ANTI-NUTRITIONAL COMPOSITION OF ETHANOL ROOT EXTRACT OF MORINGA OLEIFERA LAM

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A PROJECT WORK SUBMITED TO THE DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (HONOURS) UNIVERSITY OF BENIN, BENIN CITY.

JANUARY, 2025.

CERTIFICATION

This is to certify that this work was carried out by **Osariemen George OVENSERI** of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.

Prof. Timothy Odaro	**Date**
(Project Supervisor)	
Prof. E. D. Vwioko	**Date**
External Supervisor	**Date**

DEDICATION

I dedicate this project work to my Heavenly Father, who has been my ever present God and the source of my strenght. I also want to dedicate this project work to my parents (Rev. Barr. and Dr. Mrs G. O. Chime), I sincerely appreciate them for their enormous support, encouragement and provision throughout the course of this my final year project.

ACKNOWLEDGEMENTS

My deepest gratitude goes to God, for providing all that was needed to complete this project. I also want to use this medium to appreciate my parents (Rev. Barr. and Dr. Mrs G. O. Chime) for their love, care, prayers, provision and unwavering support. I also want to appreciate the Head of Department, Prof. E. D. Vwioko , the Staff Advisor, Prof. B. O. Edegbei and my amiable Course Advisor, Mr. Theophilus Dana for their enormous contributions to me all through these years of study. To my project Supervisor, Prof. Odaro Timothy, I sincerely appreciate you for the guidance, knowledge and suggestions that you provided to me in the course of this project work, God bless you Sir. I also owe deep appreciation to Rev. E. O. Oghama for his contributions, ideas and suggestions all through this project. More also, I want to appreciate all my Professors, Lecturers , Staff Members and Non-academic Staff Members for their support so far my journey in this institution. My project colleagues (Royce, Rhoda, Masse, Jessica and Ekene) have all been amazing, I pray the labour market favours us all. I also want to appreciate my all friends and coursemates, especially the phantom brothers (Moses, Phoenix and Emma), Ogunzie Jude and Excellent Ehizua. I also want to acknowledge the entire Students Association and my Executives for their support all through the period of this project work. I cannot fail to mention the names of these people, Mr. Daniel, Aunty Praise, Ebenezer Chime, Bros Venko, Bros Kendrick, Osarugue, Mr. Eghosa, and Mr. and Mrs. Ibeh, a big shoutout to you all for all the support and encouragements shown by you all towaards my years of study. I pray God continues to bless and increase you all.

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ABSTRACT

Moringa oleifera Lam, a plant renowned for its medicinal and nutritional properties, has been widely consumed globally. However, its ethanol root extract, though utilized in traditional medicine, has not been extensively characterized for its anti-nutritional composition. This study aimed to quantify the levels of anti-nutritional properties present in the ethanol extract of M. *oleifera* Lam and to validate the health implications of consuming this extract.

Using standardized analytical techniques, the ethanol root extract was assessed for its content of cyanide, oxalate, and phytate. The results showed significant levels of these anti-nutrients, with 0.32133333 mg/g of cyanide, 2..2 mg/g of oxalate, and 0.74255 mg/100g of phytate. These findings suggest that excessive consumption of the ethanol root extract may pose health risks due to the potential toxicity of these compounds.

However, this study also highlights the potential benefits of these anti-nutrients in moderation, including their antioxidant properties, ability to regulate calcium levels, and support blood sugar control. The results of this study provide valuable insights into the anti-nutritional composition of the ethanol root extract of M. oleifera Lam and emphasize the need for proper processing and dosage to maximize its nutritional benefits while minimizing its potential health risks.

CHAPTER ONE

1.0 INTRODUCTION

Moringa oliefera Lam. is a perennial tropical deciduous belonging to the family Moringaceae. It originated in india and has been widely cultivated in Asia, Africa, and tropical and subtropical regions of central America. *Moringa oliefera* Lam.. has a significant economic value in addition to being a species of ornamental tree, since pactically all of its parts, including the roots, leaves, seeds are used as food. Due to its abundance in active components, it is also has a variety of pharmacological functions such as anti-oxidation, anti-inflammatory , anti-diabetic, anti-lipid , anti-cancer and anti-bacterial activities (Cao *et al*., 2023).

The plant is also known as Horse- radish tree, Drumstick tree. Every part of this plant contains a valuable medicinal feature. It is a rich source of vitamin A and vitamin C. Different types of active phytoconstituents like alkaloids, protein, quinine, sapnins, flavonoids, tannin, steroids, glycosides, fixed oils and fats are present. Some other constituents are niazinin A, niazinin B and niazmicin A, niaziminin B.

