 1 with *Moringa oleifera* leaf**:**

Probable Isolates	Microbial Isolates	Colour of colony

Org. 1	*Escherichia coli*	Purple and green metallic sheen.
Org. 2	*Proteus mirabilis*	Colourless
Org. 3	*Klebsiella pneumoniae*	Pink mucoid

For EMB Agar 2 with *Moringa oleifera* Seed:

Probable Isolate	**Microbial Isolates**	**Colour of Colony**
Org. 2	*Proteus mirabilis*	Colourless

EMB = Eosin Methylene Blue
Org. = Organism

TABLE 2.2: COLONY COUNT OF BACTERIAL ISOLATES

Agar	**Sample**	**Total Colony Count(CFU/ML)**
NA 1	ML	Too numerous to count
NA 2	MS	124

NA 1= Nutrient Agar Plate 1
NA 2= Nutrient Agar Plate 2
ML= *Moringa oleifera* Leaf
MS= *Moringa oleifera* Seed

TABLE 2.3: COLONIAL MORPHOLOGY OF THE BACTERIAL ISOLATES.

For ML:

Isolates	**Form**	**Surface**	**Colour**	**Margin**	**Elevation**	**Opacity**
Org. 1	Circular	Smooth	Whitish	Entire	Convex	Translucent
Org. 2	Irregular	Glistening	Cream	Entire	Raised	Opaque
Org. 3	Circular	Glistening	Cream	Entire	Raised	Transparent

For MS:

Isolates	Form	Surface	Colour	Margin	Elevation	Opacity
Org. 2	Irregular	Glistening	Creamy	Entire	Raised	Opaque

ML= *Moringa oleifera* Leaf
MS= *Moringa oleifera* Seed

TABLE 2.4: GRAM STAINING ANALYSIS RESULTS AND MORPHOLOGICAL CHARACTERISTICS OF BACTERIA.

For ML:

Isolates	**Shape**	**Colour**	**Organism**
Org. 1	Rod Shaped	Pink colour	*E. coli*
Org. 2	Rod Shaped	Pink colour	*P. mirabilis*
Org. 3	Rod Shaped	Pink colour	*K.pneumoniae*

For MS:

Isolates	Shape	Colour	Organism
Org. 2	Rod Shaped	Pink colour	*P. mirabilis*

ML= *Moringa oleifera* Leaf
MS= *Moringa oleifera* Seed

TABLE 2.5: BIOCHEMICAL TEST RESULTS FOR BACTERIA.

For ML:

Isolates	**Ca**	**Ci**	**Cog**	**Mol**	**Glu**	**Suc**	**Mal**	**Lac**
Org.1	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve
Org.2	+ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve
Org.3	+ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve

For MS:

Isolates	**Ca**	**Ci**	**Cog**	**Mol**	**Glu**	**Suc**	**Mal**	**Lac**
Org.2	+ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve

Ca= Catalase test
Ci= Citrate test
Cog= Coagulase test
Mol= Motility test
Glu= Glucose
Suc= Sucrose
Mal= Maltose
Lac= Lactose

TABLE 2.6: QUALITATIVE ANALYSIS RESULTS.

ANTIOXIDANTS	**ML**	**MS**
Saponin	+	+
Tannin	+	+
Steroids	+	+
Flavonoids	+	+
Flavonoids	+	+
Terpenoids	+	+
Coumarins	+	+
Glycosides	+	+
Triterpenes	+	+
Anthocyanin	+	+

