CHEMICAL ANALYSIS OF *Moringa oleifera* (LAM), *Detarium microcarpum* (GUILL AND PERR) AND *Bauhinia monandra* (KURZ) SEEDS FOR NUTRIENT AND ANTINUTRIENT FACTORS

BY

ANHWANGE Benjamin Asen
(M.Sc/Sci/31599/01-02)
B.Sc (Hons) Chemistry, (Ahmadu Bello University, Zaria) 1993.

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Department of Chemistry
Faculty of Science
Ahmadu Bello University, Zaria

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DECLARATION

I declare that this work is wholly the author's and has not been part of any presentation for a higher degree.

Date:----------
Signature
Anhwange Benjamin Asen.

CERTIFICATION

This thesis entitle "Chemical analysis of *Moringa oleifera, Detarium microcarpum* and *Bauhinia monandra*" seeds for nutrient and anti-nutrient factors by ANHWANGE Benjamin Asen, meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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Dr. V.O. Ajibola **Date**
Chairman Supervisory Committee

---------- ----------

Dr. S.J. Oniye **Date**
Member Supervisory Committee

---------- ----------

Dr. E. J. Ekamen **Date**
Head of Department

---------- ----------

Dean, Postgraduate School **Date**

DEDICATION

This thesis is dedicated to my daughter Msughshima Anhwange.

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TABLE OF CONENTS

		Page
	Title page……	
	Declaration ……	ii
	Certification……	iii
	Dedication……...	iv
	Acknowledgement……..	v
	Table of Contents……	vii
	List of Tables……..	xi
	List of Figures…….	xii
	Abstract……...	xiii
CHAPTER ONE		
1.0	Introduction…….	1
1.1	Background Study ……..	1
1.2	Evaluation of Plants Nutrients……	2
1.3	Statement of Problem ……..	3
1.4	Aims and Objectives……	5
1.5	Justification for the Study……	5
CHAPTER TWO		
2.0	Literature Review……	6
2.1.0	*Moringa oleifera* (Lam) ……..	6
2.1.1	Morphology of *Moringa oleifera*…….	6
2.1.2	Uses of *Moringa oleifera*…….	7
2.2.0	*Bauhinia monandra* (Kurz) ……..	8

2.2.1 Morphology of *Bauhinia monandra*…… 8

2.2.2 Uses of *Bauhinia monandra*…… 8

2.3.0 *Detarium microcarpum* (Guill and Sperr)……... 9

2.3.1 Morphology of *Detarium microcarpum*…….. 9

2.3.2 Uses of *Detarium microcarpum*…….. 9

2.4 Proximate Analysis……. 10

2.5 Moisture Content of Seeds…… 11

2.6 Ash Content of Seeds…… 11

2.7 Organic Matter Content of Seeds……. 12

2.8 Mineral Content of Seeds……. 12

2.9 Lipids Content of Seeds…… 13

2.10 Protein Content of Seeds…….. 14

2.11 Amino acid Content of Seeds……. 15

2.12 Carbohydrate Content of Seeds…… 15

2.13 Anti-nutritional Factors in seeds…… 16

CHAPTER THREE

3.0 Materials and Methods…… 20

3.1 Sample Collection and Preparation……. 20

3.2 Reagents…….. 20

3.3 Determination of Moisture Content…….. 25

3.4 Determination of Ash and Organic Matter Content…… 25

3.5 Elemental Analysis…….. 25

3.5.1 Elemental Analysis by X-Ray Fluorescence Spectrometry……. 25

3.5.2 Determination of Sodium and Magnesium by AAS……. 27

3.5.3 Determination of Phosphate-Phosphorus……. 28

3.5.4 Determination of Sulphate-Sulphur……. 29

3.6 Determination of lipid Content……. 30

3.7 Determination of Crude Protein…… 30

3.8 Determination of Soluble Carbohydrate…….. 31

3.9 Determination of Crude Fibre…….. 32

3.10 Determination of Hydrogen Cyanide…… 32

3.11 Determination of Phytate…….. 33

3.12 Determination of Tannins……. 33

3.13 Determination of Saponins…… 34

3.14 Determination of Chemical Properties of the Oils…… 35

3.14.1 Determination of Iodine Value ……. 35

3.14.2 Determination of Acid Value……. 35

3.14.3 Determination of Free Fatty Acid…….. 36

3.14.4 Determination of Peroxide Value…….. 36

3.14.5 Determination of Saponification Value……. 37

3.14.6 Determination of Ester Value…… 37

3.14.7 Extraction of Fatty Acids from the Oils…… 37

3.14.8 Spectrometric Determination of the Oils and Fatty Acids…… 38

3.15 Determination of Amino Acids……. 38

3.16 Statistical Analysis…… 40

CHAPTER FOUR

4.0	Results discussion and Conclusion ……..	41
4.1	Results …….	41
4.2	Discussion……..……..…	51
4.3	Conclusion……	64
4.4	Recommendation…….……	64
	References ……..	66

LIST OF TABLES

Table 3.0	Operation parameters for the determination of sodium and magnesium by Atomic Absorption Spectrometer……	28
Table 4.0	Moisture, ash and organic contents of the seeds……	41
Table 4.1	Elemental composition of the seeds ……	42
Table 4.2	Nutritional and anti-nutritional content of the seeds……	43
Table 4.3	Chemical properties of the seeds oils……	43
Table 4.4	Amino acids content of the seeds…….	44

LIST OF FIGURES

Fig. 4.1	Infra red (IR) spectra of *Moringa oleifera* oil ……	45
Fig. 4.2	IR spectra of *Detarium microcarpum* oil ……	46
Fig. 4.3	IR spectra of *Bauhinia monandra* oil …….	47
Fig. 4.4	IR spectra of *M. oleifera* fatty acids ……	48
Fig. 4.5	IR spectra of *D. microcarpum* fatty acids ……	49
Fig. 4.6	IR spectra of *B. monandra* fatty acids ……	50

ABSTRACT

The seeds of *Moringa oleifera* Lam (family: Moringaceae), *Detarium microcarpum* (family: Caesalpiniodeae) and *Bauhinia monandra* (family Caesalpiniaceae) were analyzed for mineral elements, nutritional and anti-nutritional contents, and the chemical properties of their oils determined. The concentrations (ppm) of the essential elements potassium, calcium, magnesium sodium, sulphur, phosphorus and iron ranged between 77400-1190 ppm for *M. oliefera,* 105000-220 ppm for *D. microcarpum*, and 77900-1594.33 ppm for *B. monandra.* The concentrations (ppm) of the essential trace elements – iodine, copper, manganese, chromium molybdenum, selenium and zinc also ranged between 3930-11.10., 2780-35.20 and 4150.00-11.4 (ppm) for *M. oleifera, D. microcarpum* and *B. monandra* respectively. The non-essential elements (arsenic, lead, tin, nickel, bromine, vanadium, cobalt, thallium and niobium have concentrations ranging between 1100.0-108, 1960-17.4, 1690-10.70 ppm. for *M. oleifera, D. microcarpum* and *B. monandra* respectively. The protein and lipid contents were highest in *Moringa oleifera* (40.19 and 41.58% respectively). *D. microcarpum* contained 35.94 and 11.24% protein and lipid content respectively. Carbohydrate was highest *in D. microcarpum* (42.20%) followed by *B. monandra* (21.40%) and *Moringa oleifera* 9.11%. *Bauhinia monandra* contained the highest content of phytate (11.57mg/100g) followed by *Moringa oleifera* 10.18(mg/100g), while *Detarium microcarpum* had 5.25mg/100g. The hydrogen cyanide content in the seeds were (0.58, 0.25, 0.32 mg /100 g) for *M. oleifera, D. macrocarpum and B. monandra* respectively. The tannin and Saponins content in *Bauhinia monandra* were 6.0 and 2.05%; *Detarium microcarpum* had 4.75 and 0.20% while *Moringa oleifera* had 2.13 and 2.25% respectively. The iodine value of the oils of

M. oleifera, *D. microcarpum and B. monandra* were 59.48, 58.02 and 56.60 respectively. Saponification value of *M. oleifera, D. microcarpum* and *B. monandra* oils were 197.57, 220.66 and 196.12 g / KOH respectively. The acid value, free fatty acid and peroxide value were low (less than 9.0). The ester values of the oils were 173.15, 212.54 and 189.35 for *M. oleifera, D. microcarpum* and *B. monandra* respectively. The essential amino acids (lysine, phenyalanine leucine, isoleucine, methionine, valine, cystine and threonine content ranged from 5.74-1.0, 2.58-0.75, 3.77-1.11 (g/100g protein) for *M. oleifera, D. microcarpum* and *B. monandra* respectively. The non-essential amino acid were in various concentrations ranged from 14.43-2.09, 9.78-1.06, 11.75-2.36 (g/100g protein) for *M. oleifera, D. microcarpum* and *B. monandra* respectively. The high elemental, proteins, lipids, carbohydrates and amino acids composition of the seeds suggest that they could serve as supplementary sources of nutrients to man and livestock, provided the anti-nutritional content of the seeds are reduced or eliminated by further processing.

CHAPTER ONE

INTRODUCTION

1.1 Background Study

The science of nutrition deals with the study of food constituents and their effects on the body. Food constituents refer to food nutrients required to promote and sustain good health as well as restore health in times of illness. These are chemical substances found in food and can be divided into two major groups, macro and micronutrients. The macronutrients are proteins, carbohydrates and lipids, while the micronutrients are the minerals and vitamins. All these nutrients work together to build and maintain a healthy body, protect it from diseases as well as restore it to good health (Barke, 1996). It is a known fact that food does not guarantee good health; it is adequate nutrition that plays a vital role in achieving optimum health. Other factors include environmental conditions, mental health, lifestyle, heredity, diseases, education and economic status (Rao, 1994). The effects of inadequate nutrition practices are easily observed by the physical appearance of hair, skin, eyes and body weight changes. While over nutrition leads to overweight, protruding stomach, double chin and skin folds around the waist and neck. Hence adequate nutrient intake is a prerequisite for good health. (Lockett *et al*, 2000).

In most countries of Africa, including Nigeria, this prerequisite is not met owing to the neglect of the agricultural sector by the government, rising population and ban on importation of some cereals such as rice, corn e.t.c, resulting in limited food supply (Igbedioh, 1996). The consequence of this is hunger, malnutrition and increase in dietary diseases, especially for the vulnerable population groups such as children and pregnant women (Sadik, 1991). Therefore, the exploitation of the neglected and lesser-known

plants of natural bushes and forest may be one approach to attain good health and in general food security (Rao, 1994).

1.2 Evaluation of Plant Nutrients

The analysis of nutritional value of plant food materials has attracted contemporary scholarships because of the role they play as major constituents of necessary food required for the growth and survival of living organisms. Nutritional and dietetic treatment of diseases as well as research into the problem of human nutrition demand for the knowledge of chemical composition of food. Information about plant food materials are of nutritional significance in assessing the particular plant that would be given special consideration with regards to propagation and cultivation, to meet the needs of the increasing population (Rao, 1994).

The result of analysis of some edible plants of Botswana, revealed that, one hundred and fifty wild edible plants that have high nutritional value. Some of the species include *Bauhinia, Grewia, Scherocarya, Ximenia, Strychnos, Cereopegia, Ochna, Detarium* and *Silla;* but land use system are altering the distribution and abundance of such food plants (Campbell, 1986). According to Baumer (1995) over two hundred trees and shrubs of West African origin can produce products that have high nutritional value. Smith (1996) reported that wild species of some plant fruits, leaves, nuts and seeds are of high minerals. The investigation of the nutritional role of some wild plants of Niger origin (the species include *Moringa oleifera, Ximenia americana, Amaranthus viridus, Corchorus tridens, Habiscus sabdarifa, Maeria crassifolia and Leptadema hastate)* showed that all the plants contained significant amount of essential nutrients, like amino acids, fatty acids and trace minerals (Smith,1996). These results reinforced the growing

awareness that wild edible plants of the Western Sahel contribute useful amount of essential nutrients to the human diet (Freiberger, 1998). The result of the analysis of eight wild plants from this same region lead to the conclusion that the inhabitants of the Western Sahel should be encouraged to devise healthy diets from these plants especially when cereal staples are in shot supply (Sena 1998).

