CHAPTER ONE

1.0 INTRODUCTION

In Africa where protein energy gap remains an overbearing problem, plant regimens, rich in essential amino acids, are in great demand. Over decades, *Moringa oleifera* is one such essential vegetarian diet that has remained in context in the West African sub-region. *Moringa* is a drought-resistant, very fast growing tree and is available all-year round (Palada, 1995; Bhuptawat *et al.*, 2007; Aktar *et al.*, 2007). It has a multitude of uses; the leaves, the pods, flowers and growing tips of the tree are edible and nutritious (Fuglie, 1999). *Moringa* has been used to combat malnutrition especially among infants and nursing mothers (Fuglie, 1999). The leaves can be eaten fresh, cooked or stored as dried powder for many months without loss of nutritional value (Fuglie, 2001). The plant belongs to the family Moringaceae. There are about fourteen species of the tree, popular among which are *M.stenopetala*, *M.peregrina*, *M.ovalifolia* and *M.oleifera*. *Moringa oleifera* is the best known specie. It is native to sub-Himalayan parts of northern India, but is now widely distributed in the tropics and sub-tropics (Fuglie, 2001, Akhtar *et al.*, 2007; Ozumba *et al.*, 2009), because it tolerates a wide range of soil and rainfall conditions. The maximum annual rainfall requirements are over 3,000mm while the minimum is about 250mm (Fuglie, 2001). It is resistant to drought because of the presence of a long taproot (Fuglie, 2001; Ozumba, 2008). It thrives within a temperature range of 25-35 degrees centigrade, but can tolerate up to 48 degrees centigrade in the shade and can survive a light frost. *Moringa* trees flower and fruit annually, and in some regions twice annually. During the first year, it grows up to four metres in height and produces flowers and fruits. If not cut, it can

reach twelve metres in height with a trunk 30cm wide. Within three years, a tree yields 400-600 pods annually. A mature tree can produce up to 1,600 pods annually. The plant can grow from the seeds or the cuttings. It is resistant to most pests (Fuglie, 1999).

Some of the uses of the plant include in alley cropping, animal forage, as domestic cleaning agent, as dye, as fertilizer, for live fencing, as herbal remedy and as an ornamental tree (Fuglie, 1999). The recent boost in research, development, and utilization of the plant in many parts of the tropics such as East and Southern Africa, West Africa and South East Asia derived from these reported multiple uses (Fuglie, 1999). In West Africa, some countries like Senegal, Ghana, Mali and Togo have made tremendous efforts in the utilization of *Moringa* in health, agriculture, and environment. Three non-governmental organizations-*Trees for Life, Educational Concerns for Hunger Organization* and *Church World Service (CWS)* based in Senegal-have advocated the use of *Moringa* as "natural nutrition for the tropics" (Fuglie, 2001). In fact, the nutritional properties of *Moringa* leaf are well known that there seems to be little doubt of the substantial health benefit to be realized by consumption of *Moringa* leaf powder in situations where starvation is imminent. Nonetheless, the outcomes of well controlled and well documented clinical studies are still clearly of great value (Fahey, 2005).

AIMS AND OBJECTIVES OF THE STUDY

Moringa oleifera contains a lot of nutrients. Apart from its dietary importance, local folklore credits it with herbal potency. Despite these, studies on the nutritional and bioactive potentials of this plant remain scanty in Nigeria. The aim of this study is therefore:

- To determine the nutrient composition of the various parts of *Moringa oleifera* plant.
- To establish the bioactive agents and anti-nutritional factors in *Moringa oleifera* plants grown in Awka, Nigeria, and
- Conduct a dietary evaluation of *Moringa oleifera* using laboratory animals**.**

To accomplish these aims, the study is designed to achieve the following objectives:

- To evaluate the proximate composition (lipids, proteins, carbohydrates, moisture and ash) of the different parts of the plant.
- To determine the mineral composition of the different parts of the plant.
- To assess the protein quality by determining the amino acid contents of the different parts of the plant.
- To determine the anti-nutrient content in the different parts of the plant and
- To perform dietary evaluation of feed rations from the different parts of the plant using laboratory animals.

JUSTIFICATION FOR THE STUDY

This study is relevant for the following reasons:

- Most of the available literature on the nutritional value of *Moringa* are based on the evaluation of the leaves alone. The dietary potentials of other parts of the plant are largely lacking in literature. There is therefore the deliberate need to evaluate the potentials of other parts of the plant for dietary or pharmacological purposes.

- Literature suggests that most studies so far conducted on *Moringa* were conducted overseas outside the shore of Nigeria. There is paucity of information on the biochemistry and phytochemistry of *Moringa* trees cultivated in Nigeria. Since soil and physiographic factors have been shown to affect plant constituents, a study of this nature is desirous.
- Studies on the dietary constituents of the leaves credit it with essential amino acids, which is important in bridging the protein gap of poor countries like Nigeria. Empirical data is required to sufficiently support this.
- A major challenge with most novel protein sources and plant dietary regimen are the potentials of toxicity. Some plant dietary sources (particularly in unprocessed forms) have been associated with long term toxicity effects. Therefore, the determination of anti-nutritional factors in *Moringa oleifera* feed rations may present a clue to its safety.