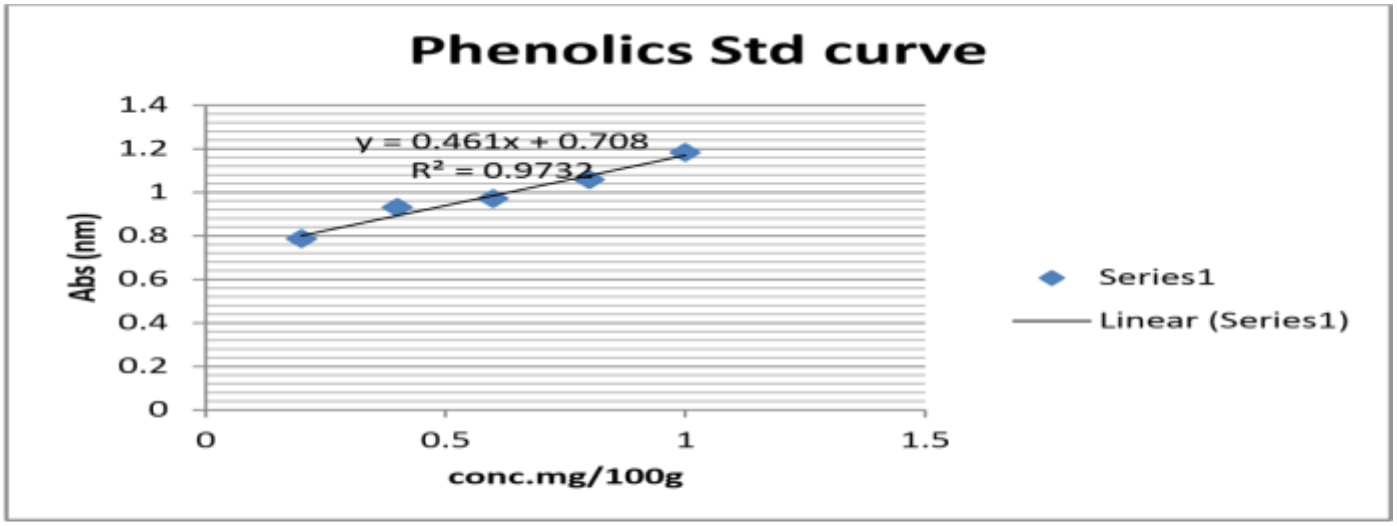
Phenolics	+	+
Amino Acid	-	-
Phlobatannin	+	-
Alkaloids	+	+

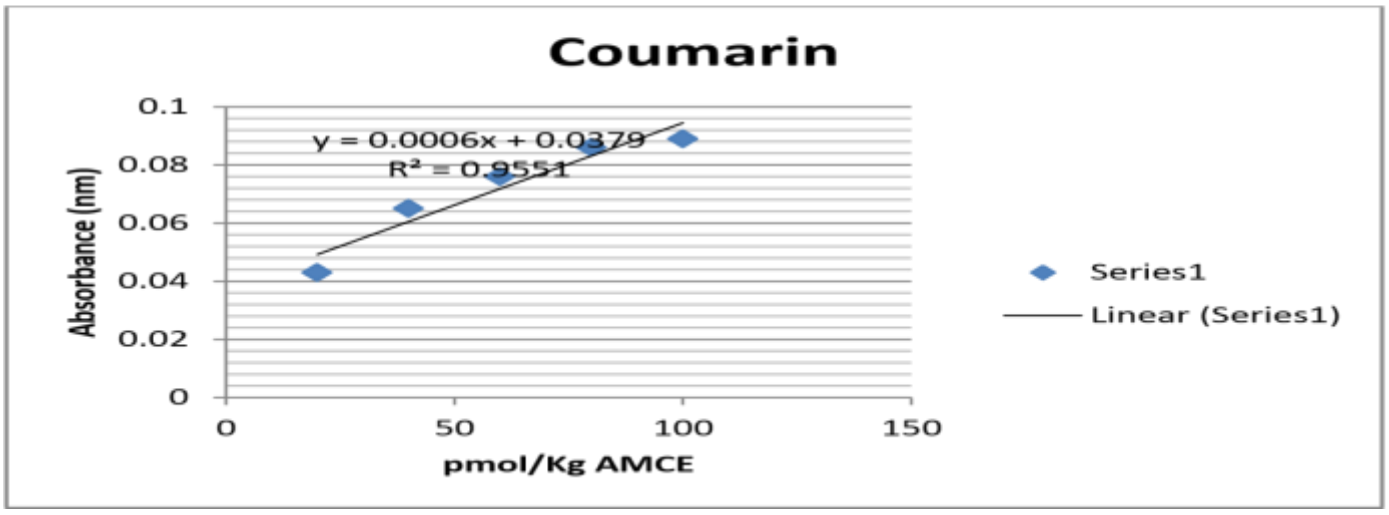
ML= *Moringa oleifera* Leaf
MS= *Moringa oleifera* Seed