Quite a number of plant food consumed by some communities are not nutritionally useful because of toxicants occurring naturally or artificially in them. The presence of such toxins could pose great hazards to the health and well being of the consumers. For example, high tannin in diet adversely affect digestibility of protein and carbohydrate, and reduces growth, feeding efficiency, metabolisable energy and bioavailability of amino acids (Chang, 1994; Sotelo, 1995a). Kamalu (1995) and Jansz (1997) stated that dietary cyanide can damage the nervous system and the thyroid gland, other effect include pancreatic diabetes, vitamin B_{12} deficiency, decrease in iodine intake, liver inflammation and hemorrhage. Kidney, adrenal, myocardial and testicular lesion are also associated with hydrogen cyanide containing foods. (Abaye, 1998). Furthermore, people who depend on high phytate diet are deficient in phosphorous, calcium, zinc, manganese, iron and magnesium, due to the binding of phytic acid to these elements, and their conversion to indigestible phytates making them unavailable, thus resulting in disease conditions (Sotelo, 1995b).

1.3 Statement of Problem

In developing countries like Nigeria, food scarcity is worsening due to increasing population, the Sahelian drought, high prices of fertilizers, restriction on the importation of foods, lack of agricultural inputs, poor loan facilities, inadequate incentives to farmers

and unstable government policies on agriculture are responsible for food shortages (Igbedioh, 1996). As a result, farmers barely produce what they need to eat with little or nothing to sell. In addition, many people cannot afford the cost of animal proteins and therefore, depend solely on carbohydrates diets. The consequences are high incidence of hunger, malnutrition and increase in dietary related diseases (Barker, 1996). In this situation, the most vulnerable groups are the children and women, especially pregnant and lactating women (Sadik, 1991). Predictions of future food needs based on the current rate of population increase and unstable political atmosphere has further compounded the problem of malnutrition and food insecurity. (Sadik, 1991).

There may be no immediate solution to the problem of food security until an interdisciplinary approach to solving the problem is achieved (Ezeagu *et al.* 1996). Therefore information on new food sources will be of value. While measures are taken to boost food production by the conventional agriculture, a lot of interest is currently being focused on the possibility of exploiting the vast numbers of unconventional plants resources that exist in the wild (Rao, 1994). Many of such plants have been identified, but lack of data on their chemical composition has limited the prospects for their utilization (Baumer, 1995). Some reports on proximate analysis of some wild fruits and seeds indicate that they could be good sources of nutrients for man and livestock (Ezeagu *et al*., 1996), without taking into account of the trace and non-essential minerals, chemistry the oils, amino acids and anti-nutritional component of the seeds.

1.4 Aims and Objectives of the Study

This study is aimed at analyzing the seeds of three plants, *Moringa oleifera* (Lam), *Detarium microcarpum* (Guill and Perr) and *Bauhinia monandra* (Kurz). The objectives are:

a. to determine the essential, trace and non-essential minerals of the seeds.

b. to determine the quality of the oils from the seeds.

c. to determine the protein and amino acids contents of the seeds.

d. to determine the soluble carbohydrate of the seeds and.

e. to determine anti-nutrient contents of the seeds.

1.5 Justification for the Study

The study is expected to provide information on the nutritional values of the seeds, which may serve as an alternative sources of nutrients. The lipids analysis will give an idea of the types and quality of oils in the seeds; and whether the oils can serve as alternative sources of energy for man and livestock. The amino acids analysis may be used in supplementing the essential amino acids needed by man and livestock. The elemental analysis will give insight to the availability of essential and trace minerals lacking in some other food products. The anti-nutritional analysis will help determine whether the concentration of anti-nutritional factors in the seed are more than man and animal threshold levels.

CHAPTER TWO

LITERATURE REVIEW

2.1.0. *Moringa oleifera* (LAM)

Moringa oleifera belongs to a monogeneric family of shrubs and trees called moringaceae. It local names includes 'Zogallangandi' (Hausa), 'Okwe' (Igbo) and 'Ewe' 'Igale' (Yoruba) (Dalziel, 1955). It is called 'Jeelagede' by the Tiv people. The tree is considered to originate from Agra and Oudh in northwest region of India, south of the Himalayan Mountain (Odee, 1998). It is now cultivated throughout the Middle East and most of the Tropical belt (Lowell, 2001). The tree was introduced to East Africa from India at the beginning of the 20th century. It has now spread to almost every part of Africa since it is a drought resistant tree. (Keay, 1989).

2.1.1. Morphology of *Moringa oleifera* (LAM).

According to Morton (1991), *Moringa oleifera* is a fast growing perennial tree that can reach a maximum height of 7.12 metres and a diameter of 20-40cm at chest height. The stem is straight but occasionally poorly formed. The stem reaches a height of 1.5-2 metres before it begins branching but can reach up to 3m. The branches grow in disorganized manner and the canopy is umbrella shaped. The alternate, pinnate leaves grow mostly at the branch tips. They are 20-72cm long, grayish-downy when young, long petiole with 8-10 pairs of pinnae each bearing two pairs of opposite elliptic or ovate leaflets one at the apex, all 1-2cm long; with gland at the bases of the petioles and pinnae. The flowers have pleasant fragrance and are 2-5cm wide producing profusely in auxiliary drooping panicles of 10 to 25cm long. They are white or cream coloured and yellow dotted at the base. It has juicy sepals and petals. The fruits are three lobed pods which

hang down from the branches and are 20 to 60cm in length. Each pod contains 12 to 35 seeds. The seeds are round with a brownish semi-permeable seed hull. The hull has three white wings that run from top to bottom at 120-degree interval. The average weight per seed is about 0.3g and the kernel to hull ratio is 75.25 (Odee, 1998).

2.1.2. Uses of *Moringa oleifera* (Lam).

The common use of the tree is as fence around compound and gardens. The leaves, fruits, flowers, roots and immature pods are edible and they form part of traditional diets in many countries of the tropics and sub-tropics (Odee, 1998). In Nigeria, apart from soup making, the leaves can be used in making salad in combination with groundnut cake. The seeds can be ground to powder and used for seasoning. The oil from the seed is pale yellow and has a sweet smelling taste (oil of Ben or Benhen). It is almost Odourless and possesses a blend appreciable taste. The oil is edible and suitable for perfuming and hair dressing (Makkar, 1997). The residual meal cake contains an alkaloid and saponin like body which causes frothing when its meal is taken. It is a suitable fertilizer but not as feedstuff. Though can be given to animals when pre-treated. The cake may be used for water treatment (Lowell, 2001). The leaves for scrubbing utensils and cleaning walls. In Sudan, the tree is planted on graves in the belief that it keeps away hyenas and its branches are used as charm against witchcraft (Folkard, 1996).

The medicinal values of the tree are:- the leaves are used for the treatment of fever, catarrh, gonorrhea, headache, blood pressure and anxiety. The root, bark and steam are used to treat epilepsy, nervous debility, hysteria, kidney pains, asthma and snake bite. While the seed and seed oil are used against fever, back pain, rheumatism and bladder troubles (Morton, 1991; Lowell, 2001).

2.2.0 *Bauhinia monandra* (Kurz)

Bauhinia monandra belongs to the caesalpiniaceae family of shrubs that is well known as ornamental trees found in tropical West Africa and India. The trees are grown around Lake Chad region. The tree is called 'Jiga or Jirga' (Hausa), 'Obafe' (Yoruba), 'Okpo-atu' (Igbo) and 'Nyihar' (Tiv) (Dalziel, 1995).

2.2.1. Morphology of the *Bauhinia monandra* (Kurz)

The tree is about eight metres in height with a spreading crown with long slender shoots drooping at the ends. The bark are light gray, conspicuous brown lenticels, fibrous when old slash spines and very pointed spines up to 10cm long (Hutchinson, 1967). The leaves are 1- 2cm long by 1-2.5cm broad and so deeply lobed as to form practically two leaflets each. The tree nerved from the base are round at apex, light blue-green or grey-green, glabrous; the veins forming a conspicuous open network. The leaf–stalk is slender and is 6-12 cm long. The flowers are white with one large anther elongated sharply pointed, the pods split open explosively.

2.2.2. Uses of *Bauhinia monandra* (Kurz)

The tree is a decorative and is grown for its seed. The leaves and pods are eaten as vegetable in China and India while in Africa the pods and seeds are sources of black and blue dyes (Hutchinson, 1967). The fruits are eaten by both human and animals. A drink is made from the pods when pounded and boiled in water. The bark of the tree is used for binding and the fibre is used for cordage in Sudan. The tree yields a gum used in India for edible and other purposes (Irvine, 1961).

Medicinally, is used as an astringent for diarrhoea and dysentery. It is believed to cure leprosy (boiling the root or bark) and small pox. The roots are used for fever and

leaves for eye troubles (Shahina, 1989). In addition, waist bam can be made from the bark. When the leaves and the pods are boiled and mixed with corn porridge and butter, it is believed to be a magic power against spear, knife or arrow! It is called 'maganin k'arfe' in Hausa (Keay, 1989).

2.3.0. *Detarium microcarpum* (Guill and perr)

Detarium microcarpum belongs to the family caesalpiniodeae. It is found mostly in the Savannah forest. The tree is called 'Taura' (Hausa), 'Ofo' (Igbo), Ogbogo (Yoruba) and 'Agalyem' (Tiv) (Dalziel 1955).

2.3.1. Morphology of *Detarium microcarpum*.

The tree grows to a height of about 10m, usually with twisted trunk and branches. Leaflets are 2-5 pairs, pale green, elliptic apex sometimes retuse, base oblique. The flowers are white, fragrant in dense panicles. The flowers are sub sessile with four sepals and no petals. The orbicular fruit (3.175cm in diameter) is dark brown and it is brittle when dry and is sweet and edible. The tree is wide-spread in Tropical West Africa in dry Savannah habitat (Irvine, 1961).

2.3.2. Uses of *Detarium microcarpum*

The fruit is edible. A sweet meal is sometimes prepared from it together with the fruits of *Vitex doniana* and *Diospyros mespiliformis* (Irvine, 1961). What the Hausas called 'madi' it is a common believe by the Hausa people that too much eating of the fruit causes vertigo (i.e. feeling of dizziness, fear and loss of balance). On the other hand, the small absorption fruit of quite different shape probably galls are produced by the tree are poisonous to persons who may inadvertently eat them when hungry (Keay, 1989). Shahina (1989), reported that, the kernels of the seeds are deep purple brown and are oily

and edible. The Nupe people bake the seeds into cake as foodstuff for cattle. The seed are used as scented necklaces and waist girdles. The burning of the seeds drive away mosquitoes; while the ash is employed in the preparation of snuff by the Liberians (Irvine, 1961).

The medicinal values of the tree are:- The bark is haemostatic and is used to heal deep cut. In Liberia, the bark decoction is used in parturition, and has quick effect in cases of retained placenta. A cold infusion of the bark, root and wood is used in Senegal as restorative for weakness and anaemia. The fruit is used as balm for chronic backache or tuberculosis of the spine. The fruit is a remedy for chest diseases in parts of West Africa (Dalziel, 1955). In Northern Nigeria, the fruits with other fruits are used as a toxin and a stimulant on a journey. The young soft seeds are usually given to people wounded by poisoned arrow (Irvine, 1961).

2.4. Proximate Analysis

In foodstuffs and agricultural products, the useful analysis is that which assess the bulk (proximate) components rather than the individual compounds. The division of food into lipids, carbohydrates and protein account for all the organic dry matter content. A typical proximate analysis include the extraction of crude lipids, protein and fibre, the rough equivalent of carbohydrates and mineral ash in plant materials. The moisture and organic contents are included (Madubuike *et al.*, 1994). Proximate analyses are the steps in search of the nutritive value of plants (Rao, 1994). Since all information on new sources of food are of value in dealing with nutritional problems, many of such analyses have shown that some wild plants could be good sources of nutrients (Badifu, 1993; Ezeagu *et al*., 1996). Most of the parameters used in the analysis of the nutritional value

of these plants are moisture, ash, organic matter, carbohydrates estimation, lipid and protein content.