1.1 TAXONOMIC CLASSIFICATION OF MORINGA OLEIFERA LAM. (As ducumented by Paikra, 2017).

Kingdom – Plantae

Sub kingdom – Tracheobionta

Super Division – Spermatophyta

Division – Magnoliophyta

Class – Magnoliopsida

Sub class – Dilleniidae

Order – Capparales

Family – Moringceae

Genus – Moringa

Species – oleifera

1.2 NUTRITIONAL AND PHARMACOLOGICAL PROPERTIES OF MORINGA OLEIFERA LAM.

Moringa oliefera Lam.is a rich source of nutrients, including vitamins A, C, and E, calcium, iron, magnesium, and potassium. The plant also contains a range of phytochemicals, including flavonoids, phenolic acids, and saponins, which have been shown to possess antioxidant, anti-inflammatory, and antimicrobial activities (Gopalakrishnan, 2016).

1.3 TRADITIONAL USES OF MORINGA OLEIFERA LAM.

Moringa oliefera Lam. has been used in traditional medicine for centuries, particularly in Ayurvedic and Unani medicine. The plant's various parts have been used to treat a range of ailments : Inflammation and infection, cardiovascular disease, cancer, diabetes and digestive disorders (Fahey *et al.*, 2005)

The plant's roots, in particular, have been used to treat a range of conditions, including rheumatism, arthritis, and gout (Anwar *et al*,, 2003)

1.4 ANTI-NUTRITIONAL PROPERTIES OF MORINGA OLEIFERA LAM.

Despite its potential health benefits, *M. oleifera* Lam. also contains ant-inutritional properties (ANPs) that can compromise its nutritional value and bioavailability. ANPs, such as phytates,

oxalates, and saponins, can inhibit the absorption of essential minerals, including calcium, iron, and zinc (Makkar & Becker, 1996)

According to Gopalakrishnan *et al.* 2016, The roots of *Moringa oleifera* Lam. in particular, have been found to contain high levels of ANPs, including phytates, oxalates and cyanides .

1.5 BOTANICAL DESCRIPTION OF MORINGA OLEIFERA LAM.

Habit and Habitat:

Moringa oliefera Lam. is a deciduous tree that grows up to 10-12 meters in height, with a straight trunk and a spreading, open crown (Gopalakrishnan *et al*., 2016). It is native to the tropical and subtropical regions of Asia and Africa, and is commonly found in dry, sandy soils (Kumar *et al*., 2013).

Stem and Bark:

The stem of *Moringa oliefera* Lam. is erect, with a smooth, grayish-brown bark that is thin and fibrous (Leone *et al*., 2015). The bark is also characterized by the presence of numerous lenticels, which are small, raised pores that allow for gas exchange (Gopalakrishnan *et al*., 2016).

The Leaves:

The leaves of *Moringa oliefera* Lam. are alternate, bipinnate, and have a distinctive odor (Kumar *et al*., 2013). They are 20-60 cm long, with 3-9 pairs of pinnae, each bearing 6-24 leaflets (Gopalakrishnan *et al*., 2016). The leaflets are elliptic to ovate, 1-3 cm long, and 0.5-1.5 cm wide, with a rounded apex and a entire margin (Leone *et al*., 2015).

The Flowers:

The flowers of *Moringa oliefera* Lam.are small, white, and arranged in axillary panicles (Gopalakrishnan *et al*., 2016). They are 1-2 cm long, with 5 sepals and 5 petals, and are

highly fragrant (Kumar *et al*., 2013). The flowers are bisexual, with 5 stamens and a superior ovary (Leone *et al*., 2015).

The Fruits:

The fruits of *Moringa oliefera* Lam. are long, narrow pods, 30-60 cm long and 1.5-2.5 cm wide (Gopalakrishnan *et al.*, 2016). They are green when immature, turning brown and dry when mature (Kumar *et al*., 2013). Each pod contains 10-20 seeds, which are arranged in a single row along the length of the pod (Leone *et al*., 2015).

The Seeds:

The seeds of *Moringa oliefera* Lam. are small, brown, and have a distinctive winged shape (Gopalakrishnan *et al*., 2016). They are 1-2 cm long and 0.5-1 cm wide, with a rounded apex and a narrow wing (Kumar *et al*., 2013).

