

**PHYTOCHEMISTRY, PROXIMATE ANALYSIS AND ACUTE TOXICITY STUDY OF ETHANOLIC *Moringa Oleifera* ROOT BARK EXTRACT.**

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**ABSTRACT.**

*Moringa Oleifera* is a fast growing deciduous tree with its different plant parts commonly used in herbal medicine, also with good socio-economic and industrial values. The ethanolic extract of *M.Oleifera* root bark is shown to be rich in nutrients, owing to the presence of essential phytochemicals in the root bark. The phytochemicals or secondary metabolites of the ethanolic extract of the root bark were screened and identified. Also, acute toxicity study of the ethanolic extract of the root bark was carried out in Albino Rats according to Institutional Animal Care and Use (IACU) guidelines. The fresh *M.Oleifera* root was harvested at the inception of the rainy season at Pil Gani District of Langtang North LGA and excess soil washed off as the root bark was properly peeled, air dried, pulverized to powdered form and stored for subsequent use. Serial extractions using water, acetone, ethanol, and ethyl acetate were carried out by Simple Maceration. The ethanolic extract was seen to have the best yield of the phytochemicals qualitatively identified as tannins, saponins, carbohydrates, flavonoids, alkaloids, steroids, terpenes, cardiac glycosides and anthraquinones. Acute toxicity test was determined by Lorke's method and the LD<sub>50</sub> was shown to be at the highest limit dose of 5000mg/kg, which signifies that the extract is non toxic at such single dosage. The ethanolic *M.Oleifera* root bark extract contains nutritive phytochemicals with vast medicinal uses. No sign of acute toxicity was observed on oral administration as the median lethal dose was shown to be safe at the highest limit single dose of 5000mg/kg.

**Key words:** *Moringa Oleifera* root bark, Phytochemical Screening, Acute toxicity test, Albino Rats.

**INTRODUCTION.**

*Moringa Oleifera* is said to be the most widely cultivated species of the monogeneric family, *Moringaceae* that is native to most tropical areas and in various parts of Nigeria. In English, it is known as Drumstick, Horseradish or Ben oil tree. Natively in Nigeria, its commonly called Zogale in Hausa language. Awe-igbale, Igi-igbale or Igi-iyanu (Miracle tree) in Yoruba, and Okwe oyibo or Odudu oyibo in Igbo. It is also known as Sahjan or Munga in Hindi and Sajina or Sajna in Bengali. Other synonymous names are Moringe à graine ailée or Morungue in French, Ángela, Ben or Moringa in Spanish, Rawag in Arab, Moringa or Moringueiro in Portuguese and Laken in Mandarin (Chinese). The scientific classification of the plant shows that it comes from the Kingdom – *Plantae*, Sub kingdom – *Tracheobionta*, Super Division – *Spermatophyta*, Division – *Magnoliophyta*, Class – *Magnoliopsida*, Subclass – *Dilleniidae*, Order – *Capparales*, Family – *Moringaceae*, Genus – *Moringa* and Species - *oleifera*. The fast growing deciduous and medium sized tree is described as 'one of the most amazing trees' as its bark, root, fruit, flowers, leaves, seed and gum can be used medicinally<sup>1</sup>. *Moringa* root is a swollen, tuberous white tap root which has a characteristic pungent odour, and very sparse lateral roots that develop from the seedlings.

However, if the trees are not planted through the seed, a deep stout tap root with a wide-spreading system of thick, tuberous lateral roots will develop<sup>2</sup> as tap roots do not develop from trees propagated from cuttings<sup>3</sup>. Several compounds of proven medicinal value have also been isolated from the roots and root bark<sup>4</sup> as they are used in folklore medicine to treat a number of ailments<sup>5</sup>.

*Moringa* tree is rich in nutrition owing to the presence of a variety of essential phytochemicals present in its different plant parts. Such phytochemicals or secondary metabolites are certain chemicals produced by the plant that tend to show pharmacological properties such as antibacterial<sup>6</sup>, antifungal, anti-inflammatory<sup>7</sup>, antioxidative<sup>8</sup>, immunomodulatory<sup>9</sup>, antinociceptive<sup>10</sup> and hepatoprotective<sup>11</sup> activities among others<sup>12</sup>. In fact, *moringa* is generally said to provide 7 times more vitamin C than oranges, 10 times more vitamin A than carrots, 17 times more calcium than milk, 9 times more protein than yoghurt, 15 times more potassium than bananas and 25 times more iron than spinach<sup>13</sup>. This is thus a report of the determination of the qualitative phytochemical composition of ethanolic *Moringa Oleifera* root bark extract and the acute toxicity in albino rats.