2.5. Moisture Content

Moisture is the water present in feed. It gives the dry matter, quality, viability and shelf-life of the food. Plant seeds contain moisture held by the connective tissue forming a mixed medium (Madubuike *et al.,* 1994). The determination of moisture in food is important for its storage purposes. The safe limit is 15%, when this is exceeded, the storage of the food becomes very difficult are it may develop moulds and fungi on storage (Sotelo *et al.*, 1995b). A knowledge of the moisture content is needed for the optimum processing of food, for example milling of cereals, mixing of dough to optima consistency and for producing bread with the best grain, texture and freshness retention (Obizoba, 1994). The moisture, content on a number of plant fruits and seeds have been reported. Nour (1980) and Obizoba (1994), reported that Baobab fruits contain an average moisture content of 6.7%. Similarly Okoh (1984), reported that the edible parts of *Adansonia digitata*, *capsicum annum* and *Hibiscus esculentus* contained an average moisture content of 5%. The result of moisture content of the seeds of *Millettia thonningii, Gliricidia sepium, Conchocarpus sericeus, Albiza zygia*, *Daneia ogea* and *Afzelia bella* are 4.71% 6.77%, 5.49%, 7.80% 9.86% and 6.81% respectively (Ezeagu *et al.,* 1996).

2.6. Ash Content

The ash content is total mineral. The ash content of some plants *Millettia thonningii* (3.70%), *Glycine max* (4.18%) *Diosyro mespiliformis* (2.89%) *Albiza zygia* (2.8%), *Afzeliu bella* (2.97%) and *Entandrophragma angolense* (2.91%) were reported .

Nour (1980) reported the ash content of *Adansonia digitata* as 5.3%. The ash content of *Mucuna tampe* seed was reported as 3.0% by Handajani (2001). Nielsen *et al.* (1996) reported a high value for soyabean (*Glycine max* L) (5.9%) while peanut (*Arachis hypogaea* L), has 3.0% as its ash content.

2.7. Organic Matter

The organic matter content of a plant material gives its value of the total lipids, proteins and carbohydrate. It is a measure of the nutritional value of the material (Ezeagu *et al.*,1996). Organic matter is determined by subtracting the ash content from 100%. This means that for any material that the ash content has been given, the organic matter can be obtained.

2.8. Mineral Content of Seeds

Several mineral elements are essential to both plants and animals. Mineral elements have three broad functions in the body, (a) they constitute the structural components of body organs and tissues, in this group we have calcium, phosphorus, magnesium and fluorine in bones and teeth; phosphorus and sulphur in muscle proteins. (b) they are constituent of body fluids and tissues, as electrolytes concerned with the maintenance of osmotic pressure, acid-base balance, membrane permeability, muscle irritability and oxygen transport (Smith *et al*., 1996). These include sodium, potassium, chloride phosphorus, calcium and magnesium in blood, cerebrospinal fluid and gastric juice, and iron in the blood (Eromosele, 1991). (c) they act as catalysts in enzymes and hormone systems, forming the integral and specific components of metalloenzymes or less specific activators of these enzymes (Reddy and Lone, 1999). Eromosele (1991, 1993), examined the mineral content of some fruits of wild plants such as *Sclerocarya*

birria, and *Adansonia digitata*) and reported high concentrations of calcium and phosphorus; while the iron contents were 2 to 5 times higher than the values for common fruits. Smith *et al.* (1996), analyzing for Cu, Fe, Mg, Mn and Zn concentrations in wild and cultivated fruits and seeds of southern Burkina Faso and Niamey, in Niger Republic. Compared them with imported, exotic reference goods reported that the wild plants contained higher mineral contents. Glew *et al.* (1997) working on *Hibiscus esculentus* seeds reported that they are excellent sources of zinc, but that *Adansonia digitata* and *Bombax constatum* seeds have higher calcium concentrations.

2.9. Lipid Content of Seeds

Fats and oils constitute a high percentage of most seeds composition. They are high sources of energy to plants and animals. They supply the body with the essential fatty acids, fat soluble vitamins and serve as a protective layer on the surface of many body organs (Close and Menke, 1996). The seeds of *Sclerocarya birra* contain about 11.0% oil (Ogbobe, 1992). Chen *et al.* (1998) stated that Torreya seeds in China contained about (42.67 – 54.39%) oil, made up of linoleic acids and oleic acid, thus making the oil a high-quality oil. Zygodlo *et al.* (1994) studied the oil content of the seeds of *Oenothera picensis*, *Oenothera indicora, Ludwigia longifolia* and *L. peruviana* (onagraceae) and reported that they contain 18.3, 16.4, 13.9 and 10.1% oil respectively. The chromatographic analysis of the oil showed high levels of linoleic acid. Physio-chemical properties of the seed oils of some wild plants (*Lophira alata, Lophira lanceolata, Sclerocarya birra* and *Bliglia sapida*) of Nigerian origin showed that the iodine values of the oils did not exceed 88 but that their saponification values were in the range of 157-261mg / KOH. The peroxide value showed little changes under the

conditions of darkness and refrigeration (Eomosele, 1993). Dufaunel *et al.* (1999) reported that 80% of oil can be extracted from sunflower seeds using twin-screw extruder. This oil has an acid value below 2mg / KOH/g and high pressing temperature and low moisture content. The analysis of lipids from hazelnuts (*Corylus avellana* L) grown in New- Zealand revealed that, monounsaturated oleic acid content was 73.8% to 80.1% of the total fatty acids, while tocopherol content was 225.8 to 552.0mg/g. The oil was stable to oxidation. Ezeagu *et al.* (1996) reported the crude lipid content of *Glycine max* as 27.77%, *Entandophragnia angolense* 59.3%, *Millettia thonningii* 30.66%, *Lonchocarpus sericeus* 34.15% and *Afzelia bella* 23.17%. Wang *et al.* (2000) reported that oil from *Ginkgo biloba* contained 10.6% myristic acid, 4.1% palmitic acid, 1.9% stearic acid, 1.1% oleic and 0.4% linoleic acid. Ibiyemi *et al.* (1995) reported that there is no significant difference in the five parameters (iodine value, acid value, peroxide value, saponification value, and polar compound) of *Thevetia peruviana* seed oils.

2.10. Protein Content of Seeds

Proteins are described as substrates of the first order in the metabolism of every living organism (Ruiz and Setelo 2001). Their functions in the body include the supply of amino acids for growth or body-building, maintenance and replacement of worn-out or damaged tissues. The amino acids also help in the formation of body enzymes, hormones, antibodies and production of energy (Pellett, 1996). Proteins occur as organic compounds in plants, but differ in elementary composition. In addition to carbon, hydrogen and oxygen, they also contain nitrogen. Protein forms the major constituent in most seeds (Pellett, 1996., Petzke, 1997). Baobab fruits contain about 2.6% protein (Nour, 1990). *Hibiscus esculentus* and *Parkiia biglobosa* seeds contain 20 to 37% protein (Glew, 1997).

The fruits and seeds of some desert plants can serve as sources of essential amino acids. Seeds of *Sclerocaryra birra* contain about 36.7% crude protein, while that of *Cambe abyssnica* contain about 50% protein (Ogbobe, 1992., Massoura, 1998). Analyzing for the protein content of cotton seed (*Gossypium* spp), groundnut (*Arachis hypogaea*) and soya bean (*Glycine max*) Close and Menke (1996) reported them as 51.5, 51.3 and 51.4% respectively. The protein content of peanut (*Arachis hypogaea)*, soya bean (*Glycine max*) were 29.3 and 53.4% respectively (Sen *et al.,* 2001). Ruiz and Setelo (2001) have reported the seeds of Mexican wild lupins can serve as a potential protein supply.

2.11. Amino Acid Content of Seeds

The quantity of protein in a given food is not its nutritive value but the quality of the amino acids, hence the quality of a given protein cannot be determined without referring to the amounts and types of essential amino acids present (Zarkadas *et al.*, 1997). The essential amino acids are not manufactured by the animal body, they are obtained from plant food materials. Wang (2000), separated fifteen different types of amino acids from matured seed of *Ginko biloba;* and lysine, aspartic acid, alanine, arginine, histidine, glutamic acid and isoleucine with concentrations of 0.28, 0.163, 0.133, 0.133, 0.123, 0.115 and 0.095% respectively. Ekandem *et al.* (2002) detected nine amino acids in mango (*Mangifera indica*) seeds, lysine, Histidine, phenylalanine, Threomine, cysteine, methionine, leucine, isoleucine and valine. Their concentrations were 5.76, 2.55, 0.06, 0.61, 0.01, 0.18, 1.01, 0.49 and 0.77 g/16gN respectively.

2.12. Carbohydrates

The term carbohydrate covers a broad spectrum of compounds ranging from simple monosaccharides and disaccharides to complex polysaccharides. Carbohydrates

are primary metabolites of animal kingdom. Over half of the food that animals consume is composed of carbohydrates. Apart from their nutritional and metabolic functions, carbohydrates are important natural sweeteners and raw materials of various fermentation products. Baobab fruit contains 23.3% total carbohydrate (Nour, 1980). *Sclerocarya birra* contained 17.2% carbohydrate (Ogbobe, 1992). Herog *et al.* (1994) reported that *Diosyro mespiliformis*, *Diosyro ogea*, *Afzeliu bella* and *Albiza zygia* have as high as 77, 74, 54, 50% carbohydrate in their seeds respectively. The difference in nitrogen retention of plant seeds influences the indigestible carbohydrate fractions of a seed Ezeagu *et al.* (1996). The total dietary fibre in *Prosopis africana, Lonchocarpus sericeus, Enterolobium cyclocarpum* and *Sesbania pachyearpa* were reported as 77.6, 32.9 57.6 and 55.5% respectively. Alvarado *et al.* (2001) reported that tomato seeds contain about 59.07g total carbohydrate. Peterbauer *et al.* (2002) reported that raffinose oligosaccharides are the soluble carbohydrate in seeds and other tissues of plants. The carbohydrate content of the seed of *Ginkyo biloba* contained 84% starch, 6.7% glucose, 4.2% fructose, 0.02% polysaccharide and 0.01% disaccharides. Mulberry seeds contain about 4.5% neutral sugar (Yeasmin *et al.,* 2001) while mango seed contain 62.12% starch (Ekandem *et al*., 2001).

2.13 Anti-nutritional Factors in seeds

Anti-nutritional factors are nutritional inhibitos and toxic substances associated with foodstuffs. They are classified as those naturally present and those due to contamination which may be of fungal origin or maybe related to soil and other environmental influence (Grela, 2000). These factors modify the nutritional value of the individual foodstuffs, and some have serious consequences. The frequently occurring

anti-nutritional substances in seeds and grains are phytates, tannins, saponins, hydrogen cyanides, digestic enzyme inhibitors, oxalate and alkaloids. (Grela, 2000).

Phytate represents a complex class of naturally occurring phosphorus compounds that can influence the functional and nutritional properties of foods. Phytic acid, myo-inositol 1,2,3,4,5,6 – hexakis (dihydrogen phosphate), is the main phosphorus store in mature seeds. Phytic acid has a strong binding capacity, readily forming complexes with multivalent cations and proteins. Most of the phytate metal complexes are insoluble at physiological pH. Hence phytate binding renders several minerals biologically unavailable to animals and man (Radositits *et al*. 1997). Mercedes, *et al*. (1999) determined the phytate content of some varieties of *Phaseolus vulgaris* and found that it ranged between 2.9 to 5.0g/kg. Mohammed *et al.* (1986) observed that soybean has a higher content of phytic acid (23.35mg/g) than the black-eyed pea (8.74mg/g). The phytic acid content of several varieties of sorghum had been reported to ranged from 170 to 380mg per 100g while that of millet varied from 172mg per 100g. Other foodstuff like Hard red wheat, refined corn bran, split peas and Brown rice are 68.8, 22.45, 16.79 and 15.55(mg/g) respectively (Camire *et al*., 1982). Adekunle, (2004) reported that the phytate content of some indigeous seed of Nigeria ranged between 3.27 to 0.17g/kg of dry matter. Chitral *et al*. (1995) state that the phytic acid content of soybean was 36.4mg/g, mung bean 12.0mg/g) and chickpea (9.6mg/g). Similarly, Grela *et al*. (2001), found that raw seeds of *Lathyrus sativus* contained 3.4g/kg of dry matter phytic phosphorus. Thompson, (1993) reported that the phytate content of soy bean meal was 3.2 to 3.8%, rape seed meal 6.0 – 7.3%, sunflower 6.2 to 9.2% and peanut meal 3.2 to 4.3%.