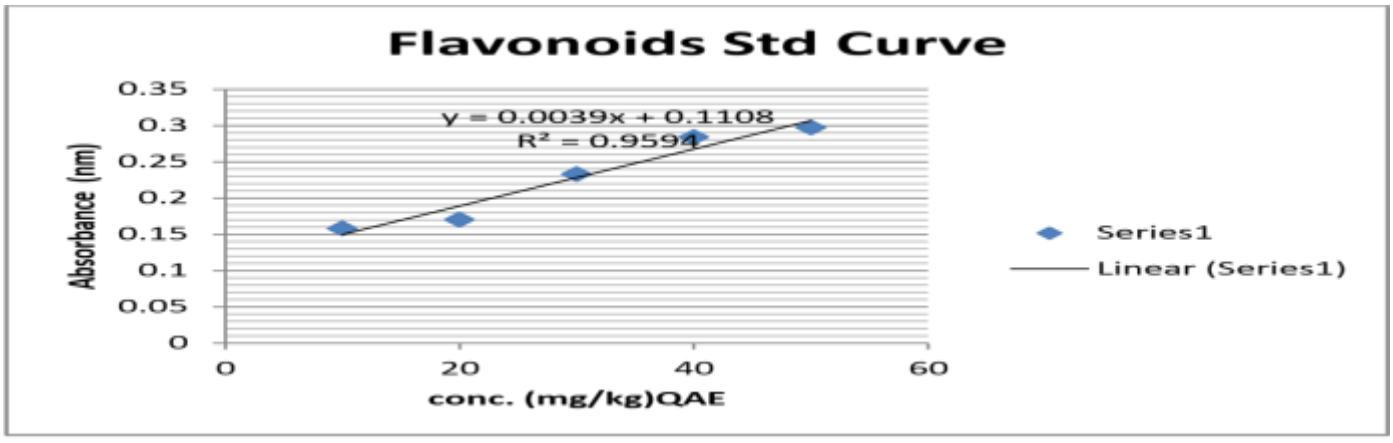
TABLE 2.7: QUANTITATIVE ANALYSIS RESULTS.

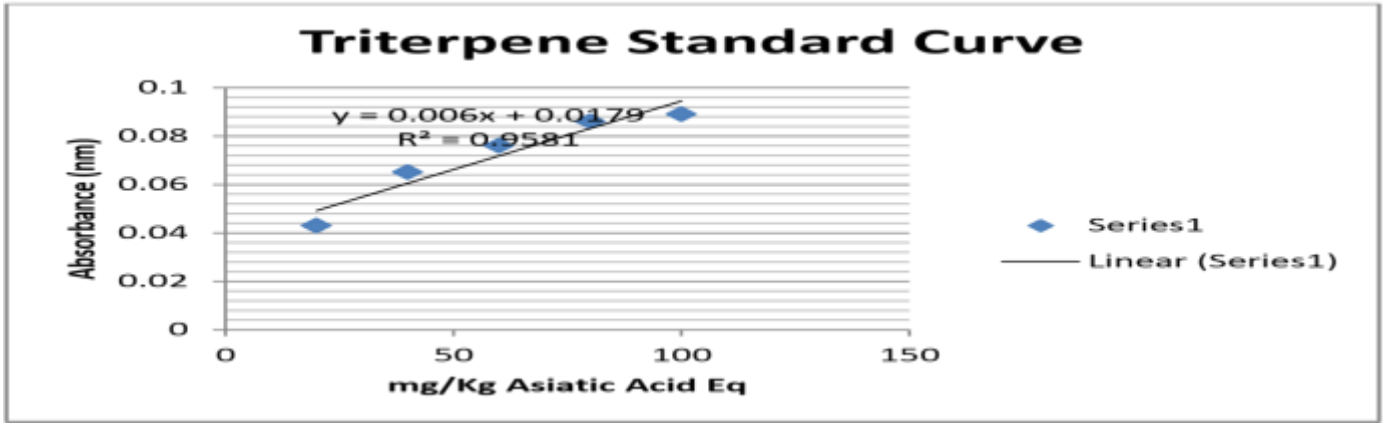
Antioxidants	**ML**	**MS**
Saponin	0.851175	0.562142
Tannin	5.744431	45.45455
Phenolics	69.05041	52.86713
Phlobatannin	73.38804	NIL
Steroids	45.51667	65.18333
Flavonoids	38.0776	140.0513
Coumarins	11.79592	5.9954
Anthocyanin	0.256819	0.152767
Terpenoids	44.69796	31.02449
Glycosides	8.93592	5.03532
Triterpenes	329.6833	69.0167
Alkaloids	21.24444	12.02222