## MATERIALS AND METHODS.

### Plant Collection And Extract Preparation.

Fresh *Moringa oleifera* roots were obtained from Pil-Gani District (Latitude 9°11'30"N and Longitude 9°52'35"E) of Langtang North LGA, Plateau State at the inception of the rainy season in the month of May, and immediately transported to the Laboratory in Biochemistry and Chemotherapy Division, Nigeria Institute of Trypanosomiasis Research (NITR), Vom, Jos South LGA of Plateau State after the family and specie of the plant was authenticated by the Herbarium, Department of Plant Sciences, Faculty of Natural Science, University of Jos. The whole root was washed thoroughly to remove traces of soil/sand and the root bark was separated (peeled off) from the whole roots and dried to a constant weight under shade at room temperature for approximately five weeks. Proper care was taken in the drying process to avoid any fungal growth of the fresh root bark. The dried sample was then adequately pulverized using a good dry mechanical blender to reduce particle size and stored in polythene bags until required for preparation of the extract.

### Extraction Process.

The dried *Moringa oleifera* root bark was reduced to powdered form so that the Aqueous, Ethanolic, Acetonic and Ethyl acetate extracts of the pulverized form were obtained by Simple Maceration. Solutions obtained were dried with the rotary evaporator to obtain the pure form of each extract before phytochemical screening and the relevant assays were conducted.

### Qualitative Phytochemical Analysis.

The ethanolic extract of the *Moringa oleifera* root bark was evaluated for the presence of carbohydrates, anthraquinones, flavonoids, tannins, alkaloid, saponins, cardiac glycosides, steroids and terpenes at Pharmacognosy Department, University of Jos, Plateau State using prescribed qualitative methods and standard operating procedures<sup>14</sup>.

0.5 gram of the extract was dissolved in a test tube with 10 ml of distilled water. This was then shaken vigorously for 30 seconds and allowed to stand. Frothing which persisted on warming was taken as preliminary evidence for the presence of Saponins<sup>15</sup>.

100mg of the plant extract was dissolved in 1ml glacial acetic acid containing one drop of ferric Chloride solution. This was then underplayed with 1ml of conc. Sulphuric acid. A brown ring colour observed at the interface indicates the presence of cardiac glycosides (deoxysugar characteristic of cardenolides)<sup>16</sup>.

0.5 gram of the plant extract was taken into a dry test tube and 5ml of chloroform was added and shaken for

5minutes. The extract was filtered, and the filtrate was shaken with an equal volume of 100% Ammonia Solution. Pink-violet or violet or red colour in the ammonical layer (lower layer) indicates the presence of free anthraquinones<sup>16</sup>.

0.1 gram of the plant extract is dissolved in 1ml of chloroform. 1ml of acetic anhydride and 2 drops of concentrated Sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) was added. A pink colour which changes to bluish green on standing is indicative of the presence of steroids and terpenes.

2g of the plant extract was completely detanned with acetone. The residue was extracted in warm distilled water after evaporating the acetone on a water bath. The mixture was filtered while hot and the filtrate was cooled. For the lead acetate test, 5mls of lead acetate solution was added to 5ml of the detanned water extracted. A yellow-coloured precipitate indicates the presence of flavonoids. For the sodium hydroxide test, 5mls of 20% sodium hydroxide was added to equal volume of the detanned water extracted. A yellow solution indicates the presence of flavonoids.

To 0.5 gram of the plant extract, 1ml of distilled water is added and shaken. Filter the solution and add equal volume of ferric chloride solution. Blue-black, green or blue-green precipitate indicates the presence of Tannins. 0.5g of the plant extract was stirred with 3mls of 1% aqueous Hydrochloric acid (HCl) on steam bath. 1ml each of the filtrate was treated with a few drops of Mayer's reagent, Dragendorff's reagent, and picric acid solution. Precipitation with either of these reagents is a preliminary evidence of the presence of alkaloids.

### Quantitative Phytochemical Screening.

The ethanolic extract of the *Moringa oleifera* root bark was also evaluated to obtain the quantity or percentages of Flavonoids, Tannins, Saponins, Steroids and Alkaloids present using various methods by Harbone (1973) and Pearson (1976) as updated in 2018 by the Toxicology Laboratory of the National Veterinary Research Institute (NVRI), Vom, Nigeria as follows:

For Gravimetric determination of Flavonoids<sup>17</sup>, 5g of the powdered sample is weighed and dissolved in 50mls (20mls) of distilled water. 2mls of conc. HCl solution is added and allowed to boil for 30minutes. The solution is allowed to cool filtering with whatmann's filter paper (No. 42). 20ml of ethyl acetate is added to extract the flavonoids. After partitioning, the flavonoid with the ethyl acetate layer is recovered while the aqueous layer is discarded. The flavonoid with the ethyl acetate layer recovered is put in an evaporating dish of known weight (W<sub>1</sub>) and dried in the oven at 60°C, cooled in a dessicator, then weigh. The weight of sample + evaporating dish (W<sub>2</sub>). Percentage Flavonoids is calculated thus;

$$\% \text{Flavonoids} = \frac{W_2 - W_1}{\text{Weight of Sample}} \times \frac{100}{1}$$