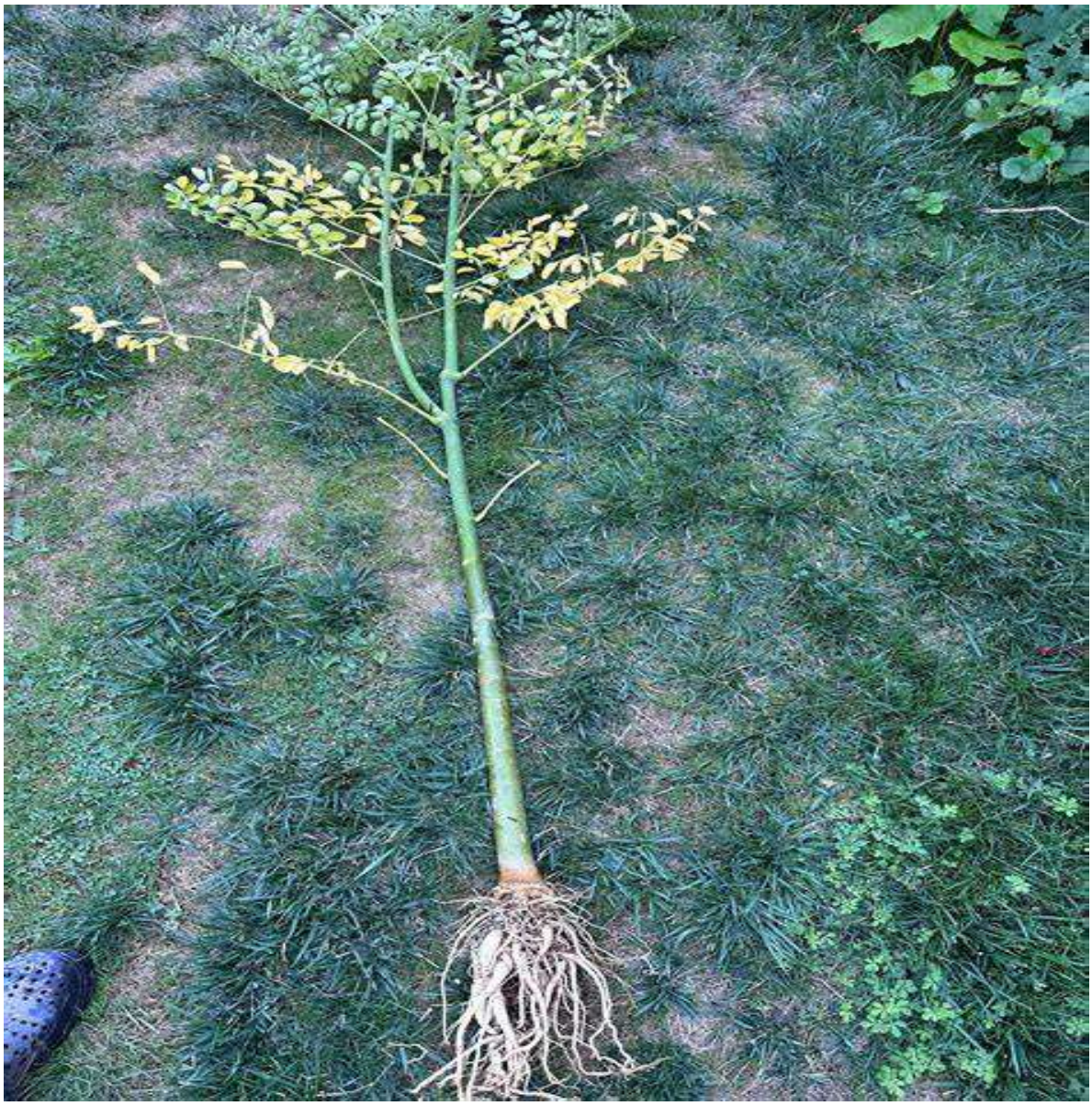

Plate 1: shows a young *M. oleifera* Lam, plant

Plate 2: shows a mature *Moringa oleifera* lam. tree

Plate 3: shows a sample of the roots of *Moringa oleifera* lam.

1.6 DESCRIPTION OF THE ROOTS OF MORINGA OLEIFERA LAM.

The Root System:

The root system of *Moringa oliefera* Lam. is a taproot system, characterized by a large, central root that grows straight down into the soil (singh *et al.*, 2013). The taproot is thick and fleshy, with a smooth and hairless surface (leone *et al.*, 2015).