CHAPTER TWO

2.0 LITERATURE REVIEW

2:1 Taxonomy

Moringa belongs to the family *Moringaceae* with about fourteen species. The botanical classification of the *Oleifera* sp. is provided hereunder.

Classification of *Moringa oleifera*

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnolipsida
Order:	Brassicales
Family:	Moringaceae
Genus:	*Moringa*
Species:	*Moringa oleifera*.

Vernacular names of *Moringa oleifera* are:

English:	Horse-radish tree, radish tree, mother's best friend.
French:	Ben aile, benzolive.
Senegal:	Neverdie, nebeday.
Nigeria:	It has different vernacular names:
Igbo:	Okwe oyibo, okochi egbu, okughara ite, odudu oyibo.
Yoruba:	Ewe ile, ewe igbale, idagba maloye.
Hausa:	Zogalla, zogalla-gandi, bagaruwar maser.
Fulani:	Gawara, rinimaka.

Sources: Fuglie, (1999); ASICUMPON-Checklist of medicinal plants of Nigeria and their uses, (2005); Ozumba, (2008).

The following Plates 1 and 2 show the nursery forms and full grown *Moringa* plant. Plates 3 and 4 show the pods and seeds respectively while the leaves and the flowers are shown in Plates 5a and 5b. The stems are shown in Plate 6.

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Plate 1: *Moringa* nursery.

Plate 2: *Moringa* plant.

Plate 3: *Moringa* pods.

Plate 4: *Moringa* seeds.