For Spectrophotometric Determination Of Tannins By Folin Denis [18], 2g of the powdered sample is weighed and dissolved in 50mls of distilled water in a clean beaker. The solution is shaken for 30minutes and filtered with whatmann's filter paper. 5mls of the filtrate is pipetted into a clean 50mls volumetric flask and diluted with 3mls of distilled water. 1ml of Folin Dennis reagent is added and 2.5mls of 35% saturated sodium carbonate solution is also added to make up to mark. Solution is incubated for 90minutes at room temperature for colour development. Absorbance is read at 760nm wavelength with reagent blank zero. Standard solution is prepared with 5mls of tannic acid solution and diluted with 3mls of distilled water. Percentage of Tannins is calculated thus;

$$\% \text{Tannins} = \frac{AS-W}{2 \times AY} \times \frac{100}{1}$$

Where W = weight of sample, AS = Absorbance test sample, AY = concentration of standard in mg/ml.

For Gravimetric Determination of Saponins by Double Extraction, 5g of the powdered sample is mixed with 50mls of 20% aqueous ethanol in a clean flask and heated with periodic agitation in a water bath for 90minutes at 55°C. The solution is filtered with whatmann's filter paper (no. 42) and the residue is extracted with 20% ethanol, combined together. The extracts is reduced to about 40ml at 90°C and transferred to a separating funnel. 40mls of diethyl ether is added and shaken vigorously. Separation is allowed by partitioning as the ether layer is discarded while the aqueous layer is recovered. Saponins are extracted with 60mls of normal Butanol. The combined extract is washed with 5% aqueous sodium chloride solution and evaporated to dryness in a pre-weighed evaporating dish (W<sub>1</sub>) at 60°C in the oven. Allow to cool in a dessicator and then reweigh (W<sub>2</sub>). Percentage Saponins is calculated thus;

$$\% \text{Saponins} = \frac{W_2 - W_1}{\text{Weight of sample}} \times \frac{100}{1}$$

For Gravimetric Determination of Steroids. 5g of the powdered sample is weighed in cleaned beaker. Hydrolyze by boiling in 50mls of concentrated Hydrochloric acid solution for 30minutes. Filter with whatmann's filter paper (no. 42) and transfer the filtrate into a separating funnel. Add equal volume of ethyl acetate, mix well and allow to separate into two layers. The ethyl acetate layer (extract) is recovered and dried in evaporating dish of known weight (W<sub>1</sub>), while the aqueous layer is discarded. The extracted steroid is dried at 100°C on a steam bath for 5minutes and conc. amyl alcohol is added. Heat the steroid, extract for few minutes. Dry in the oven, then cool in a dedicator.

Reweigh (W<sub>2</sub>). The concentration of steroid is determined and expressed as a percentage thus:

$$\% \text{Steroids} = \frac{W_2 - W_1}{\text{Weight of sample}} \times \frac{100}{1}$$

For Gravimetric Determination Of Alkaloids, weigh 5g of the plant sample (powder). Dissolve in 40mls of 10% ethanolic acetic acid, allow to stand for 4hours at room temperature. Filter with whatmann's filter paper (No. 42). Concentrate by evaporation over a steam bath to ¼ of the original volume. Precipitate the alkaloid with conc. Ammonia solution in drops until in excess. The resulting alkaloid precipitate is recovered by filtration using previously weighed filter paper (W<sub>1</sub>). The precipitate is washed with 9% ammonia solution, dried in the oven at 60°C for 30minutes and cooled in a dessicator, then reweighed (W<sub>2</sub>). The concentration of steroid is determined and expressed as a percentage thus:

$$\% \text{Alkaloids} = \frac{W_2 - W_1}{\text{Weight of sample}} \times \frac{100}{1}$$

#### **Proximate Analysis.**

Proximate analysis of the Crude *Moringa Oleifera* root bark was carried out to evaluate Moisture content, Crude Protein, Crude Fats, Crude Fibre, Crude Ash content, Nitrogen Free Extract (Carbohydrate), Calcium and Phosphorus contents using various methods in synchronization with the Commission Regulation (EC) (2009)<sup>19</sup> as highlighted:

For Gravimetric Determination of Dry Matter/Moisture Content, dry an aluminium dish with cover at 103±2 °C for at least 2 hours, cover dishes and move to a desiccator, immediately cover desiccator and allow covered dishes to cool to room temperature. Do not allow dishes to remain in the desiccator for more than 2 hours. Weigh dishes with cover (W<sub>1</sub>) to the nearest 0.1 mg, removing one at a time from the desiccator and keeping the dessicator closed between dish removals. Use tongs to handle beakers. Add approximately 2g of ground sample to each dish. Record weight of the dish with cover and sample (W<sub>2</sub>) to the nearest 0.1g. Shake dish gently to uniformly distribute the sample and expose the maximum area for drying. Insert samples (with lids removed to the side) into a pre-heated oven at 103±2 °C and dry for at least 2 hours, start timing once the oven has reached temperature (dry to constant weight, may need to check this for various sample types. Once confirmed, use that drying time). Move samples to a desiccator, place cover on each dish, seal the desiccator and allow to cool at room temperature. Do not allow samples to remain in the desiccator for more than 2 hours. Weigh dish with cover and dried sample (W<sub>3</sub>), recording weight to the nearest 0.1g.