Tannins are polymers resulting from the condensation of flavan-3-ols. They are referred to as procyanidins because cyanidin was thought to be the sole anthocyanidin involved in seeds and grains tannins (Grela, *et al*., 2001). Tannins in diet impart astringent taste, which affect palatability, reducing food intake and consequently body growth. It binds to both exogenous and endogenous protein including enzymes of the digestive tract, affecting the utilization of the proteins (Chang, 1994; Jansz, 1997). The mean tannins content in the seeds of pea cultivars was 0.92mg/g and that of the unprocessed field peas varied between 1.8 to 2.1mg/100g (Zdunczyk *et al*., 1997). Grela, *et al*. (2001) reported an average tannin content in seeds of *Lathyru sativus* as 2.41g/kg. *Vitellaria. paradoxa* and *C. hitea* were 1.78 and 13.0g/kg dry matter respectively. Saponins are glycoside containing a polycyclic aglycone moiety of either C_{27} Steroid or C_{30} triterpenoid (known as Sapongenins) attached to a carbohydrate. They are found naturally in plants especially desert plants. They consist of a fat-soluble nucleus, having either a steroid or triterpenoid structure, with one or more side chains of water-soluble carbohydrates (Mercedes *et al.* (1999). They have a characteristic bitter taste, foaming properties, and can cause injuries to the digestive mucosa and haemolytic changes in blood (Ukpali, 2003). Ogbobe, (1992) reported the crude saponin in *sclerocarya birra* seeds as 0.9%. The average saponin content of *phaseolus vulgaiis* seed was 0.44g/kg (Mercedes *et al*., 1999). Duhan, (2001) reported the saponin content of pigeon pea cultivars as ranging between 2164 to 3494mg/g; while Ukpabi (2003), reported that the saponin content of *Napoleona imperialis* in Nigeria as 20%.

Hydrogen cyanide is a colourless liquid or gas with a faint, bitter almond odour. Cyanides are found naturally in foods, different varieties of fruits, seeds, vegetables and

grains (Ukpabi, 2003). Hydrogen cyanide diet can damage the nervous system and the thyroid gland, (Kamalu, 1995, Jansz, 1997). Thompson, (1993) stated that the various cassava roots, contained hydrogen cyanide content of over 100mg/kg while that of linseed meal was 250mg/kg, rape seed (65.5mg/kg) and *Camdina sativa* seed 4.4mg/kg. The hydrogen cyanide content of *Napoleona imperialis* seeds is 135mg/g (Ukpabi, 2003). Adekunle (2004) reported hydrogen cyanide content of some wild indigeneous seeds in Nigeria to range between 12.40 to 0.80g/kg of dry matter.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample Collection and Preparation.

The seeds of *Moringa oleifera, Detarium microcarpum and Bauhinia monandra* were collected from villages around Samaru-Zaria, Kaduna state. The seeds, flower, leaves and stem were deposited at the Herbarium in the Department of Biological sciences, Ahmadu Bello University, Zaria, for identification and filling. *Moringa oleifera* was given file number 571, *D. microcarpum* 900676 and *B. monandra* 1600.

The seeds were obtained by breaking the pods and removing the seeds coats. They were dried at $60^{o}C$ in an air-circulating oven, ground in porcelain mortar with pestle to fine particles and were stored in screw capped plastic containers. Chemical analysis were carried out on the ground samples.

3.2 Reagents.

All reagents used were AnalaR grade reagents. They were used without further purification and all solutions were prepared with distilled water.

- Concentrated Hydrochloric acid (sp. gr. 1.18) 35.4% v/v
- Concentrated Perchloric acid (sp. gr. 1. 54) 60% v/v
- Concentrated Sulphuric acid (sp. gr. 1.84) 98% v/v
- Concentrated Nitric acid (sp. gr. 1.42) 69-70.5% v/v
- Orthophosphoric acid 85.0% v/v
- Organic binder (10% styropore in toluene).
- 52% perchloric acid ($87cm^3$ of acid was diluted to 100 cm^3 with water).

- 0.25 mol/dm^3 H_2 SO_4 (13.32 cm^3 of acid was diluted to 1000 cm^3 with water).
- 0.5 Mol/dm^3 HCl (8.74 cm^3 of acid was diluted to 100 cm^3 with water.
- 2% HCl (5.65 cm^3 of acid was diluted to 100cm^3 with water).
- 6 mol/dm^3 HCl (524.27 cm^3 of acid was diluted to 1000 cm^3 with water).
- Petroleum ether (40-60^oC)
- Antidumping granules
- Carbon tetrachloride
- Chloroform
- Acetic acid
- 50% acetic acid (50 cm^3 of acid was diluted to 100cm^3 with water).
- Iodine crystals (resublimed).
- Potassium iodide (10%) w/v 10g of KI crystals was dissolved in water and diluted to 100 cm^3.
- Potassium iodide (5%) w/v 5g of KI crystals was dissolved in water and diluted to 100cm^3.
- Diethyl ether
- Ethanol 95% v/v
- Ethanol 20% (21.0 cm^3 of ethanol diluted to 100cm^3 with water.
- 0.1 mol/dm^3 sodium thiosulphate (13.21g of the salt was dissolved in water and diluted to 1dm^3).
- 0.1 mol/dm^3 potassium hydroxide (5.6g of KOH crystals was dissolved in water and diluted to 1dm^3).

- 1% phenolphthalein (1g of phenolphthalein crystals was dissolved in 50cm^3 of ethanol and diluted to 100cm^3 with distilled water).
- 0.5 mol/dm^3 alcoholic potassium hydroxide (28g of KOH crystals was dissolved in 20cm^3 of water and made to 100cm^3 with ethanol (95% v/v).
- Wijs reagent (0.2 mol/dm^3 ICl solution). 8.67g of iodine crystals was dissolved in 100cm^3 of methanol, 7. 96g of iodine trichloride (ICl_3) was dissolved in glacial ethanoic acid. The separate solutions were carefully warmed in a water bath, mixed in 100cm^3 volumetric flask and made up to mark with glacial ethanoic (acetic) acid and stored in dark brown bottle (Pearson, 1976).
- Potassium sulphate crystals
- Copper sulphate crystals
- 40% w/v sodium hydroxide (40g of NaOH pellets was water dissolved in water and diluted 100 cm^3).
- 0.312 mol/dm^3 NaOH. (12.48g of NaOH pellets was dissolved in water and diluted to 1000cm^3).
- Methyl red in indicator (100mg of methyl red was dissolved in 60cm^3 of ethanol and diluted to 100cm^3 with water).
- Starch solution 1g of starch dissolved in 10cm^3 of water and stirred. Boiling water was added to 200cm^3. The starch solution was heated until translucent fluid was obtained).

- Anthrone reagent: 380cm^3 of Sulphric acid (sp. Gr. 1.84) was diluted with 165cm^3 of water, 0.5g of anthroner was dissolved in the acid. The solution was store in dark brown bottle.
- 4% Boric acid (4 g of the acid diluted to 100cm^3 with water).
- D-glucose
- 0.02 silver nitrate (3.4g of $AgNO_3$ dissolved in water and diluted to 100cm^3.
- 1% HCl in methanol (2.8cm^3 of conc HCl diluted to 100cm^3 with methanol.
- Vanillien-HCl reagent. 1g of vanillien dissolved in 100cm^3 of 1% HCl

  Catechin (standard solution) 100mg of catechin dissolved was in 10cm^3 of ethanol to give a concentration of 1mg/cm^3.
- 0.025 mol/dm^3 iron (III) chloride (4.063g of $FeCl_3$ was dissolved in water and diluted to 1000cm^3.
- 0.3% Ammonium thiocyanate (0.3g of Ammonium thiocyanate dissolved in 100cm^3 of water.
- Phosphate standard solution (4.393 of dry potassium dihydrogen-orthophosphate (KH_2P0_4) was dissolved in water and diluted to 1dm^3 (1cm^3=1mg P).
- Ammonium molybdate sulphuric acid reagent. 25g of Ammonium molybdate $(NH_4)_6$ $Mo_7O_{24}\cdot 4H_2O$) was dissolved in 200cm^3 of distilled water in a beaker and warmed slightly. 280cm^3 of conc. Sulphuric acid was diluted to 400cm^3 with water and the molybdate solution was then

filtered into the acid with thorough mixing. The solution was made up to 1000cm^3 after cooling with water and stored in the dark.

- Stannous chloride reagent: this was prepared by dissolving 0.5g $SnCl_2 \cdot 2H_2O$ in 250cm^3 of 2% v/v hydrochloric acid and made to 1000cm^3 with water.
- Standard sulphate solution (1cm^3=1mg S) This was prepared by dissolving 7.688g of magnesium tetraoxosulphate heptahydrate ($MgSO_4 \cdot 7H_2O$) in water and made to 1000cm^3.
- Standard magnesium solution (1cm^3=1mg) (10.0135g of magnesium tetraoxoshulphate (IV) heptahydrate $MgSO_4 \cdot 7H_2O$) in water, add 1.5cm^3 of conc. HNO_3 and diluting the solution to 100cm^3 with water.
- Standard solution of sodium. (25.42g of dry NaCl was dissolved in water and 1.5cm^3 of HNO_3 (sp. gr. 1.42) was added. The solution was diluted to 1dm^3 with water (1cm^3=1mg Na)).
- Gum –acacia 0.5% w/v (0.5g of Gum acacia was dissolved in 100cm^3 of warm water.
- n-butanol
- 5% sodium chloride solution (5g of sodium chloride crystals was dissolved in water and diluted to 100cm^3.

  6 mol/dm^3 ammonium hydroxide (112.28cm^3 of ammonia (sp.gr.0.91) was diluted with water to 100cm^3).

3.3 Determination of Moisture Content. (Allen, 1974).

A crucible was dried to constant weight in oven at 105^{o}C. A known weight of the sample was placed into the crucible and dried in the oven at 105^{o}C to constant weight. The crucible and its content was cooled in a dissicator and weighed. The moisture content was calculated and expressed in percentage. Three replicate determination were made in each case and the mean value reported.

3.4 Determination of Ash and Organic Matter Content of the Seeds (A.O.A.C., 1980)

The crucible was preheated in a muffle furnace at 550^{o}c for one hour, cooled in a dessicator and weighed. 2g of the oven dried ground sample was transferred into the crucible and weighed. The crucible and its content was placed in the muffle furnace and the temperature was allowed to rise to 550^{o}C. After maintaining the temperature at 550^{o}C for twelve hours, the crucible with its residue was allowed to cool to 200^{o}C before transferring it into the dessicator to cool to room temperature and then weighed. The organic matter content was calculated by subtracting the percentage ash content from 100. The experiment was repeated several times.

3.5 Elemental Analysis

3.5.1 Elemental Analysis by X-Ray Fluorescence Spectrometer (XRFS).

(Funtua, 1999; Ogunleye *et al*, 2002).

X-ray fluorescence spectrometer is an instrument which consist of annular cadmium 109 as an isotopic excitation X-ray source. The measuring system consists of silena model 12170 lithium drifted selenium detector with a resolution of 170eV for 5.90keV line coupled to a computer controlled analog digital converter (ADC) Card.

There is the preamplifier and the main amplifier, which is the spectroscopy amplifier. The voltage of 240V is applied and it utilizes the MAESTRO software for spectral acquisition, peak location, energy assignment, elemental identification, smoothening background subtraction and normalization and the AXIL software for quantification of the acquired spectra.

The instrument is non-destructive and is used for qualitative and quantitative determinations of elements, based on measurement of the wavelength, energy or intensity of X-ray spectral lines excited by a suitable energy source (Goulden, 1978).

Preparation of Pellets.

Pellets of 19mm diameter and weight 0.2843g *(Moringa oleifera)*, 0.3150g *(Detarium microcarpum)* and 0.2983g (*B. monandra*) were made from the ash samples-using three drops of an organic binder (10% solution of styropore in toluene). The pelletisation was done using a pressure of about 10 tons (204081. 60Nm^{-2}) on a hydralic press.