Root Morphology:

The roots of *Moringa oliefera* Lam. are white or yellowish in color, with a cylindrical shape and a rounded apex (Kumar *et al.*, 2013). The root bark is smooth and thin, with a few scattered hairs as reported by Gopalakrishnan *et al.*, 2016.

Root Chemical Composition:

The roots of *Moringa oliefera* Lam. contain a range of bioactive compounds, including alkaloids, glycosides, and phenolic acids (Rao *et al*., 2018). The roots also contain high levels of dietary fiber, protein, and minerals such as potassium, magnesium, and iron (Kumar *et al*., 2013).

Medicinal Properties:

The roots of *Moringa oliefera* Lam. have been used in traditional medicine for centuries, particularly in Ayurvedic and Unani medicine (Singh *et al.*, 2013). The roots are said to have anti-inflammatory, antioxidant, and antimicrobial properties, and are used to treat a range of ailments, including arthritis, rheumatism, and digestive disorders (Rao *et al*., 2018).

1.7 CULTIVATION OF MORINGA OLEIREA

Climate and Soil Requirements:

Moringa oleifera Lam. is a tropical plant that thrives in warm climates with average temperatures ranging from 25-35°C (Gopalakrishnan *et al*., 2016). It can grow in a wide range of soils, but prefers well-drained, sandy loam soils with a pH between 6.0 and 7.0 (Kumar *et al*., 2013).

Propagation:

Moringa can be propagated through seeds, stem cuttings, or tissue culture. Seeds are the most common method of propagation, and should be sown in well-draining soil with a temperature of around 25°C (Gopalakrishnan *et al*., 2016).

Irrigation and Water Management:

Moringa oliefera Lam. requires consistent moisture, especially during the first year after planting. However, it is also drought-tolerant and can survive with minimal watering (Leone *et al*., 2015).

Fertilization:

Moringa oliefera Lam. is a low-input crop and does not require high levels of fertilization. However, applying organic fertilizers such as compost or manure can improve soil fertility and promote healthy growth.

Pruning:

Regular pruning is necessary to promote bushy growth and prevent the plant from becoming leggy. Pruning should be done during the dormant season, and the plant should be trained to a central leader or trellis system (Leone *et al*., 2015).

Pest and Disease Management :

Moringa oliefera Lam. is generally a low-maintenance crop, but can be susceptible to pests such as aphids, whiteflies, and spider mites (Kumar *et al.*, 2013). Regular monitoring and integrated pest management strategies can help prevent infestations

1.8 PROCESSING OF MORINGA

Most plants lose their nutritive properties when processed. When compared, the nutritive content of raw, germinated and fermented moringa seed flour was found to have phytochemicals higher in raw seed flour and amino acid content was at its peak in fermented and germinated seed flour as seen in Ijarotimi *et* al., (2013). This could be a result of the biochemical activities during germination and microbial activity during fermentation.

The presence of phytate and other anti-nutrients can reduce the bioavailability of certain nutrients and processing can hence be done for maximum utilization of required nutrients from the seeds and leaves according to Sallau B. *et al*. (2012).

Yang *et al*. In 2006 reported that boiling increases the availability of iron and antioxidant content. Hence, the processed moringa seed flour can be used to treat malnutrition problems. However, some studies have shown that children refuse to take in moringa due to its slight

bitter taste. Kiranawati *et al*. (2014) designed Moringa noodles by three methods of cooking noodles, sautéing, steaming and boiling.

1.9 PRESERVATION METHODS

Moringa oliefera Lam. can also be preserved for a long time without loss of nutrients. Drying or freezing can be done to store the leaves. A report by Yang *et al*. (2014) shows that a low temperature oven used to dehydrate the leaves retained more nutrients except vitamin C than freeze-dried leaves. Hence, drying can be done using economical household appliance like stove to retain a continuous supply of nutrients in the leaves. Preservation by dehydration improves the shelf life of Moringa without change in nutritional value. An overdose of moringa may cause high accumulation of iron. High iron can cause gastrointestinal distress and hemochromatosis. Hence, a daily dose of 70g of moringa is suggested to be good and prevents over accumulation of nutrients. (Yang *et al*. 2014)

1.10 MEDICINAL APPLICATIONS OF MORINGA OLIEFERA.

- **Antioxidant** :

M. Oleifera Lam. root is a rich source of antioxidants, which are compounds that help protect cells from damage caused by free radicals. Free radicals are unstable molecules that can cause oxidative stress, leading to cell damage and potentially contributing to chronic diseases such as cancer, diabetes, and heart disease.