$$\% \text{Dry Matter (DM)} = \frac{(W3-W1)}{(W2-W1)} \times 100$$

$$\% \text{Moisture} = 100 - \% \text{DM}$$

In Kjeldahl Method Of Determination Of Nitrogen And Calculation Of Crude Protein, digestion of the sample is done by weighing approximately 1g of the sample, recorded to the nearest 0.1mg (W) and transferring to the digestion tube. In each batch, use a tube without sample as blank test. Add 2 Kjeldahl tablets and 20mls sulphuric acid. If fuming is a problem, add a few drops of anti-foaming agent. Place the tubes in a digestion unit and connect to the fume removal manifold. Digest the sample at least 1 hour at  $420 \pm 20^\circ\text{C}$ . Turn the digestion off, remove the tubes and allow to cool for 10 – 20 minutes. Add distilled water to each tube to a total volume of approximately 80mls. Distillation and Titration is then carried out by placing a conical flask containing 25 – 30mls of conc. Boric acid under the outlet of the condenser of the distillation unit in such a way that the delivery tube is below the surface of the Boric acid solution. Add 50mls NaOH and distill the Ammonium by following the instructions of the manufacturer. Titrate the content of the conical flask with Hydrochloric acid standard solution after adding a few droplets of indicator solution using a titration unit and read the amount of the titrant used. The endpoint is reached at the first trace of pink colour in the contents. Record the amount of acid used to the nearest 0.05ml for the blank test (Vb) and for each sample (Vs).

$$\% \text{Nitrogen (N)} = \frac{(Vs-Vb) \times M(\text{HCl}) \times 1 \times 14.007}{W \times 10}$$

Where Vs = ml HCl needed to titrate the sample, Vb = ml HCl needed for the blank test, M(HCl) = molarity of HCl, 1 = acid factor, 14.007 = molecular weight of Nitrogen, 10 = conversion from mg/g to %, W = weight of the sample (g)

$$\% \text{Crude Protein} = \% \text{N} \times F$$

Where F = 6.25 for all forages, feeds and mixed feeds, 5.70 for wheat grains, 6.38 for milk and meat products.

In the Determination of Crude Fat – Ether Extract, extraction is done by weighing at least 5g of the sample to the nearest 0.1mg (W1) into the extraction thimble and cover with a fat-free wad of cotton wool. This step should only be performed for the determination of fat without hydrolysis. Transfer some silicon carbide chips to a dry flask and weigh to the nearest 0.1mg (W2). Add 95ml of petroleum ether. Place the thimble in the extractor and connect it to the dry flask and reflux unit. Extract for 6 hours with petroleum ether and regulate the heating apparatus to obtain at least 10 siphonings per hour. Distill the solvent until the flask is nearly free from the solvent, leave overnight in a fume hood to ensure all solvent is evaporated. Dry the flask with residue for 1.5 hours in a

vacuum oven at  $80 \pm 2^\circ\text{C}$ . Cool in a dessicator and weigh to the nearest 0.1mg (W3). Percentage crude fibre with or without Hydrolysis:

$$\% \text{Crude fat} = \frac{(W3-W2)}{W1} \times 100$$

For the Determination Of Crude Fibre - Filtration Method, Pretreatment of the sample weighing 1g of the sample to the nearest 0.1mg to each P100 crucible. Place the crucibles in the filtration equipment and add approximately 30mls of petroleum ether to each crucible and filter using vacuum. Repeat the washing two times. Dry the residue in air and transfer quantitatively to a beaker. The sample is then Digested by adding to each beaker 150mls Sulphuric acid and boiling for  $30 \pm 1$  minutes. If foaming occurs, add a few drops of anti-foaming agent. Filter the mixture through a crucible using a vacuum. Wash the residue 5 times, each time with 10ml of hot distilled water. Add a volume of acetone to just cover the residue. Remove the acetone after a few minutes by applying slight suction. Transfer the residue quantitatively to a beaker. Add to each beaker 150mls potassium hydroxide and boil for  $30 \pm 1$  minutes. Filter the mixture through a crucible using a vacuum. Wash the residue with hot distilled water until the rinsings are neutral. Wash the residue 3 times under vacuum, each time with 30ml of acetone. Dry the residue by suction after each washing. Drying And Incineration is then carried out by putting the crucibles in an oven adjusted to  $103 \pm 2^\circ\text{C}$  and dry for 4 hours. The drying time starts when the oven has reached  $103^\circ\text{C}$ . Place the crucibles in a dessicator and allow to cool. Weigh the crucible directly after removing from the dessicator to the nearest 0.1mg. Place the crucibles in a muffle furnace and incinerate the samples for 2 hours at  $550 \pm 20^\circ\text{C}$ . The incineration time starts when the furnace has reached  $550^\circ\text{C}$ . Place the crucibles in a dessicator and allow to cool. Weigh the crucible directly after removing from the dessicator to the nearest 0.1mg (W3).