Procedure

The pellets were introduced into the X-ray fluorescence (XRF) generator (model SL 12170) and analysed. Measurements are performed using an annular 25 mCi ^{109}Cd as the excitation source, that emits Ag-K X-rays (22.1 keV) and ^{55}Fe that emits Mn-K X-rays (5.89 keV), in which case all elements with lower characteristic excitation energies are accessible for detection in the samples. The spectra for the samples are collected for 3000s with the ^{109}Cd source and the spectra were then evaluated using the AXIL-QXAS program

3.5.2 Determination of Sodium and Magnesium by Atomic Absorption Spectrometer (AAS). (Allen, 1974)

3.5.2.1 Digestion of samples

1.0g of sample was weighed into a 100cm^3 Kjedahl digestion flask. 2cm^3 of $HClO_4$, 10cm^3 of nitric acid and 1cm^3 of sulphuric acid was added into the flask. Digestion was done slowly at moderate heat, and then increased later until white fumes were obtained. This was cooled and filtered quantitatively into a 100cm^3 volumetric flask and made up to volume with water

3.6.2.2 Working Standard Solutions

a. Sodium (Na)

1000mg/l stock solution of sodium was diluted by pipetting 10.00cm^3 of the solution into 100.00cm^3 volumetric flask and was made up to volume with water to give 100mg/l of the solution. This was further diluted by pipetting 2.00cm^3, 4.00cm^3, 6.00cm^3, 8.00cm^3 and 100.00cm^3 of the solution into different 100cm^3 volumetric flask and making up to volume with water to give 0.20mg/l, 0.4mg/l, 0.6mg/l, 0.8mg/l and 1.0mg/l working standard solutions of sodium respectively.

b. Magnesium (Mg)

1000mg/l stock solution of magnesium was diluted by pipetting 10.00cm^3 of the solution into 100cm^3 volumetric flask and made up to volume with water to give 100mg/l of the solution. This was further diluted by pipetting 1.00cm^3, 2.00cm^3, 3.00cm^3, 4.00cm^3 and 5.00cm^3 of the solution into different 100cm^3 volumetric flasks and were made up to volume with water to give 0.10mg/l, 0.20mg/l,

0.30mg/l, 0.40mg/l and 0.50mg/l working standard solutions of magnesium respectively.

3.5.2.3 Operation parameters for the determination of sodium and magnesium by Atomic Absorption Spectrometer.

Table 3.0

Element	Wavelength	Flame	Bandpass (nm)
Sodium	598.0	AAL	0.02
Magnesium	285.2	AAL	0.02

Key:

L = Fuel Lean

AA= Air/Acetylene

3.5.2.4 Method (Goulden, 1978)

The operating parameters in Table 3.1 were used to calibrate the instrument for each element determined by setting the top working standard to a suitable scale deflection and the standard (distilled water) to zero and then aspirated the inter-mediate working standard and solution prepared for the element being determined. The calibration curve for the absorption of the elements was plotted and the concentration of each element was read. Blank determinations were carried out and the spray and burner flush with water after each run.

3.5.3 Determination of Phosphate-Phosphorus (Allen, 1974)

Digestion of Sample

1.0g of sample was weighed into 100cm^3 Kjedahl flask 2cm^3 of $HClO_4$, 10.00cm^3 of HNO_3 and 1.0cm^3 of H_2SO_4 were added to the sample; stirred gently and digested slowly at moderate heat at the beginning and later increased. Digestion was completed in 15 minutes after the appearance of white fumes. It was cooled and diluted and was filtered into a 100cm^3 volumetric flask and was made to mark with water.

Method

Working standards of 0 to 0.15mg P were prepared by pipetting between 0 to 15cm^3 standard solution into 100cm^3 volumetric flasks. Solutions were diluted to two third full and 2cm^3 each of ammonium molybdate reagent and stannous chloride solution were added and mixed thoroughly. The solutions were diluted to mark and kept for thirty (30) minutes samples were prepared using the same procedures and the absorbance of the elements were read at 700nm using water as reference. A calibration curve was plotted using the standard solution. The amount of phosphorus in the sample read off from the graph and the percentage concentration calculated as:

$$\text{Phosphorus (\%)} = \frac{\text{Cmg x Solution Volume x 100}}{\text{Aliquot Volume x sample weight}}$$

Where Cmg is the amount of phosphorus obtained from the calibration curve.

3.5.4 Determination of Sulphate-Sulphur (Allen, 1974).

Digestion of Sample

1.0g of dry sample was weighed into an evaporating dish and 4.0cm^3 of HNO_3 (sp. gr. 1.42) was added and left to stand overnight. The solution was evaporated at 70^0C to dryness and then ashed at 500^0C for 6 hours. 10.0cm^3 of 25% HNO_3 was added and it was filtered into a 100cm^3 volumetric flask and made up to volume with water.

Procedure

Working standards containing 0 to 0.05mg sulphur was prepared by pipetting 0 to 5cm^3 standard solution into a 100cm^3 volumetric flask and was diluted to two third full. 10cm^3 of sample solution was pipetted to another 100cm^3 volumetric flask. 10.0cm^3 of 25% nitric acid was added to both sample and standard. 5cm^3 of 50% acetic acid and 1cm^3 H_3PO_4 were added to both standards and sample and was stirred to mix. The

mixture was diluted and stirred again. 1g of $BaCl_2$ crystal was added, mixed and left to stand for 10 minutes. The flasks were inverted twice and left for another 5 minutes. This process was repeated five times and $1cm^3$ of gum acacia solution was added and was made to mark with water. The flasks were inverted for another five times and left for one and half hour. The turbidity was measured at 470nm using water as the reference. A calibration curve was plotted using the standard solutions. The amount of sulphur in the sample read from the graph.

$$S\ (\%) = \frac{Cmg \times Solution\ Volume}{Aliquot\ volume \times Sample\ weight.}$$

Cmg is the amount of sulphur obtained from the calibration curve.

3.6 Determination of Lipid Content. (Pearson, 1976)

10.00g was weighed into pre-weighed fat free thimble. $350cm^3$ petroleum ether (40-60^oC) was poured into a previously weighed $500cm^3$ round bottom flask containing few antibumping granules. The soxhlet extractor was fitted into the $500cm^3$ flask and the extraction carried out for six hours. The petroleum ether was then distilled off using a rotary evaporator leaving the lipid in the flask. This was dried in a water bath to constant weight. The percentage lipid was calculated. Three replicate determinations were made for each sample and the average determined.

$$\%\ Lipid = \frac{Weigh\ of\ lipid}{Weight\ of\ Sample} \times 100$$

3.7 Determination of Crude Protein (AOAC, 1980)

2.0g Kjedahl digestion flask and few antibumping granules (1.0g of K_2SO_4 and anhydrous $CuSO_4$) were added to the flask. $25.0cm^3$ of sulphuric acid (98 v/v) was added. The flask was fixed on the Kjedahl digestion rack and was heated slowly at first until

frothing subsided and then vigorously with occasional rotation (to ensure even digestion and avoid overheating of the content) until a clear solution was obtained. The solution was diluted to 250cm^3 and transfered in to the Kjedahl distillation flask. Few drops of phenolphthalein and 70cm^3 of 40% (w/v) sodium hydroxide solution were added. The ammonia liberated was trapped in 4% (w/v) boric acid solution (100cm^3) contained in a 500cm^3 conical flask to which two drops of methyl red indicator was added. Distillation was stopped after the colour change. The content of the conical flask was titrated with 0.1 mol dm^{-3} hydrochhloric acid. The percentage nitrogen was calculated as

$$\text{Nitrogen (\%)} = \frac{0.014 \times C \times VA \times 100}{\text{Weight of sample (s)}}$$

Where C = molarity of HCl

V = titre value of HCl

Crude protein = % nitrogen x 6. 25

Three replicate determination were made in each case and the average determined

3.8 Determination of Soluble Carbohydrate (Pearson, 1976)

0.5g of sample was weighed into a 100cm^3 digestion flask. 10cm^3 of water was added and the mixture was stirred. 13cm^3 of 52% perchloric acid was added and the resulting mixture was diluted with water and was filtered into 250cm^3 volumetric flask and was made up to the mark. 10cm^3 of the solution was diluted to mark with water in a 100cm^3 volumetric flask. 1cm^3 of the diluted solution and standard glucose solution were transferred into separate test tubes. To each test tube, 5.0cm^3 of anthrone reagent was added and the content of the tubes was mixed thoroughly. The test tubes were placed in a boiling water bath for 12 minutes after which they were quickly cooled to room temperature. The absorbance of the solutions were measured at 630nm using water as blank. A calibration curve was plotted and the amount of carbohydrate in the sample was

determined. Three replicate determinations were made in each case and the average determined

$$\text{Soluble carbohydrate (\%)} = \frac{\text{Cmg x extracted volume}}{\text{aliquot Volume x sample weight}}$$

Where C = mg of carbohydrate obtained from the graph.

3.9 Determination of Crude Fibre (Pearson, 1976)

2.0g of ground sample was placed in a round bottom flask 100cm^3 of 0.25 $moldm^3$ H_2S0_4 was added and the mixture boiled under reflux for 30 minutes. The hot solution was filtered under suction. The insoluble matter was washed several times with hot water until it was acid free. Thereafter, it was transferred into a flask containing 100cm^3 of hot (0.312 $moldm^{-3}$) NaOH solution.

The insoluble residue was washed with hot water until it was base free. It was dried to constant weight at 100^oC was cooled in a dessicator and weighed (X_1). The weighed sample was incinerated in a muffle furnace at 525^oC for two hours, cooled in a dessicator and re-weighed (X_2). The crude fibre was calculated as

$$\text{Crude fibre (\%)} = \frac{X_1 - X_2 \text{ x } 100}{\text{Weight of sample}}$$

Where X_1-X_2 = the loss in weight on ashing.

Three replicate determinations were made in each case and the average determined

3.10 Determination of Hydrogen Cyanide (HCN). (A.O.A.C., 1980)

(Alkaline titration method)

10g of sample was soaked in a mixture of 200cm^3 of water and 10cm^3 of othophosphoric acid. The mixture was left for 12 hours to release all bounded hydrocyanic acid. A drop of anti-forming (paraffin) and antibumping agents were added and the solution distilled until 150cm^3 of the distillate was collected. 20cm^3 of distillate

was taken into a conical flask and diluted with 40cm^3 of water, 8.0cm^3 of 6.0 moldm^{-3} ammonium hydroxide (NH_4OH) and 2.0cm^3 5% (w/v) potassium iodide (KI) solutions were added. The mixture was titrated with 0.02 moldm^{-3} silver nitrate ($AgNO_3$) using a microburette until a faint but permanent turbidity was obtained (1cm^3 0.02 moldm^{-3} $AgNO_3 \equiv$ 1.08mg HCN). Three replicate determinations were made in each case and the average determined

3.11 Determination of Phytate (Reddy, 1982).

4.0g of sample was soaked into 100cm^3 of 2% hydrochloric acid for five hours and was filtered. 25.0cm^3 of the filtrate was taken into a conical flask and 5.0cm^3 of 0.3% ammonium thiocyanate solution was added. The mixture was titrated with a standard solution of iron (III) chloride until a brownish-yellow colour persists for five minute (1cm^3 of 0.025mol/dm^3 $FeCl_3 \equiv$ 6.601mg phytate). Three replicate determinations were made in each case and the average determined

3.12 Determination of Tannins (Vanillin – HCl Method; Bagepallis *et al,* 1982).

500mg of sample was placed in a centrifuge tube; 10cm^3 of 1% HCl in methanol was added and shaken for 20minutes on a flask shaker. The tubes were then centrifuged for 10 minutes at 6000 RPh and the supernatant transferred into a 25cm^3 volumetric flask. To the residue in the tube, another 5cm^3 of 1% HCl – methanol was added and shaken. The tubes were centrifuged for 20 minutes at the same speed and the supernatant added to the first extract in the 25cm^3 volumetric flask, the volume was made up to mark with 1% HCl – methanol reagent and the content was thoroughly shaken to ensure proper mixing. To estimate the tannin content of the samples, 1cm^3 aliquote of the extract was pipetted into a test tube and 5.0cm^3 of vanillin- HCl reagent was added to each of the catechin

standard, sample solutions and blank. The absorbance of the samples and blank as well as standard catechin at different concentrations were determined at 500nm. A calibration curve was plotted using the standards solutions of catechin. The concentrations of catechin were determined by extrapolation and tannin content of the sample calculated using the formula below. Three replicate determinations were made in each case and the average determined

$$\text{Tannin (\%)} = \frac{\text{Cmg catechin x volume made up x 100}}{\text{Volume of extract x weight of sample}}$$

Cmg = amount of catechin obtained from the graph.

Working standard:

The working standard were prepared by pipetting out 0.2, 0.4, 0.6, 0.8 and 1.0cm^3 of the catechin stock solution into separate tubes and their volume made up to 10cm^3 with methanol. The standard solution gave a concentration of 0.02, 0.04, 0.06, 0.08 and 0.10 mg/cm^3 of catechin respectively.