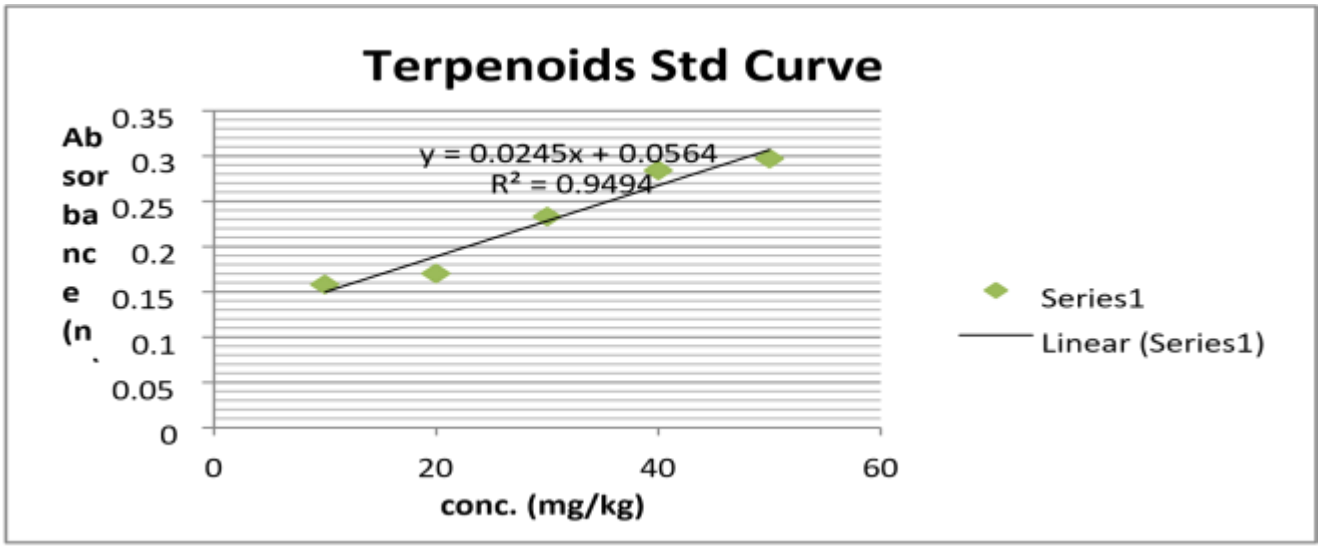
ML= *Moringa oleifera* Leaf
MS= *Moringa oleifera* Seed