Percentage Crude fibre

$$(\% \text{CF}) = \frac{(W3-W2)}{W1} \times 100$$

Gravimetric Determination Of Crude Ash is done by drying the incinerating dish at  $103^\circ\text{C}$  for at least 2 hours, remove from the oven and cool in the dessicator. Weigh the empty dish to the nearest 0.1mg (W1). Add approximately 5g of the sample to the dish and weigh to the nearest 0.1mg (W2). Place the dish in preheated muffle furnace at  $550 \pm 20^\circ\text{C}$  for 3 hours. Inspect visually if the residue is free from carbonaceous particles. Transfer dish into a desiccator and allow to cool at room temperature (approximately 45 minutes). Weigh the dish to the nearest 0.1g (W3).

$$\text{Percentage Ash (\% ASH)} = \frac{(W3-W1)}{(W2-W1)} \times 100$$

In the Determination Of Nitrogen Free Extract, the carbohydrate content was determined by difference (that is, the addition of all the percentages of moisture, ash, crude lipid, crude protein, and crude fiber was subtracted from 100%). This gave the amount of nitrogen-free extract otherwise known as carbohydrate:

$$\%NFE = 100 - (\%Moisture + \%Crude\ Protein + \%Crude\ Fibre + \%Crude\ Fat + \%ASH + \%Calcium + \%Phosphorus)$$

For Spectrophotometric Determination of Calcium, weigh approximately 1g of the sample to the nearest 0.2mg (W) in a beaker and place in a cold muffle furnace. Close the furnace and gradually raise the temperature to 550°C over about 90 minutes. Maintain this temperature for 16 hours (e.g. overnight) to remove carbonaceous material and then open the furnace and allow to cool. Add 10mls 6M hydrochloric acid to each beaker and place on a preheated hot plate (approximately 250°C), cover the beakers with a glass plate, digest for 20 minutes. Allow the beakers to cool and remove from the hot plate. Transfer quantitatively the content of the beakers to a 25ml volumetric flask, make up to mark with distilled water and mix well. Measure calcium in the solutions and standards by the CPC method by following the instructions of the manufacturer of the test kit, measure the absorbance at 578 nm. Calculate calcium content in the measured solution by linear regression. Percentage Calcium is calculated as:

$$\% Calcium = \frac{(C \times V \times DF)}{(W \times 10)}$$

Where C = Concentration of calcium in measured solution (mg/litre), V = Volume of the solution ( in litres, i.e. 0.025L), DF = Dilution factor ( normally, i.e. 1), W = weight of the sample (g) and 10 = Factor to convert g/kg to %.

In Spectrophotometric Determination Of Phosphorus, sample preparation was by weighing approximately 1g of the sample to the nearest 0.2mg (W) in a beaker and place in a cold muffle furnace. Close the furnace and gradually raise the temperature to 550°C over about 90 minutes. Maintain this temperature for 16 hours (e.g. overnight) to remove carbonaceous material and then open the furnace and allow to cool. Add 10mls 6M hydrochloric acid to each beaker and place on a preheated hot plate (approximately 250°C), cover the beakers with a glass plate, digest for 20 minutes. Allow the beakers to cool and remove from the hot plate. Transfer quantitatively the content of the beakers to a 25ml volumetric flask, make up to mark with distilled water and mix well. Let the solution stand overnight. Measure calcium in the solutions and standards by the CPC method by following the instructions of the manufacturer of the test kit, measure the absorbance at 578 nm. To measuring Phosphorus Content, dilute an

aliquot of the solution with distilled water to obtain a phosphorus content not exceeding 40µg/ml. Transfer 10ml each of the diluted solution and standard solutions to separate test tubes. Take a test tube with 10ml water (blank). To each tube, add 10ml molybdovanadate reagent, mix and leave to stand for 10 minutes at 20°C. Measure the absorbance of the solution at 430nm within 45 minutes using a spectrophotometer against the blank. Calculate phosphorus content in the measured solution by linear regression. Percentage of phosphorus is calculated as :

$$\% Phosphorus = \frac{(C \times V \times DF)}{(W \times 10)}$$

Where C = Concentration of phosphorus in measured solution (mg/litre), V = Volume of the solution ( in litres, i.e. 0.025L), DF = Dilution factor ( normally, i.e. 1), W = weight of the sample (g) and 10 = Factor to convert g/kg to %.