3.13 Determination of Saponins (Hudson and El – Difrawi, 1979)

10g of sample was taken into 100cm^3 of 20% aqueous ethanol in water and mixture agitated with a magnetic stirrer for twelve (12) hours at 55^oC. The solution was filtered using whatman No.1 filter paper and the residue was re-extracted with 200 cm^3 of 20% aqueous ethanol. The extracts were combined and reduced to about 40cm^3 under vacuum using a rotary evaporator. The extract and 20cm^3 diethyl ether were transferred into a 250cm^3 separatory funnel and was shaken vigorously. The aqueous layer was discarded. The purification process was continued until a colourless aqueous extract was obtained. The pH of the aqueous solution was adjusted to about 4.5 by adding 4.0g of sodium chloride, and the solution was shaken with butanol. The butanoic extract was

washed twice with $10cm^3$ of 5% sodium chloride and was evaporated to dryness in a fume cupboard, to give the saponin, which was weighed and expressed in percentage. Three replicate determinations were made in each case and the average determined

3.14 Determination of Chemical Properties of the Oils

3.14.1 Determination Iodine Value (Wijs Method; A.O.A.C., 1980)

0.20g of oil sample was weighed into a $250cm^3$ conical flask and was dissolved with $15.0cm^3$ carbon tetrachloride $25cm^3$ of Wijs reagent was added to the mixture. The flask was then stoppered and gently shaken and the solution placed in the dark for 30 minutes. The excess iodine was determined by adding $20cm^3$ 10% (w/v) potassium iodide solution and $150cm^3$ water and titrating with $0.1moldm^{-3}$ sodium thiosulphate using starch as indicator. The titration was continued until blue colour just disappeared after a vigorous shaking. A blank determination was carried out and the iodine value was determined using the formula below. Three replicate determinations were made in each case and the average determined

$$\text{Iodine value} = \frac{12.69 \times C \times (V_1 - V_2)}{\text{Weight of sample}}$$

Where C = Concentration of sodium thiosulphate used

V_1 = Volume of sodium thiosulphate used for blank

V_2 = Volume of sodium thiosulphate used for sample.

3.14.2 Determination of Acid Value. (A.O.A.C., 1980)

5.0g of oil was weighed into a $250cm^3$ conical flask containing $50cm^3$ solvent mixture (v/v) 95% ethanol and diethyl ether. The solution was titrated with 0.1 $moldm^{-3}$ potassium hydroxide using $1cm^3$ of 1% (w/v) phenolphthalein as indicator until pink colouration persisted. The acid value was expressed in milligram per gram of oil.

Acid value (mg/KOH) = $\frac{56.1 \times C \times V}{\text{Weight of sample}}$

Where C = Concentration of KOH used

V = Volume of KOH used

56.1 = Molecular mass of KOH.

3.14.3 Determination of Free Fatty Acid

Using the procedure described in 3.14.2, the free fatty acid was calculated

as Free fatty acid = $\frac{C \times V \times M}{\text{Weight of sample}}$

Where M = Molecular weight adopted for the sample oil (one tenth of the molecular weight (28.2) of oleic acid)

C = Concentration of KOH used

V = Volume of KOH.

3.14.4 Determination of Peroxide Value (A.O.A.C., 1980)

0.5g of oil sample was weighed into 250cm^3 conical flask, containing 10cm^3 of chloroform and 15cm^3 of acetic acid. The mixture was stirred after which 1cm^3 of (10% w/v) potassium iodide was added and the solution covered with a stopper and shaken for one minute. The flask was place in a dark placed for five minutes. 75 cm^3 of water was added and the liberated iodine was titrated with standard sodium thiosulphate solution. Three replicate determinations were made in each case and the average determined

Peroxide value (mg/kg) $\frac{V_o\text{-}V \times C}{\text{Weight of sample}}$

Where V_o = Blank volume

V = Sample volume

C = Concentration of sodium thiosulphate

3.14.5 Determination of saponification value (A.O.A.C, 1980)

20g of oil sample was weighed into 250cm^3 conical flask and 25.0cm^3 of freshly prepared 0.5moldm^{-3} alcoholic potassium hydroxide was added. The mixture was boiled under reflux for an hour and was titrated with 0.5moldm^{-3} hydrochloric acid using phenolpthalein indicator. A blank determination was carried out and the saponification value determined using the formula below. Three replicate determinations were made in each case and the average determined

$$\text{Saponification value} = \frac{56.1 \times C \times (V_o - V)}{\text{Weight of sample}}$$

Where C = Concentration of HCl

V_o = Volume of HCl required for blank

V = Volume of HCl required for sample.

3.14.6 Determination of Ester Value (Pearson, 1976).

The ester value was determined from the expression

Ester Value = Saponification Value – Acid Value.

3.14.7 Extraction of Fatty Acids from the Oils

5cm^3 of oil was taken into a 250cm^3 conical flask and 25cm^3 of alcoholic KOH was added. The mixture was boiled under reflux for 1 hour. 100cm^3 of water was added and evaporated on a steam bath to remove the alcohol. This was transferred into a 500cm^3 separatory funnel. Water was added to bring the total value to 250cm^3. The mixture was neutralized with 10cm^3 solution of 6 moldm^{-3} hydrochloric acid to separate the fatty acid and 75cm^3 of diethyl ether was added. The mixture was shaken and the aqueous layer was decanted off and the ether layer washed until the aqueous layer was neutral to methyl

orange. The diethyl ether extract containing the fatty acid was withdrawn, filtered and evaporated on a steam bath and kept in the freezer.

3.14.8 Spectrophometric Determination of the Oils and Fatty Acids.

Using the ATI Mattson Genesis series FTIR Unicam Infrared Spectrophotometer, the IR Spectra of the crude oils and the fatty acids extracts were run and the results expressed as percentage transmittance against wave number as shown in Figure 4.1 – 4.6.

3. 15 Determination of Amino Acids (Spackman, 1958; Pearson, 1976)

(i) Defatting of samples

2g of sample was weighed into the extraction thimble and the fat was extracted with petroleum ether (40-60^{o}C) using soxhlet extraction apparatus for 6 hours.

(ii) Hydrolysis of the samples

40mg of the defatted sample was weighed into the glass ampoule. 7cm^3 of 6 mol/dm^3 hydrochloric acid was added and oxygen expelled by passing nitrogen into the ampoule (to avoid possible oxidation of some amino acids during hydrolysis). The glass ampoule was sealed with a Bunsen burner flame and was kept in an oven preset at 105^{o}C for 22 hours. The ampoule was cooled and broken at the tip and the content was filtered to remove the residue. The filtrate was evaporated to dryness at 40^{o}C under vacuum in a rotary evaporator. The residue was dissolved with 5cm^3 acetate buffer (pH 2.0) and was stored in plastic specimen bottles.

(iii) Loading of the hydrolysate into the TSM Analyzer

The amount loaded was between 5 to 10 microlitres. This was dispensed into the cartridge of the analyzer. The TSM analyzer is designed to separate and analyze acidic, neutral and basic amino acids of the hydrolysate. The analysis lasted for 76 minutes.

(iv) Calculation of amino acid values

The net height of each peak produced by the chart record of the TSM (each representing an amino acid) was measured. The width at half height was measured and recorded. The approximate area of each peak was obtained by multiplying the height and width at half- height. The norleucine equivalent (NE) for each amino acid in the standard mixture was calculated (using the formula below)

$$NE = \frac{\text{Area of norleucine peak}}{\text{Area of each amino acid}}$$

A constant S was also calculated for each amino acid in the standard mixture.

Standard = NE standard x Molecular weight x µmol of amino acid std.

The amount of each amino acid present in the sample was calculated in g/100g protein using the formula below.

$$\text{Where } C = \frac{\text{Dilution factor x 16}}{\text{Wt of sample x N \% x 10 x Vol. Loaded}} \text{ x NH x weight of (nleu)}$$

Where NH = Net Height

W = Width

Nleu = Norleucine

3.16 Statistical Analysis

The one-way analysis of variance (ANOVA) was used to compare the means of the variables. Through the general linear model program SAS and the Fisher multiple comparison procedures, the least significance different (LSD) at $p<0.05$ was determined (Adama, 1996). This procedure gave an insight as to which plant seeds could better serve as a source of nutrients.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

The results of moisture, ash and organic content of the seeds are as presented in Table 4.0. The results of the elemental composition of the seeds (concentrations in ppm) using ED-XRF are presented in Table 4.1. The result gives the concentrations of the essential bulk, essential trace and non-essential elements of the seeds. The results of nutritional and anti-nutritional content of the seeds are presented in Table 4.2. The results of the iodine, acid, free fatty acids, peroxide, saponification and ester values of the seed oils are as showing in Table 4.3. While the results of amino acid composition of the seed were presented in the Table 4.4.

Table 4.0 The moisture, Ash and Organic Contents of the seeds

	Moringa oleifera	*Detarium microcarpum*	*Bauhinia monandra*
Moisture (%)	3.76 ± 0.01c	4.69 ± 0.06b	7.55 ± 0.04a
Ash (%)	3.60 ± 0.03b	2.77 ± 0.10c	4.11 ± 0.04a
Organic Matter (%)	96.40 ± 0.03a	97.23 ± 0.10b	95. 56 ± 0.04c

Values are Mean ± SE, means followed with the same alphabet are not significantly different ($p<0.05$)

Table 4.1 Elemental Content of the Seeds (Concentrations in ppm) using ED-XRF

	Moringa oleifera	*Detarium micro- carpum*	*Bauhinia monandra*
a)	Essential Bulk elements		
Potassium	77400 ± 246	105000 ± 299	74200 ± 246
Calcium	20500 ± 203	23000 ± 269	77900 ± 298
Magnesium	1190 ± 330	220 ± 20.00	2870 ± 20.00
Sodium	1999 ± 70.00	2384 ± 270	2319 ±70.00
Sulphur	3752. 33 ± 4.00	16248. 33 ±18.00	4166. 67 ± 5.00
Phosphorus	1365 ± 7.00	1252 ± 4.00	1594. 33 ± 9.00
Iron	1480 ± 90.0	3120 ± 136	1710 ± 115
(b)	Essential Trace Element		
Iodine	3930.00	5420.00	4150.00
Copper	127 ± 27.00	177 ± 35.00	374 ± 36.00
Manganese	374 ± 101	2780 ± 177	352 ±0.00
Chromium	460. 00	496.00	516. 00
Molybdenum	11.10	670 ± 12.00	11.4 ± 4.00
Selenium	22.80	35. 20	26. 10
Zinc	510 ± 27.00	635 ± 33	535 ± 30.00
(c)	Non-essential trace Elements		
Arsenic	41.80	51.40	57.60
Lead	60.70	73.90	85.40
Tin	35.0	362.0	364.0
Nickel	83. 1 ± 35.00	176 ± 47.00	96. 30
Bromine	19.30	22.80	20. 10
Vanadium	519. 00	753.0	769.0
Cobalt	120.00	187.0	186.0
Thallium	1100.00	1960.0	1690.0
Rubidium	308 ± 12.00	914 ± 18.00	342 ± 12.00
Strontium	70.2 ± 80.00	83.1 ± 9.00	654. ± 15.00
Yttrium	50.90	62.0	67.80
Zirconium	22.90	25.30	25.20
Niobium	10.80	17.4 ± 60.00	10.70

Table 4.2 Nutritional and Anti-nutritional Content of the Seeds.