- **Anti-allergic** :

Moringa root has been found to possess anti-allergic properties, which may help to alleviate symptoms associated with allergic reactions. (Rani *et al*.,2019)

- **Anti-atherosclerotic :**

The roots of *M. oleifera* Lam. has been studied to possess anti-atherosclerotic properties, which may help to prevent or reduce the progression of atherosclerosis, a condition characterized by the buildup of plaque in the arteries. (Chumark *et al*.,2008)

- Anti-constipation :

As seen in Tang et al.,(2021), the roots of *M. oleifera* Lam. Can be used to treat constipation

- Anti-helminthic :

Helminths are parasitic worms that can infect human and animals, causing a range of health problems and the use of the roots of *M. oleifera* . can help combart this medical problem |(Prabhu *et al*., 2011)

1.11 RESEARCH AIMS AND OBJECTIVES

The objectives of this study are:

1. To quantify the levels of anti-nutritional properties (ANPs) present in the ethanol root extract of *Moringa oleifera* Lam.

2. To evaluate the potential health implications of consuming the ethanol root extract of *Moringa oleifera* Lam.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 SAMPLE COLLECTION AND PROCESSING

The *M. oleifera* Lam. Sample was gotten from Obeh quarters, Sakponba road, and brought to the Department of Plant Biology and Biotechnology, University of Benin for identification. it was thereafter chopped into pieces, then shade-dried and further dried in an oven at 65℃. The sample was then pulverized using a British-milimachine. 5 g of the sample was thereafter macerated in absolute ethanol (997%) and finally concentrated in a rotary evaporator.

2.2 ANTI-NUTRIENT'S ASSAAYS

2.3 DETERMINATION OF CYANIDE CONTENT

The cyanide content s determined using the method of Onwuka (2005)

Materials needed:

Whatman no 1 filter paper, Potasium cyanide (KCN), and Alkaline picrate solution - dissolved 1g of picrate and 5g of Na_2CO_3 in 200ml of distilled H_2O

Procedure

Weighed 5 g of dried sample and dissolved it in 50 ml of distilled H_2O. It was allowed to stay overnight and then filtered with Whatman no 1 filter paper and then moved on to Prepare different concentrations of KCN solution containing 0.1 - 1.0 mg/ml.

2.4 DETERMINATION OF OXALATE CONTENT

Materials Needed:

The following materials used to determine the oxalate content in the plant *M. oleifera* lam. As reported by Mahmood *et al* 2020 are: 3M of H_2SO_4, Magnetic stirrer, Whatman no 1 filter paper and 0.05M of $KMnO_4$

To prepare 600ml of 3M of H_2SO_4 solution gravity = 1.85

Given that, Molarity = $\frac{\text{No of moles}}{liter}$

$\frac{600}{1000}$ = 0.6 L

No of moles = molarity × liter

= 3 × 0.6

= 1.8 moles

Wt_g = No of moles × mm

= 1.8 × 98

= 176.4 g of pure H_2SO_4 needed.

But stock was 98% pure (0.98 purity)

= $\frac{176.4}{0.98}$ = 180 g of stock needed

Volume = $\frac{Wtg}{Lg/mol}$ = $\frac{180}{1.85}$ = 97.3 ml

Therefore, I measured 97.3 ml of stock solution of H_2SO_4 in 600ml of distilled H_2O.

To prepare 0.05M of $KMnO_4$ (1 liter)

Molar mass = 158.034

No of moles = 0.05moles

Wtg = 0.05 × 158.034

=7.9 g

Then dissolved 7.9 g of $KMnO_4$ salt in 100 ml of distilled H_2O.

Procedure

Weighed 1g of sample into 100 ml conical flask ,then added 75 ml of H_2SO_4 ,Therafter, Stirred for 1hr with a magnetic stirrer, and then Filtered using a Whatman no 1 filter paper. I measured out 25 ml of the filtrate and titrated while it was hot againt 0.05 M $KMnO_4$ solution until a faint pink colour became persistent for at least 30seconds . The oxalate content was then calculated by taking 1ml of 0.05 M $KMnO_4$ as equivalent to 2.2 mg oxalate (Chinma and Igyor, 2007 ; Ihekoronye and Ngoddy, 1985). 1ml = 2.2mg oxalate

2.5 DETERMINATION OF PHYTATE CONTENT

Materials Needed

The materials needed to carry out this experiment are 2% of HCl solution, 0.3% Ammonium thiocyanate and fercic chloride (0.00195g/ml)

To prepare 100ml = 1.95g in 100ml distilled H_2O

Procedure.