#### **Establishing LD<sub>50</sub>.**

Lethal dose (LD<sub>50</sub>) is known as the amount of an ingested substance that kills 50 percent of a test sample. It is expressed in milligrams of substance per kilogram of body weight (mg/kg). The determination of the median Lethal Dose (LD<sub>50</sub>) of extracts was carried out using albino rats in two stages [20]. In the initial investigations, the range of doses producing the toxic effects is established. Based on these results, further specific doses are administered to calculate an LD<sub>50</sub> by Geometric Mean. Thus, in the initial phase, 9 male rats of both sexes were divided into three groups of 3 rats each. Groups 1, 2 and 3 were orally administered with crude ethanolic *M. Oleifera* root bark extract at 10, 100 and 1000 mg/kg respectively. The rats were then observed for signs of intoxication and mortality in minutes, hourly, daily and terminated after 14 days. In the second phase of the study, 3 rats were assigned into 3 groups of 1 rat each. The animals were individually administered 1600, 2900, and 5000mg/kg of the crude ethanolic extract and also observed for the same time duration as the initial stage for signs of intoxication and mortality. Results were properly documented as observed. All animals that died or remained alive for the duration of the experiment were autopsied *in-situ* for any lesions. The liver, kidneys, heart, lungs, and spleen were harvested, and samples were taken for histopathological examination.

#### **Ethical Clearance.**

The Albino rats were properly housed and maintained at the Biochemistry and Chemotherapy Division Laboratory, Nigeria Institute for Trypanosomiasis Research (NITR), Vom in relation to the use of Laboratory animals with protocol as approved by the Institutional Animal Care and Use (IACU) in

collaboration with the Office of Laboratory Animal Welfare (OLAW), Reference number: F17-00379 authenticated by the Ethical Committee, Animal Experiment Unit, Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos, Nigeria. The animals were reared in spacious rat cages using clean sawdust as bedding, which was routinely changed to maintain cleanliness of the cages. The animals were also fed on commercial Vital super starter poultry feed as clean drinking water was provided ad libitum. They enjoyed twelve hours of light and same hours of darkness.

**RESULTS AND DISCUSSION.**

**Preliminary Results From Extraction Procedure.**

The percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The Percentage yield of the aqueous, ethanolic, acetonic and ethyl acetate extract of *Moringa Oleifera* root bark was calculated as shown;

Table 1. Percentage Yield from extraction procedure.

EXTRACTION SOLVENTS	Aqueous (H <sub>2</sub> O)	Ethanol	Acetone	Ethyl Acetate
$\frac{\text{Weight of extract}}{\text{Weight of crude}} \times 100$	$\frac{9.4128}{60} \times 100$	$\frac{3.4056}{60} \times 100$	$\frac{3.1782}{60} \times 100$	$\frac{2.0340}{60} \times 100$
%Yield	15.7	5.5	5.3	3.4

The results in Table 1 showed that the aqueous solvent gave the highest extraction efficiency at 15.7% compared to the Ethanolic and Acetonic solvents at 5.5% and 5.3% respectively. The Ethyl Acetate solvent gave the least efficient at 3.4%. This indicates that extraction efficiency

is dependent on polarity of solvents used as polar solvents tend to yield higher extraction percentages than non-polar solvents. Thus, decrease in solvent polarity leads to decrease in percentage yield of extracts and vice versa.

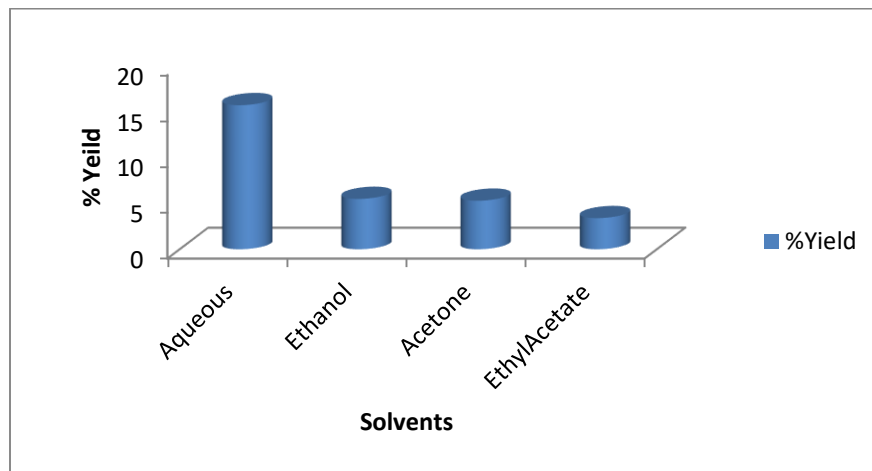


Fig. 1 Percentage Yield of *M. Oleifera* root bark

**PRELIMINARY RESULTS FROM QUALITATIVE PHYTOCHEMICAL SCREENING**

Table 2. Phytochemical constituents of the different extracting solvents.