	Moringa oleifera	*Detarium microcarpum*	*Bauhinia monandra*
Lipid (%)	41. 58 ± 0.32a	11.24 ± 0.09c	28.79± 0.21b
Protein (%)	40.19 ± 0.85a	35.94 ± 0.06b	32. 76 ± 0.51c
Carbohydrate (%)	9.11 ± 0.13c	42.20 ± 0.25a	21. 40 ± 0.10b
Fibre (%)	3.29 ± 0.42a	3.42 ± 0.28a	3.25 ± 0.14a
Phytate (mg/100g)	10. 18 ± 0.46b	5.25 ± 0.03c	11. 57 ± 0.47a
Hydrogen cyanide (mg/100g)	0.58 ± 0.04a	0.25 ± 0.04b	0.32 ± 0.00b
Tannins (%)	2.13 ± 0.14c	4.75 ± 0.12b	6. 00 ± .09a
Saponins (%)	2.25 ± 0.07a	0.20 ± 0.01c	2. 05 ± 0.00b

Values are Mean ± SE, means followed with the same alphabet are not significantly different ($p<0.05$)

Table 4.3 Chemical Properties of the Seeds Oils

	Moringa oleifera	*Detarium microcarpum*	*Bauhinia monandra*
Iodine value	59.48 ± 0. 22a	58.04 ± 0.21b	56.60 ± 0.29c
Acid value	6.25 ± 0.10c	8.12 ± 0.04a	6.77 ± 0.10b
Free fatty acid	3.52 ± 0.06b	4.58 ± 0.02a	3.80 ± 0.15b
Peroxide value (mg/kg)	2.35 ± 0.01a	2. 18 ± 0.01b	1.99 ± 0.02c
Saponification value (g/KOH)	179.75 ± 0.47c	220.66 ± 0.62a	196. 12 ± 0.46b
Ester value	173. 51 ± 0.55c	212. 54 ± 0.62 a	187. 35 ± 0.50b

Values are Mean ± SE , means followed with the same alphabet are not significantly different ($p<0.05$)

Table 4.4 Amino Acid Content (g/100g protein) of the Seeds

Amino acids	*Moringa oleifera*	*Detarium microcarpum*	*Bauhinia monandra*
Lysine	3.21	2. 14	2. 86
Phenylalamine	4.24	2.58	3.77
Leucine	5.74	2.35	2.13
Isoleucine	4.01	2.35	2.31
Methionine	1.00	0.75	1.54
Valine	2.05	2.13	3.54
Cystine	2.09	1.07	1.11
Threonine	3.03	2.17	2.70
Glutamic acid	14.43	9.78	11.75
Arginie	8.00	5.66	6.74
Aspartic acid	6.22	4.79	6.02
Serine	4.22	3.12	4.58
Glycine	4.96	2.41	3.09
Alamine	3.22	2.10	2.99
Histidine	2.20	1.13	2.36
Proline	2.09	2.09	2.37
Tyrosine	2.37	1.06	3.18

4.2 DISCUSSION

From Table 4.0 the observed moisture content *B. monandra* was highest (7.55%) that of *D. microcarpum* was 4.69% while *M. oleifera* moisture content was 3.76%. There was a significant difference ($p<0.05$) in the moisture content of the seeds. The high moisture content of *B. monandra* falls below the 15% required as safe storage limit for plant food (Sena *et al*, 1998). The moisture contents of the seeds are low; this is an indication that they can be stored for a long time without the development of moulds. Our results are in agreement with those of Akpata and Miachi (2001) who reported a value of 3.5% moisture for *D. microcarpum* while Campden and Chlolerywood (1998), gave a higher value of 4.5% for *M. oleifera*. The moisture content of any material is dependent on the seasonal variation, therefore, the difference in moisture contents could be due to environmental factors and the time the experiments were carried out.

The ash content of *B. monandra* was 4.44% (Table 4.0). This value is higher compared to that of *D. microcarpum* (2.77%) and *M. oleifera* (3.60%). There was significant difference ($p<0.05$) in the ash content of the seeds. *Detarium microcarpum* ash content (2.77%) is lower than the 3.5% reported by Akpata and Miachi (1998). The ash content of the seeds was inversely proportional to the organic matter contents (Table 4.0). The ash content of a plant material is a measure of its inorganic matter content. However due to volatilisation of some volatile inorganic elements at high temperature ($525^{o}C$) the ash content is a direct measure of the total mineral content.

The organic matter contents of *D. microcarpum*, *M. oleifera* and *B. monandra* were 97.23%, 96.4% and 95.56% respectively. There was a significant difference ($p<0.05$) in the organic matter composition of the seeds. Organic matter measures the

nutritional value (lipids, proteins and carbohydrates) of a plant material. The higher the organic matter content, the higher the nutritional value. The results indicate that the seeds have high nutritional value. The seeds of *D. microcarpum* are nutritious and are used as feed for cattle by the Nupe people of Niger State (Dalziel, 1955).

The results of the essential and bulk elements (K, Ca, Na, Mg, S, P and Fe) Table 4.1 show that the concentrations of potassium (105,000 ppm), sulphur (16,248.33 ppm) and Iron (3120 ppm) were highest in *D. microcarpum*. Calcium (77,900 ppm), magnesium (2870 ppm) and phosphorus (1594.33 ppm) concentrations were highest in *B. monandra*. Sodium concentrations rang between 2319 – 2999 ppm) with the highest value occurring in *M. oleifera*. These elements are needed in significant quantities by the body. They constitute parts of the rigid body structure soft tissue and body fluids. Reddy and Love (1999), reported that these elements are needed for growth, production of bones, teeth, hair, blood nerves, skin, vitamins, enzymes and hormones. The healthy nervous transmission, blood circulation, fluid regulation, cellular integrity, energy production and muscle contraction are influenced by these essential elements, deficiency in any of these elements can lead to diseases and their high concentration can be toxic (Schauss, 1995).

The essential trace elements (iodine, copper, manganese, chromium, molybdenum, selenium and zinc) Table 4.1 were in various concentrations with the highest observed by iodine (5420 ppm) in *D. microcarpum* and the lowest by molybdenum 11.10 ppm. This class of elements are required in minute quantities. Iodine is essential for the development and functioning of the thyroid gland and its deficiency can cause an enlargement of the gland (Wasantwisut, 1997). Copper is essential for

blood, nerves joints, heart, skin, liver and the immune and nervous systems. Chromium is necessary for glucose metabolism, while selenium and molybdenum are necessary for vitamin E, supports critical antioxidant enzyme functions and general operation of several key enzymes in the body respectively (Clark *et al.*, 1996; Sardesai, 1993). Zinc supports the health of the immune system, normal synthesis of protein, and the health of reproductive organs. Its deficiency affects normal physical growth, skin and nerve health, natural healing ability and immune function especially in infants (Shils *et al.*, 1999). Manganese is necessary for the normal bone metabolism, important enzyme reactions, maintain normal nerve, brain and thyroid functions (Shils *et al.,* 1999; Keen *et al.*, 1999).

The non-essential elements (Arsenic, lead, tin, Nickel, Bromine, Vanadium, Cobalt, Thallium, Rubidium, Strontium, Yttrium, Zirconium and Niobium have concentrations ranging between (1960.0ppm – 10.70 ppm). These class of minerals are not essential to human nutrition, but have some valid health benefits (Nielsen, 1996), for example, Naghii and Samman (1993), reported that boron is involved in the metabolism of other minerals, especially calcium and magnesium, regulation and function of steroid hormones. Silicon is important in the composition and calcification of cartilage to produce bone, while vanadium contributes to fat metabolism (Shils, 1999).

The oil content of *Moringa oleifera* was 41.58%, *B. monandra* 28.70%, while *D. microcarpum* was11.24% (Table 4.2). There was significant difference ($p<0.05$) in the oil content of these plants. The seeds of *M. oleifera* was found to released the highest quantity of oil compared to the other two seeds. The results from *M. oleifera* and *D. microcarpum* are comparable to those obtained by Campden (1998) and Akpata (2001) respectively. In addition, the oil content of *B. monandra* compared with other known oil

seeds like soya beans 27% (Ezeagu *et al.,* 1996). The low oil content of *D. microcarpum* relegates it as a commercial source. The search for new sources of oil is of interest because, among the various foodstuffs, they provide the body with maximum energy, twice that for an equal amount of protein or carbohydrate (Dreon, 1990). Lipids also facilitate the intestinal absorption and transport of fat soluble vitamins A, D, E and K. In certain climates, subcutaneous body fats helps to insulate the body from the cold and prevent heat loss through the skin. Lipids lend a pleasant taste and texture to foods, the essential fatty acids prevent skin dryness and scaling, regulate the action of hormones and facilitate transmission of nerve impulses (Rao, 1994; Sanchez-Vioque *et al.,* 1998).

The protein content of *M. oleifera* 40.31%, is followed by that of *D. microcarpum* 35.94% and lastly *B. monandra* 33.09% (Table 4.2). The protein content of *Moringa oleifera* is higher than the 38.4% reported by Lockett (2000), who also stated that the seeds of the plant are good sources of protein. In addition, Okuda (2001), reported that the high protein content of the seed is responsible for their coagulation and flocculatory properties. Forlkard (1999), reported that the seeds are used in the water treatment. The seeds are capable of removing about 90 – 99.9% bacteria which are attached to the solid particles as well as clearing the water. Ndabigenesere (1998), observed that *Moringa oleifera* seeds has no effect on the quality of the treated water but has a considerable increase in the concentration of the organic matter. Therefore, the active proteins may be purified before used in the treatment of water and waste water. In contrast, De-Olivera, *et al* (2001), reported that *Bauhinia bauhiniodes* seeds protein has been implicated in inhibiting cruzipian, a cysteine proteinase from trypansoma cruzi. The appreciable protein contents of the seeds can serve as a supplement to animal proteins because it

compares favourably with other proteinous plants like soya bean (*Glycine max*) 51.4%, groundnut (*Arachis hypogae*) 51.3%, sunflower (*Helianth annuus*) 42.9%, cottonseed (*Gossypium spp*) 41.7% and Linseed (*Linum usitatissim*) 38.7% (Close and Menke, 1996). A significant different ($p<0.05$) was observed in the protein content of the seeds. Protein deficiency causes growth retardation, muscle wasting and edema (abnormal swelling of the belly, and collection of fluids in the body (Zarkada *et al.*, 1997).

The carbohydrate content of *D. microcarpum* was 42.20%, *B. monandra* has 21.45% while *M. oleifera* is 9.11%. The carbohydrate contents of *D. microcarpum* compare well with the value (39.0%) reported in literature (Akpata and Miachi, 2001). The high carbohydrate content of *D. microcarpum* indicates that its seed will provide more heat and energy to the body than the other two seeds. Statistical analysis of variance showed that there is significant difference ($p<0.05$) in the carbohydrate content of the seeds. Onyechi (1998), observed that vegetable flour prepared from the seeds of *D. microcarpum* a rich source of water-soluble non-starch polysaccharides. These components are responsible for the reduction of plasma insulin and glucose level in human beings. Oneweluzo *et al.* (1999), reported that the water soluble polysaccharide content of *Detarium microcarpum* may improve moisture retention properties of bread and reduce crumb firming. *Detarium microcarpum* seed flour have good nutritional quality and the functional properties confirmed their suitability for use in various food preparations (Apata and Miachi, 2001). Horan (1999), observed that, the binding nature of some extract from *Bauhinia* species is a result of their carbohydrate content. Deficiency in carbohydrates causes the body to divert proteins and body fat to produce the needed energy and can lead to depletion of the body tissue (Barker, 1996).

The fibre content of *M. oleifera*, *D. microcarpum* and *B. monandra* was 3.29%, 3.42% and 3.25% respectively. There was no significant difference ($p>0.05$) in the fibre contents of the seeds. Fibre is that part of the diet (usually from plants) that cannot be broken down by the human digestive system. Fibre diet is necessary because, it promotes the wave-like contraction that move food through the intestine. High fibre foods expand the inside walls of the colon, easing the passage of waste by absorbing large amount of water, resulting in softer and bulkier stool. Increase in the amount of fibre diet not only treat constipation but also lowers cholesterol level in the blood, reduces the risk of various cancers, bowel diseases, improve general health and well being. The recommended amount of fibre in an average adult is 18 – 32g per day (Barker, 1996). When this limit is exceeded, the absorption of certain minerals including iron, zinc and calcium may be hindered.

Table 4.2 The highest phytate content of 11.57 mg/100g) was observed in *B. monandra*. *Moringa oleifera* contained 10.18 mg/100g of phytate, while *D. microcarpum* phytate level was 5.57 mg/100g (Table 4.2). There is a significant difference ($p<0.05$) in the phytate composition of these seeds. The phytic acid in food has the ability of binding phosphorus and converting it to phytate while other mineral elements like calcium, zinc, manganese, iron and magnesium are converted to the phytin complexes, which are indigestible substances, thereby decreasing the bioavailability of these elements for absorption (Erdman 1979; Biehl *et al.* 1997). Phytic acid has a negative effect on amino acid digestibility, thereby posing problem to non-ruminant animals due to insufficient amount of intrinsic phytase necessary to hydrolyse the phytic acid complex (Makkar and Becker, 1998; Turner *et al*., 2002).