According to Lucas and Markakas, 1975 the following are the procedures emplyed in determining the phytate content in *M. oleifera* Lam.

2g of sample was weighed into different 250ml conical flasks and 100ml of 2% HCl was added to soaked sample for 3hour. Then proceeded to filtering using a Whatman no 1 filter paper. 50ml of each filtrate was taken into 250ml beaker and 10ml of distilled H_2O was added to each sample to give proper acidity. 10ml of 0.3 ammonium thiocyanate solution was then added as indicator and titrated with standard iron(iii) chloride solution contaiing 0.00195 iron/mol. The end point was observed to be yellow which persisted for 5 minutes

% phytic acid = titre value × 0.00195g × 1.19 × 100

were

2.6 DATA ANALYSIS

Data obtained from the cyanide, oxalate and phytate analysis were expressed using descriptive statistics. They were expressed as graphs and percentage composition using microsoft excel (2013) by microsoft corporation software.

CHAPTER THREE

3.0 RESULTS

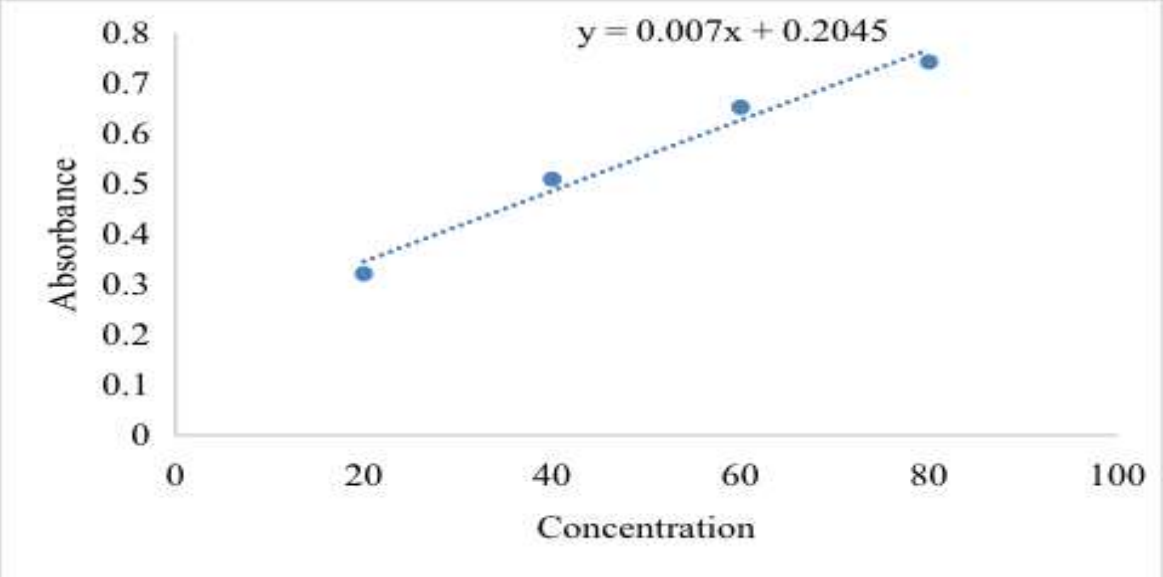

Figure 1: Determination of *Moringa oleifera* Root Cyanide Content from Potassium Cyanide Standard Calibration Graph