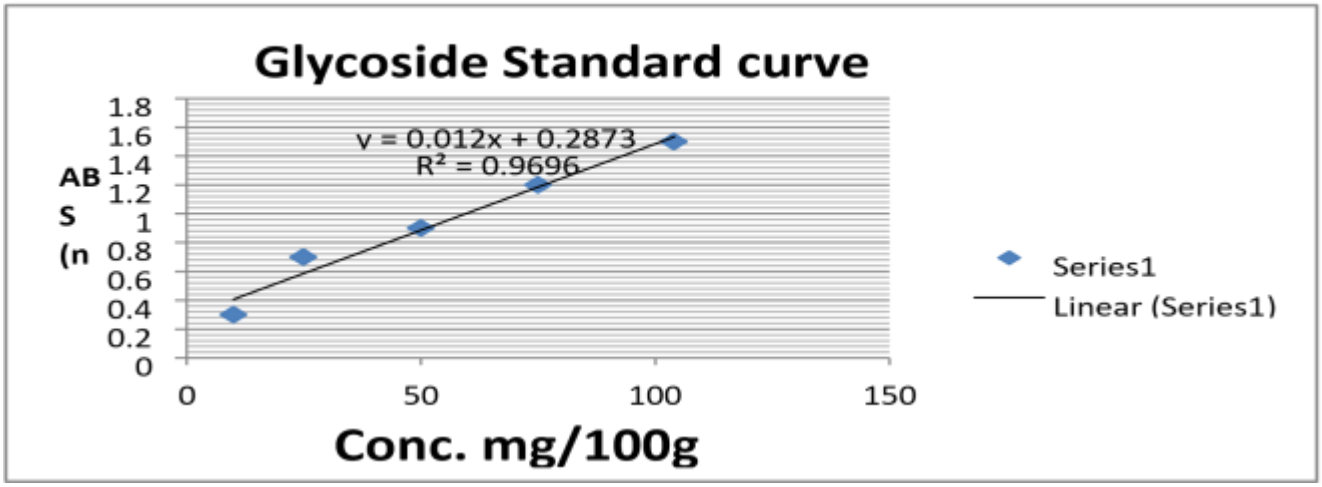

Phenolics Std curve
y = 0.461x + 0.708
R² = 0.9732
Abs (nm)
conc.mg/100g
Series1
Linear (Series1)