Constituents	Aqueous	Ethanol	Acetone	Ethyl Acetate
Tannins	++	+	+	-
Saponins	++	++	++	-
Carbohydrates	+++	++	+++	+
Flavonoids	+++	++	++	+
Alkaloids	+	++	++	+
Steroids	-	+	+	++

Terpenes	-	++	+++	+
Cardiac Glycosides	+	++	+++	+
Anthraquinones	-	+	+	++

**Key: +++ High Concentration ++ Moderate Concentration + Low concentration - Not detected**

Qualitative phytochemical screening helps to understand a variety of chemical compounds produced by plants and quantification of those metabolites will also help to extract, purify and identify the bioactive compounds for useful aspects to human beings [21]. The results obtained from the Qualitative analysis of the crude *Moringa Oleifera* root bark extract obtained from Pil Gani District in Langtang North LGA of Plateau State showed the presence of various phytochemicals for the different solvents used. The Aqueous extract, though with the highest percentage yield, didn't contain steroids, terpenes and anthraquinones whereas the Ethanolic extract contained a moderate concentration of all stated phytochemicals except for Tannins, Steroids and Anthraquinones which were present but in lower concentrations. This concurs with the research by Lambole and Kumar (2011)[22]. The Acetonic and Ethyl Acetate extracts were both shown to be void of Saponins

and also, there was absence of Tannins in the Ethyl Acetate extract. It was thus concluded from the results of the Qualitative phytochemical analysis that the Ethanolic Extract of *Moringa Oleifera* root bark is the most suitable extract to be primarily used, being the semi-polar solvent that yielded most of the needed phytochemicals with conducive extraction efficacy and appropriate quality.

### Results From Quantitative Phytochemical Screening of Crude Ethanolic Extract of *Moringa Oleifera* Root Bark.

Quantitative phytochemical analysis of the crude ethanolic extract of *M. Oleifera* root bark was carried out in triplicates and the results presented as mean  $\pm$  Standard Deviation (S.D);

Table 3. Quantitative estimation of Phytochemicals.

PHYTOCHEMICALS	TRIPLICATES			MEAN $\pm$ S.D
	1	2	3	
%ALKALOIDS	13.62	13.63	13.82	13.69 $\pm$ 0.09
%SAPONINS	15.04	15.01	15.09	15.05 $\pm$ 0.03
TANNINS (mg/100g of sample)	0.581	0.582	0.581	0.581 $\pm$ 0.0005
%FLAVONOIDS	3.90	3.90	3.96	4.00 $\pm$ 0.09
%STEROIDS	0.15	0.20	0.15	0.16 $\pm$ 0.02

The results indicate a higher percentage of Saponins (15.05 $\pm$ 0.03%) and Alkaloids (13.69 $\pm$ 0.09%) than Flavonoids (4.00 $\pm$ 0.09%) and Steroids (0.16 $\pm$ 0.02%). Tannins (0.581 $\pm$ 0.0005mg/100g of sample) were also seen to be in low amount. Saponins are known as active expectorants and are very useful in the treatment of upper respiratory tract inflammations; they also have anti-diabetic and anti-fungal properties<sup>23</sup>. Saponins, also often referred to as natural detergent due to their foamy nature, possess anti carcinogenic properties, immune modulation activities and regulation of cells proliferation as well as inhibition of the growth of cancer cells and cholesterol lowering activity<sup>24</sup>. The Alkaloids account for a wide range of pharmacological properties including antimalaria, antiasthma, and anticancer properties<sup>25</sup>. It was also reported to have vasodilatory, antiarrhythmic, antihyperglycemic activities<sup>26</sup>, analgesic and antibacterial properties<sup>27</sup>, though they can be toxic due to the presence of the potential nerve-paralyzing alkaloid called sporadin in the root bark<sup>28</sup>. A lower percentage of Flavonoids may account for antioxidant, anti

inflammatory, anti allergic, anti carcinogenic, anti microbial, hepatoprotective and anti viral properties<sup>29</sup>. Flavonoids also help to prevent platelets sickness and hence platelets aggregation<sup>30</sup>. However, The result of the quantity of Tannins and Steroids were seen to be quite insignificant though the slight quantity of tannins is said to possess physiological, astringent and haemostatic properties, which hasten wound healing and ameliorate inflamed mucus membrane<sup>31</sup>. Tannins can also be effective in curbing hemorrhages as well as restrict bare swellings. While tannins are proved haemostatic, they are also beneficial when applied on mucosal coating in the mouth. Hence, herbs possessing tannins are widely used as mouthwashes, eyewashes, snuff and even as vaginal douches and also treat rectal disorders<sup>32</sup>. Steroids on the other hand, increase protein synthesis, promoting the growth of muscles and bones. They also reduce the recovery time needed between training sessions and enable athletes to train more intensively for longer periods.