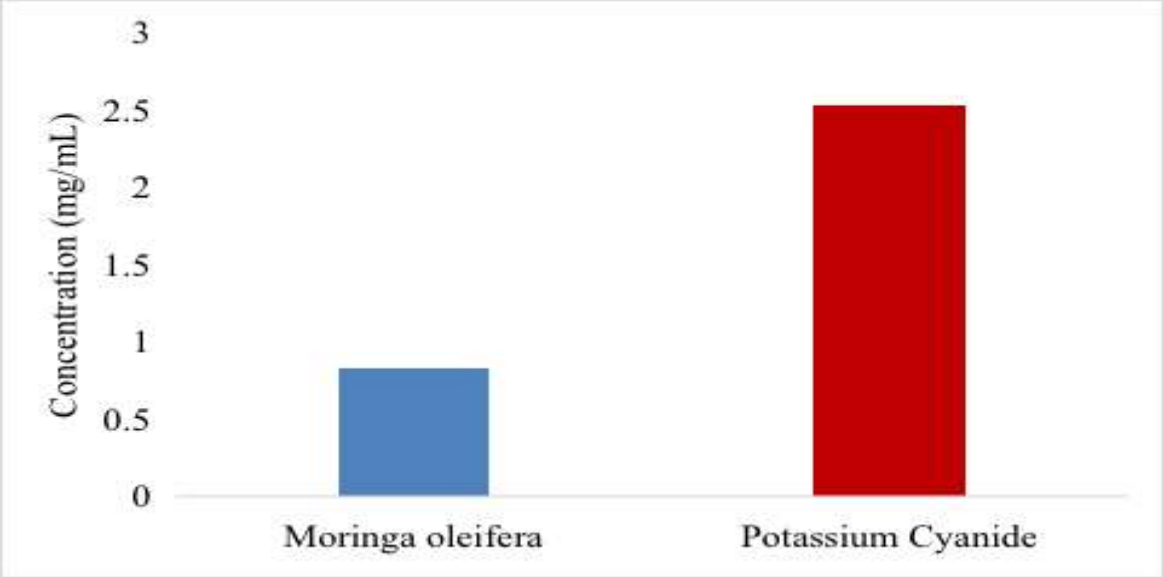

Figure 2: Comparative Analysis of the Concentration of Cyanide in *Moringa oleifera* Root and Potassium Cyanide (Standard)