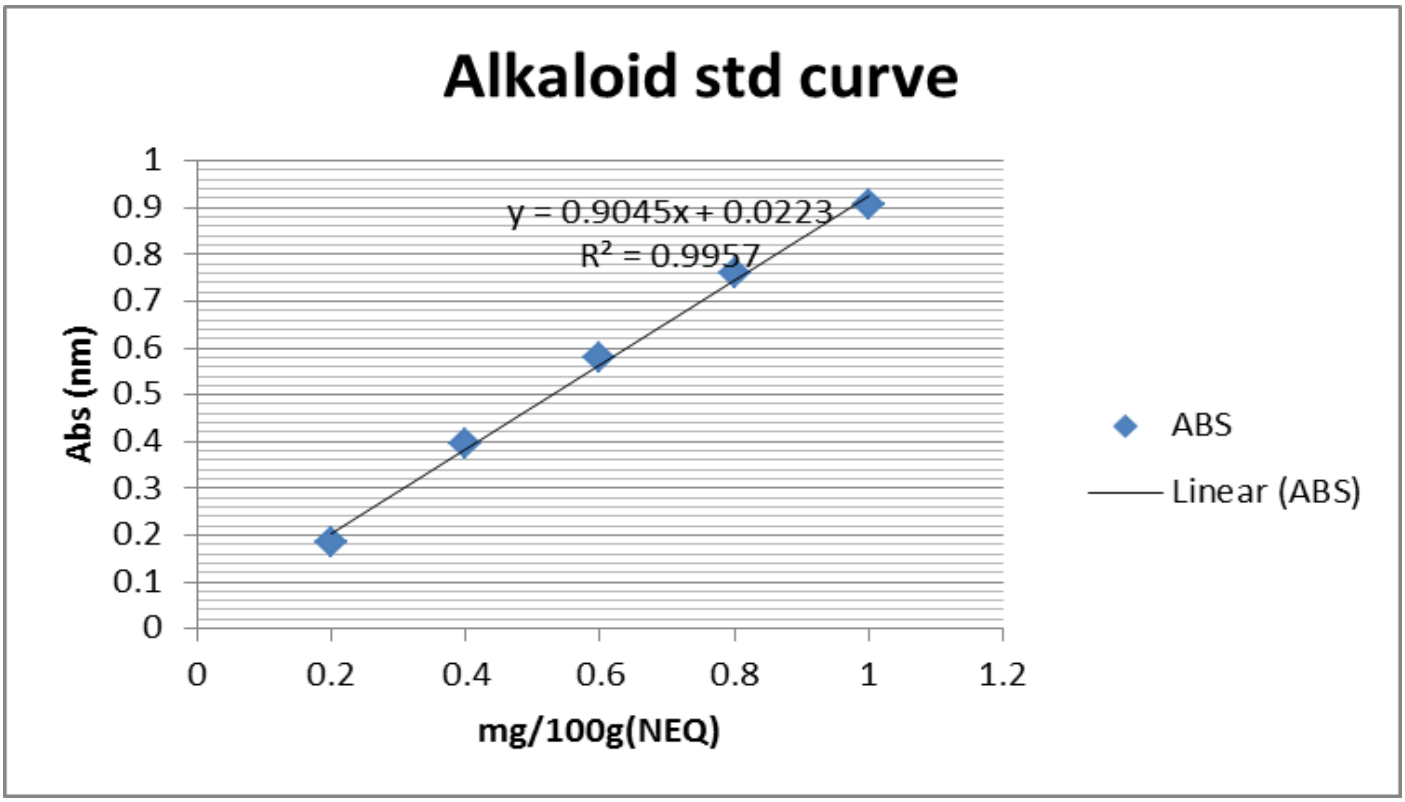

Coumarin
y = 0.0006x + 0.0379
R² = 0.9551
Absorbance (nm)
pmol/Kg AMCE
Series1
Linear (Series1)


Flavonoids Std Curve
y = 0.0039x + 0.1108
R² = 0.9594
Absorbance (nm)
conc. (mg/kg)QAE
Series1
Linear (Series1)


Triterpene Standard Curve
y = 0.006x + 0.0179
R² = 0.9581
Absorbance (nm)
mg/Kg Asiatic Acid Eq
Series1
Linear (Series1)


Terpenoids Std Curve
y = 0.0245x + 0.0564
R² = 0.9494
Absorbance (n
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
20
40
60
conc. (mg/kg)
Series1
Linear (Series1)


Glycoside Standard curve
y = 0.012x + 0.2873
R² = 0.9696
ABS (n
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
50
100
150
Conc. mg/100g
Series1
Linear (Series1)


Alkaloid std curve
y = 0.9045x + 0.0223
R² = 0.9957
Abs (nm)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.2
0.4
0.6
0.8
1
1.2
mg/100g(NEQ)
ABS
Linear (ABS)

Discussion and Conclusion

For hundreds of years, traditional healers have prescribed different parts of *M. oleifera* for the treatment of skin diseases, respiratory illnesses, ear and dental infections, hypertension, diabetes, cancer treatment, and water purification, and they have promoted its use as a nutrient-dense food source (Anwar *et al*., 2007).

The bacterial probable isolates from the study were indicated to be *Escherichia coli, Proteus mirabilis,* and *Klebsiella pneumoniae.* The biochemical tests carried out were the coagulase test, catalase test, motility test, Simmons citrate test, and sugar fermentation test using standard procedures. The microscopic examination showed that all the bacterial isolates were gram-negative.

Moringa leaf and seed contain high amounts of glycosides, phenols, steroids, flavonoids, alkaloids, saponins, tannins, etc which are medicinally important because of their antimicrobial, antihypertensive, antiinflammatory, and hypoglycemic activity. Moringa seed has high nutritional value as reflected in the appreciable amount of nutrients. The high inhibitory activity of moringa leaf against some selected gastrointestinal pathogens implies that it may be useful in treating gastrointestinal infections. The leaf of *Moringa oleifera* has a higher potential for use as an antimicrobial agent against gastrointestinal pathogens than the seed. Conclusively, the seed and leaf can be used as a food supplement to increase the nutritional composition of foods lacking protein, carbohydrates, and lipid. It can be included in diets to supplement human's daily nutritional needs. The leaf also has wound healing properties, as they are anti-inflammatory and analgesic.

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