The hydrogen cyanide composition of the seeds was low. *Moringa oleifera*, *B. monandra* and *D. microcarpum* had 0.58 mg, 0.32 mg and 0.25 mg of hydrogen cyanide respectively. Significant difference ($p<0.05$) was observed in the hydrogen cyanide content of *M. oleifera* and that of the other two seed. While there was no significant difference between *D. microcarpum* and *B. monandra*. FAO/WHO has recommended that the safe limit of HCN for human consumption is 10 mg/kg (Makkar and Becker 1998). Jansz and Uluwaduge (1997), reported that people eating food that contains low level of cyanide for a long time develops damage to the central nervous system and the thyroid gland, while Kamalu (1995) stressed that hydrogen cyanide can lead to deafness, vision problems and loss of muscle co-ordination, and that the effect on the thyroid gland causes cretinism or enlargement and over activity of the gland. Similarly, Chandra *et al.* (1980), noted that chronic exposure to hydrogen cyanide diet causes neurological, respiratory, cardio vascular and thyroid related debilities. Other side effects of cyanide include pancreatic diabetes, vitamin B_{12} deficiency and decrease in iodine uptake (Makkar and Becker, 1998) liver inflammation and haemorrhage, kidney, adrenal, myocardial and testicular lesion have been observed in dogs that consumed cassava containing low concentrations of cyanide (Abaye *et al.*, 1998; Okolie and Osagie, 1999).

The highest tannin content was observed in *B. monandra* (6.90), followed by *D. microcarpum* (4.75%) while *M. oleifera* had 2.13%. Statistical analysis of variance indicates that there was significant difference ($p<0.05$) in the tannin composition of these seeds. Tannins are water-soluble polypeptide glucosides, which belong to the polyphenolic compounds present in many plants. Tannin in diet impacts an astringent taste which affect palatability, reduce food intake and consequently body growth (Makkar

and Becker 1998). Tannin had been implicated as a binder to both exogenous and endogenous proteins, including enzymes of the digestive tract, thereby affecting the utilization of proteins (Bagepallis *et al,* 1982). Studies carried out on rats, chicks and livestock revealed that high tannin in diet adversely affects digestibility of proteins and carbohydrates, reduces growth, feeding efficiency, metabolizable energy and bioavailability of amino acids (Aletor, 1993; Sotelo *et al.*, 1995b).

The saponin content of *M. oleifera*, *D. microcarpum* and *B. monandra* were found to be 2.25. 0.20 and 2.05% respectively. A significant difference ($p<0.05$) was observed in the saponin content of the seeds. The results were comparable with those reported by Mawson *et al.* (1995). Oakenfull and Sidhu (1990), reported that, the status of saponins in nutrition is fairly diversed, Radositits *et al.* (1997), observed that saponins are haemolytic and are known to interfere with the metabolism of vitamin E and causes gastroenteritis, manifested by diarrhoea and dysentery. In contrast, saponins reduce the body cholesterol by preventing its re-absorption increasing its excretion, thereby reducing blood pressure. It suppresses rumen protozoan by reacting with cholesterol in the protozoan cell membrane, causing it to lyse (Hudson and El-Difrawi, 1979). Saponins are excellent foaming agents; as a result, they are used in beverages such as root beer and slurpies to provide the foamly 'head' (Thompson, 1993). In excreta, saponins bind to ammonia and certain other odiferus compounds and prevent their release into the air (Oakenfull and Sidhu, 1990).

The iodine value of oil or fats is the member of grams of iodine absorbed by 100 gram of the oil. The iodine value of oil indicates the class to which each belongs. There are three classes of oils based on the iodine value.

(i) Non-drying oils: This class have iodine value below 100 (e.g cocoa butter oil 32-41, olive oil 78-88, palm oil 51- 5 – 57, coconut oil 8-10, peanut oil 83 – 100 etc). These oils are slow to oxidation and therefore remain liquid for a long time. This is a useful property required in the soap, cosmetics, lubricants, leather, and candle industries. Such oils are used in the manufacture of wool, especially carding. They are employed in making tin plate and in foundry works (Rao, 1994; Le Poole, 1996). The iodine values of the seeds under investigation all fall within this category. The iodine values of the oils of *M. oleifera* was 59.53, *D. microcarpum* 58.04 and *B. monandra* 56.60.

(ii) Semi-drying oil: This class has iodine value between 100 – 150 (e.g. corn oil (111-130), cotton seed (108 – 110, sesame oil (103 – 108), soya bean oil (137 – 143) etc. These possess the property of absorbing oxygen though do not do so sufficiently to qualify them as drying oils. On exposure to the atmosphere, they thicken and remain sticky but do not form a hard dry film (Rao, 1994).

(iii) Drying oils: These have iodine value between 150 – 200. The oils absorb oxygen on exposure and are oxidized thereby converting into plastic, elastic resin like substances. Therefore, on exposure as in painting, they form a tough, elastic waterproof film, which adheres tightly to the painted surface, and protect it from the weather. This type of oils include linseed oil (173 –201), tung oil (170.6) etc. The oil is used in the manufacture of

paints, varnishes, artificial rubber, linoleums, and other coverings (Ogbobe, 1992).

Detarium microcarpum has the highest acid value of 8.12% compared to *B. monandra* (6.77%) and *M. oleifera* (6.25%). These values are lower than the minimum safe limit (15%) meant for consumption (Ekpa, 1996). They may therefore be stored with low deteriorating rate. Ekpa (1996), The acid value of an oil is the number of milligrams of KOH required to neutralize the free fatty acids in one gram of the oil. It measures the extent to which the glycerides have been decomposed by lipase action. The decomposition is usually accelerated by heat and light. Deterioration of grains and milled products have been reported to be accompanied by increasing acidity and the acids formed include free fatty acids, acid phosphates and amino acids. Free fatty acids are formed at a faster rate than the other types of acids (Savage *et al.,* 1997).

Free fatty acid is the amount of acid formed on hydrolysis or by the hydrolytic cleavage of the ester bonds of the glyceride molecule of an oil when expose to air. The free fatty acid values of an oil is the measure of the amount of the acid formed on exposure to air. Generally, high free fatty acid value indicates the deteriorating conditions and low edibility of the oil because of the development of objectionable flavour and odour and hence reducing the consumer acceptance of the oil. The results showed that the free fatty acid content of *M. oleifera*, *D. microcarpum* and *B. monandra* were 3.52, 4.58 and 3.74 respectively (Table 4.3). The low values of the free fatty acid content in oils of these seeds shows that they have low deterioration rate and high edibility. (Chen *et al.,* 1998).

The peroxide value of the oils (Table 4.3) showed that, *M. oleifera*, *D. microcarpum* and *B. monandra* were 2.35, 2.18 and 2.99 respectively. The peroxide value is the measure of oxidative rancidity of oil Ekpa (1996). Oxidative rancidity is the addition of oxygen across the double bonds in unsaturated fatty acids in the presence of enzyme or certain chemical compounds. The odour and flavour associated with rancidity are due to liberation of short chain carboxylic acids. High peroxide values are associated with higher rate of rancidity. Variation of peroxide value could be due to the number of unsaturated fatty acid content, since rate of autoxidation of fats and oils increases with increasing level of unsaturation. The low peroxide values of the oils indicate that they are less liable to oxidative rancidity at room temperature (Ekpa, 1996; Savage *et al.,* 1997). Tsaknis *et al.* (1999), observed that, *M. oleifera* seed shows high stability to oxidative rancidity and this agrees with result in this work. The oil contains high levels of unsaturated fatty acids, especially oleic acid 75.39%, while the dominant saturated acid was benenic acid (6.73%).

Saponification value is the amount of milligrams of potassium hydroxide required to convert one gram of the oil into glycerol and soap. The result showed that *D. microcarpum* has the highest value of 220.60, followed by *B. monandra* 196.12, while *M. oleifera* 179.75. Booth and Wickens (1988), reported higher value ranging between 182.2 – 187.9 for *Monriga oleifera*. The Saponification values indicate the average molecular weight of an oil Booth and Wickens (1988). A high saponification value implies greater proportion of fatty acids of low molecular weight. The saponification value of an oil gives information about the properties of the fatty acids of the oil particularly the solubility of its soap in water Folkard and Sutherland (1996). The high saponification

values of *D. microcarpum* oil showed that the oil has higher proportions of low molecular weight fatty acids than the others. The values compared well with saponification values of palm oil (196 – 205), olive oil (185 – 196), soya bean oil (193) and linseed oil 193 – 195) (Baumer, 1996).

The ester value of *D. microcarpum* was highest (212.54), *B. monandra* was 187.35, while *M. oleifera* had 173.51. The ester value of an oil measures the amount of tricylglycerols in the oil. The high ester values of these oils is an indication that high proportion of the triacylglycerol is still intact and that the oils are of appreciable quality.

Statistical analysis of variance (ANOVA) of iodine value, free fatty acid, saponfication value and ester value indicate a significant difference ($p<0.05$) in these properties of the oils. Peroxide values of the oil however showed no significant difference between the *B. monandra* and *M. oleifera* oils.

The infra red (IR) spectra analysis of the crude oils (Fig 4.1 – 4.3) indicate the carbon- oxygen double bond (-C=0) stretching absorption observed at 1746 cm^{-1} in all cases. A range of 1162 – 1163 cm^{-1} absorption recorded is characteristic of carbon oxygen single bond (-C-0) stretching. The oxygen hydrogen absorption range between 3463 – 3474 cm^{-1}; while the 3000 cm^{-1} absorption is likely to be the carbon hydrogen (–C–H) stretching.

Similar absorptions were noted in the IR spectra of the fatty acids. The carbon – oxygen double bond (-C=0) stretching is responsible for the range of absorption recorded between 1745 – 1754 cm^{-1} in the spectra. While the carbon oxygen bond (-C-0) was observed between 1163 – 1164 cm^{-1}. The oxygen hydrogen variation was recorded at 3448 cm^{-1} in *B. monandra* fatty acid spectra, while 3478 cm^{-1} absorption was observed

in *M. oleifera* fatty acid spectra. Similarly, the carbon-hydrogen (-C-H) stretching absorption was at about 300 cm^{-1} in all spectra, (Fig. 4.4 – 4.6).

The amino acid contents of the seeds are presented in Table 4.5. The concentrations (g/100g protein of the essential amino acids lysine, phenylalamine, leucine, isoleucine, methionine, valine threonine and cystine are: *M. oleifera; D. microcarpum* and *B. monandra* are shown in Table 4.4. Among the non essential amino acids, *Moringa oleifera* has (g/100g protein) of glutamic acids, arginine, aspartic acid and serine respectively. On the other hand, *B. monandra* has (g/100g protein) *glumatic*, arginine aspartic acid and serine respectively. While glumatic acid in *D. microcarpum* was (9.78g/100g protein), arginine (5.66g/100g protein) aspartic acid (4.79g/100g protein) and serine (3.12g/100g protein). Statistical analysis of variance (ANOVA) showed that there was no significant difference in the mean values of the total amino acids composition of the seeds. The results of amino acids indicate that these seeds can serve as supplementary sources of essential amino acids to man and livestock. The results compared well with those reported by Ram, (1994) and Di-Ciero *et al.,(*1998).

The amino acids make up the specific proteins required by the body's specialized tissues (Close e*t al.*, 1996; Zarkada *et al.* 1997). With the lack of any one amino acid production of the needed proteins cannot occur. Although, the body is able to make most of the amino acids it needs, some are not made in sufficient quantities and must be obtained from the diet. These are essential amino acids. The amino acid arginine and histidine are important in infants who are unable to synthesize sufficient protein for their growth requirements. Deficiency of arginine can result to impaired insulin production

and possible hair loss. Similarly, the deficiencies of the essential amino acids can result to various ailments (Rozan *et al.,* 2001) .

4.3 Conclusion

The result of this study revealed that the seeds of these wild plants have high concentrations of essential bulk and essential trace elements, lipids, proteins and amino acids which are readily available to solve some of these problems. The products of the seeds are capable of playing a substantial role in alleviating food shortage, malnutrition and hunger if given adequate attention. The proximate analysis of the seeds encourage their uses as supplementary protein, lipids and energy sources man and animal nutrition.

The chemical studies of the oil indicate that they could be employed in soap making, cosmetics, leather dressing and candle industries. The low anti-nutritional factors also support the high nutritional value of the seeds.

4.4 Recommendations

- That more research should be carried out on the constitution of other toxic substances that maybe present in the seeds.
- A concerted effort should be made to educate vulnerable populations in the value of these plants as sources of nutrition such that the preservation of the species in their natural ecosystem will be encouraged.
- Domestication of the seeds is essential and their cultivation in large scale should be embarked upon.
- The consumption of the seeds could be encourage through appropriate processing, packaging and marketing.

- The production of animal feeds and other industrial uses are recommended. This will go a long way in providing employment, increase supplement and provide affordable nutrients for livestock. On the other hand, the socio-economic life of many rural dwellers could be improved if the availability of these seeds species are enhanced. The sales of some of the seeds and their products among them will be increased.

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