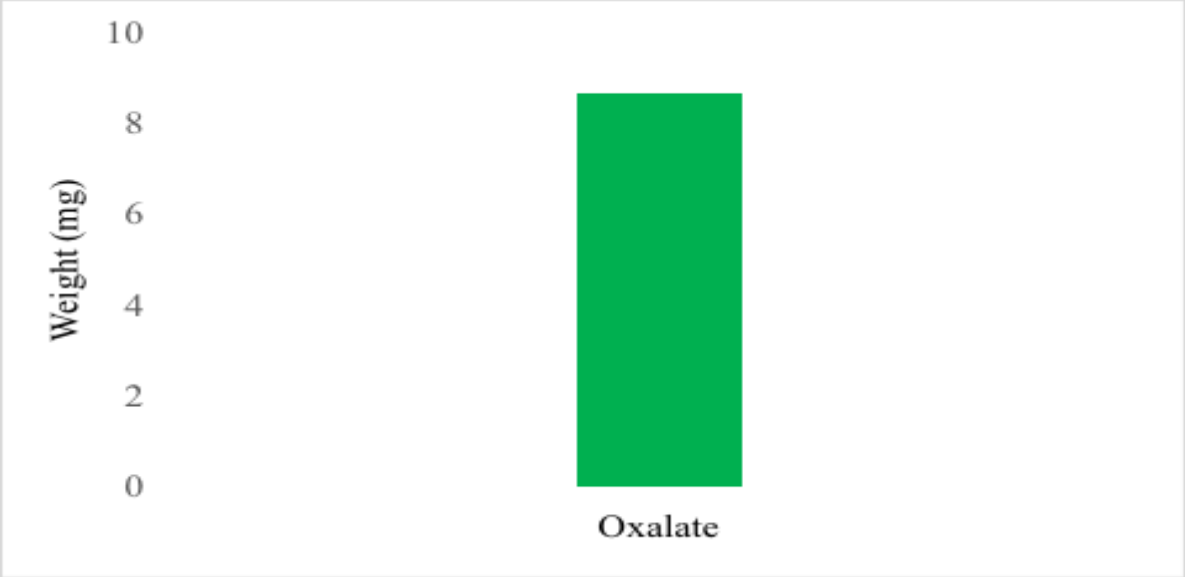

Figure 3: Oxalate Content in *Moringa oleifera* Root

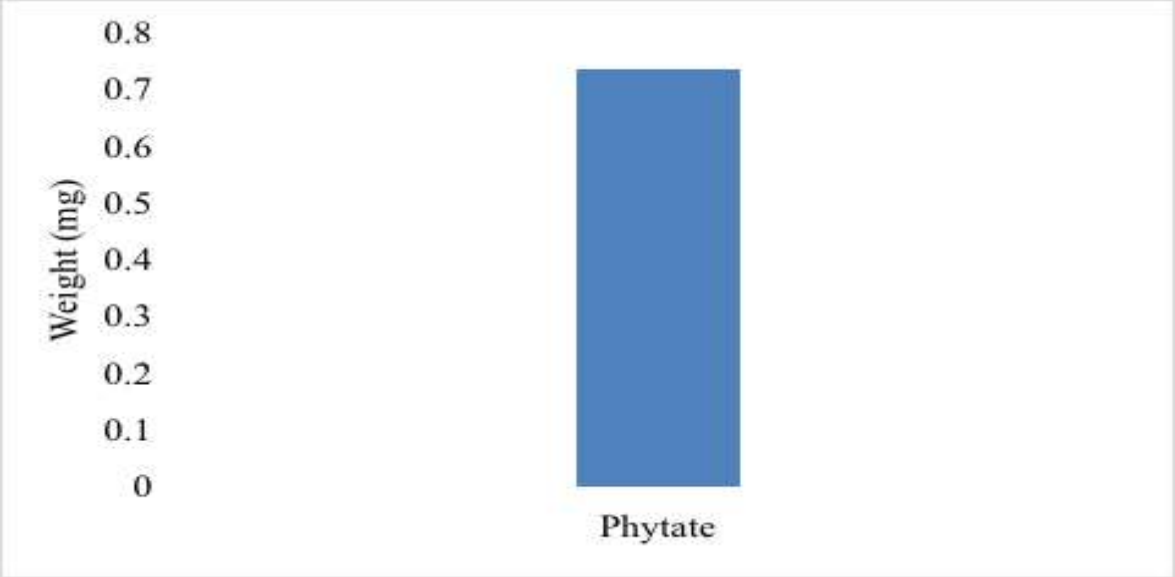

Figure 4: Phytate Content in *Moringa oleifera* Root

CHAPTER FOUR

4.0 DISCUSSION

High levels of some antinutrients e.g oxalate and phytates in human food are considered undesirable. High level of oxalate, decreases Cu bioavailability by binding with it to form insoluble salt (Umar *et al*., 2007).

The phytate content of *Moringa oleifera* Lam. root is 0.74255mg/100g, the results were very low is compared to 1326 mg/100g reported for Amaranthus viridis (Umar *et al*., 2007) and 1214 mg/100g for Tribulus terestris leaves (Umar *et al.*, 2007). This showed that the phytate content in *M. oleifera* Lam. would not cause any nutritional problems when consumed..

The formation of calcium-zinc-phytate complexes in the intestines were as a result of inhibitory effect of calcium over zinc. Consumption of animal proteins in Africa is so low that it may not significantly alter the zinc bioavailability. Undesirable levels of oxalate and phytates as recorded by (Borquaye *et al*., 2017). Oxalates cause nephrotic lesions in the kidney (Kumar Jha *et al.*, 2013). Anti-nutrients like tannins, oxalate, and phytates may be harmful when consumed in unrefined food. Presence of plants with some anti-nutritional content like cyanogenic glycosides and oxalates may reduce the bioavailability of the essential nutrients in plant foods. When soluble oxalate content is present in higher amount in the body, it blocks the absorption of soluble calcium ions because the oxalate and the calcium ions bound together to form insoluble calcium oxalate complexes (Kumar *et al*., 2013). Consequently, people were advised to avoid food that are rich in oxalate as it has a propensity to cause the formation of kidney stones (Kumar Jha *et al*., 2013).

According to Makkar *et al* in 1996, *M. oleifera* Lam. has been found to contain varying levels of cyanide and oxalate compounds. Research has shown that the cyanide content in *M. oleifera* Lam. roots ranges from 0.003% to 0.15% of the plant's dry weight. In comparison,

Vernonia amygdalina, also known as bitter leaf, has been reported to contain higher levels of cyanide, ranging from 0.2% to 0.5% of the plant's dry weight .

On the other hand, the oxalate content in *M. oleifera* Lam. roots have been found to range from 0.3% to 1.3% of the plant's dry weight (Abuye *et al*, 2017). In contrast, Vernonia amygdalina has been reported to contain lower levels of oxalate, ranging from 0.1% to 0.3% of the plant's dry weight (Okwu, 2004).

Other plants, such as Cassava (*Manihot esculenta*) and Sorghum (*Sorghum bicolor*), have also been found to contain significant levels of cyanide and oxalate compounds. For example, Cassava has been reported to contain up to 1.4% cyanide and 2.5% oxalate in its roots (Montanac, 2009), while Sorghum has been found to contain up to 0.6% cyanide and 1.2% oxalate in its grains (Selle, 2017).

4.1 CONCLUSION

Anti-nutrients in *Moringa oleifera* Lam., such as oxalates, phytates and cyanide have important benifts and roles to play in the lives of humans; regulating calcium levels, potentially reducing the risk of kidney stones, helping to fight againt stress and free radicals in the. When consumed moderately, these anti-nutrients can provide valuable health benefits.

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