**Proximate Analysis.**

The results obtained indicates that the Ethanolic extract of *Moringa Oleifera* Root bark is rich in Carbohydrate (59.88%), Crude fibre (23.72%), Ash

content (12.01%) and Moisture content (10.76%) with a lower percentage of proteins and crude fat. Calcium and phosphorus were also observed in trace amounts.

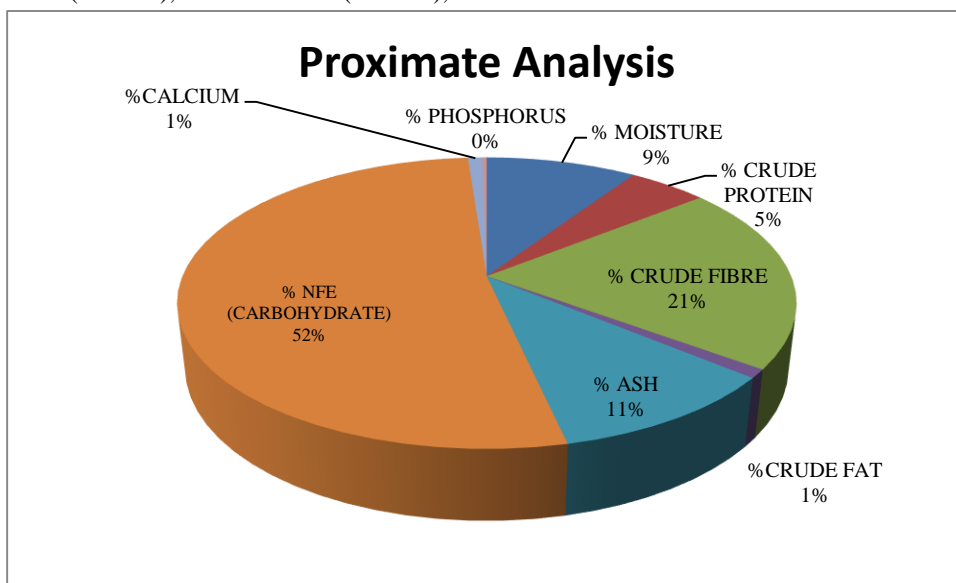


Fig. 2 Proximate Analysis of Crude Ethanolic Extract of *M.Oleifera* Root Bark

**Median Lethal Dose (LD<sub>50</sub>) Of Ethanolic *M.Oleifera* Root Bark Extract.**

All animals were carefully observed for the development of any toxic signs or symptoms such as loss of appetite, hyperventilation, weakness, itching, circling, restlessness, neurological effects, etc at different time intervals of 0minute, 15minutes, 30minutes, hourly and daily for a period of 14days for each of the 1<sup>st</sup> and 2<sup>nd</sup> stages. No significant abnormality or mortality was observed in any of the animals orally administered with the crude ethanolic extract of *Moringa Oleifera* root bark at the lowest to highest dose range of 10mg/kg to 5000mg/kg body weight respectively. The different body weights of the animals showed no significant difference as compared to the control group, though, depending on the different sexes of the rats, the male rats were seen to be naturally bigger than the females. The weight of the internal organs also showed no significant difference compared to the positive control, indicating that there was no organ toxicity since no enlargement of organs or visual lesions were observed post mortem. The median lethal dose of the ethanolic extract of *M. oleifera* root bark is shown to be above 5000mg/kg by Lorke's method. This indicates that the crude ethanolic *M. Oleifera* root bark extract is non toxic and safe for oral administration at the higher limit dose of 5000mg/kg Body weight as also reported by Lambale and Kumar (2011)<sup>22</sup>.

**Post Mortem Findings**

Post mortem examination showed no significant difference in the sizes of the different harvested organs based on individual body weights and compared to control. Also, no congestion or swelling or lesions in the harvested organs of the animals was observed, signifying that no toxic effect was prominent from the oral administration of the crude ethanolic *M.Oleifera* root bark extract in the acute toxicity test.

**CONCLUSION.**

The Ethanolic extract of *Moringa Oleifera* root bark was shown to generally contain Tannins, Saponins, Carbohydrates, Flavonoids, Alkaloids, Steroids, Terpenes, Cardiac glycosides and Anthraquinones. The oral administration of the ethanolic root bark extract was also shown to be safe with no adverse effects post mortem on the Body and internal organ weights of the Laboratory animals as compared to the control used to determine the median lethal dosage which was shown to be at 5000mg/kg as the highest single dose. This indicates that the plant extract is non toxic and belongs to class 5 or unclassified substances as per Globally Harmonized Classified System (GHC) for chemical substances indicative of very high LD<sub>50</sub> value.



**RECOMMENDATION.**

Further studies should be conducted using other extractive solvents in order to establish their respective quantitative phytochemical screening and to compare their Acute toxicity to that of the ethanolic extract of *M. Oleifera* root bark.

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