Plate 5a: *Moringa* flower and leaves

Plate 5b: *Moringa* flower and leaves

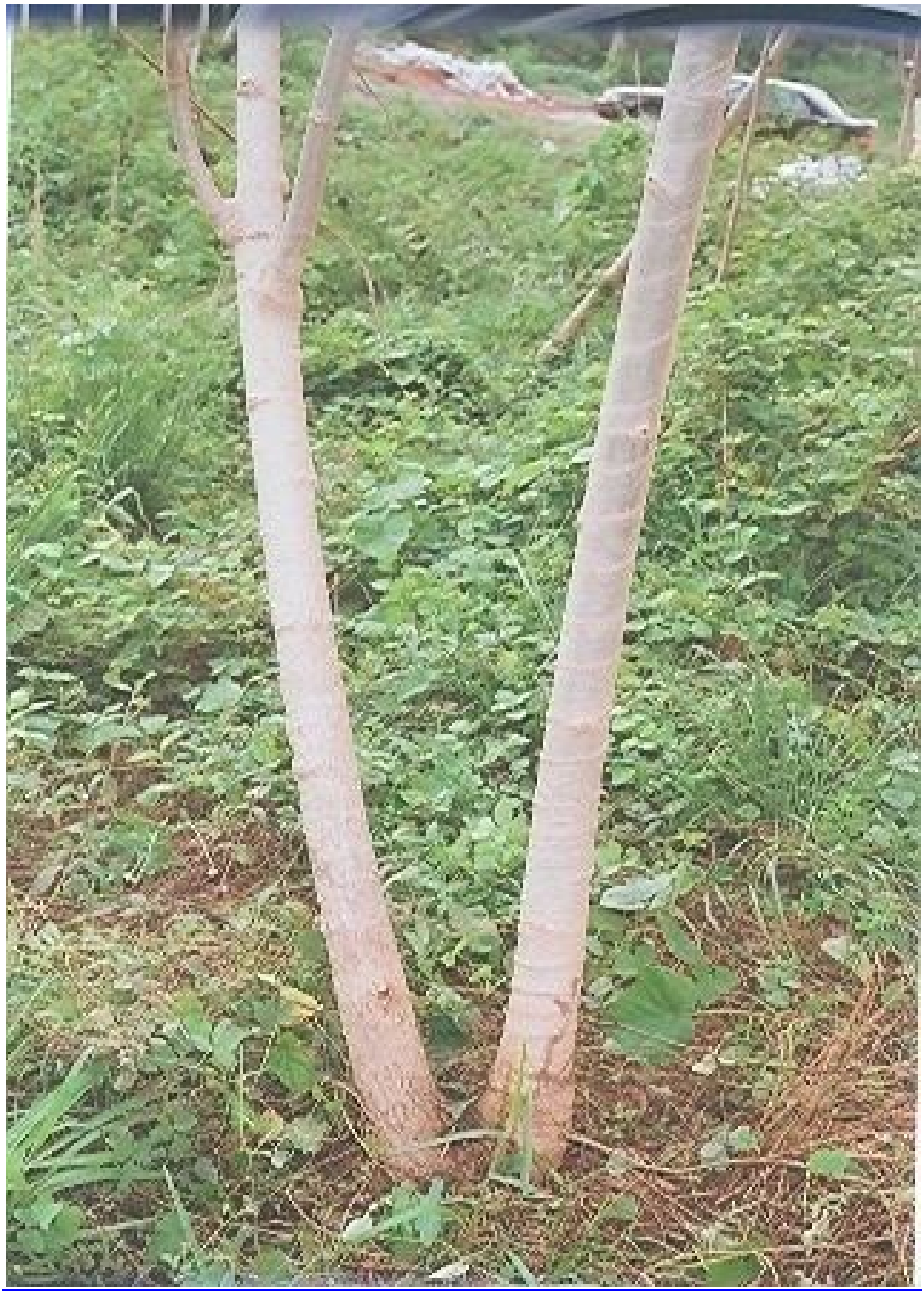


Plate 6: *Moringa* stem**.**

2:2 **Morphology**

Moringa oleifera is a short, slender, deciduous perennial plant which is about 12m tall and 30cm in diameter. It is slender with drooping branches and stems are brittle with corky bark. The leaves are alternate, oddly bi- or tri-pinnate, triangular in outline and 20-70cm long. The leaflets are variable in size and shape but often rounded elliptic. Each pinna has 3-9 pairs of 1-2 cm long orate leaflets, soft dark green above and whitish below. Its flowers are fragrant, white to creamy-white, about one inch in diameter and borne in sprays. The stamens are yellow, the pods pendulous, green to brown, triangular, splitting lengthwise into 3 parts when dry, 15-30 inches long, containing about 20 seeds embedded in the pith. The pod is tapering at both ends and is 9-ribbed. The seeds are dark brown, with 3 papery wings. The bark is smooth, dark grey, slash thin and yellowish. Its main root is rather thick (Fuglie, 2001*). Moringa* tree has the tendency to grow up straight and tall, putting out leaves and pods at its crown. It is a fast growing tree planted on a large scale as a potential source of wood for paper industry (Verma *et al.*, 1996).

2:3 **Cultivation**

Moringa oleifera is now cultivated worldwide in the tropics and sub-tropics (Fahey, 2005; Ahktar *et al.*, 2007). It grows best in direct sunlight under 500m altitude. It tolerates a wide range of soil conditions but prefers a neutral to slight acidic (pH.6.3-7.0), well-drained sandy or loamy soil. The minimum annual rainfall requirements are estimated at 250mm with maximum at over 3,000mm. However, the roots have the tendency to rot in waterlogged soil. The presence of a long taproot makes it resistant to periods of drought.

2:4 **Growing/propagation**

The shrub can be grown from the seeds or from vegetative propagation (cuttings). *Moringa oleifera* seeds have no dormant period so can be planted as soon as they are mature and can retain the ability to germinate for up to one year (Fuglie, 1999).

In vegetative propagation, hard wood is used, not green wood, for cuttings. The cuttings can be 45cm to 1.5m long and 4-16cm wide. Cuttings can be planted directly or planted in sacks in the nursery. It is advised that the cutting ends must be left to dry in a shady, dry place for the three days before planting. When planting the cuttings directly, they are planted in light, sandy soil. Too much water should not be added; if the soil is too wet or heavy, the roots may rot. Planting directly is a preferred method, because, when the cuttings are planted in the nursery, the root system is slower to develop. Cuttings planted in the nursery can be out-planted after 2-3 months.

Trimming

Moringa tree can grow up straight and tall like a mast if left alone, putting out the leaves and pods at its crown. The central growing tip of the tree should be cut off (about 1.5-2 m high), to encourage production of many branches and pods within easy reach of the ground (Fuglie, 2001). When the growing tips of the branches are regularly cut off, the tree will become bushier. The growing tips are edible. For pod production, it is recommended that the flowers be removed the first year as this will increase the pod yield the second year. Older trees which are unproductive or too high for easy harvesting can be cut down to a stump from which one or two shoots are allowed to grow.

Harvesting

In harvesting leaves, especially in making leaf sauces, seedlings, growing tips or very young leaves are used. Older leaves are ideal for making dry leaf powder, since the tough and wiry stems can be removed during the sifting process. Pods are harvested for human consumption when they are still young and pliable for eating whole. Older pods develop a tough exterior, but their pulp and immature seeds remain edible until shortly before the ripening process begins. For oil extraction, the pods are allowed to dry and turn brown on the tree. The pods are harvested before they split open and fall to the ground (Fuglie, 2001).

2:5 **Uses of *Moringa***

In the ancient world, *Moringa* was well known and has now been 're-discovered" as a multi-purpose tree with a tremendous variety of potential uses (Fuglie, 1999). The edible and pleasant-tasting oil of *Moringa* was highly valued by the ancient Roman, Greek, and Egyptian civilizations for use in making perfumes and in protecting the skin. During the 19th century, *Moringa oleifera* plantations in the West Indies were exporting the oil as Ben oil to Europe for perfume making and as a lubricant for fine machinery (Anon, 1904). In the Indian sub-continent, *Moringa* has long been cultivated for its edible fruit, and today it is being exported fresh and in tins, to Europe and Asia (Fuglie, 2001). The edible leaves are very nutritious and are consumed throughout West Africa including Nigeria as well as in some parts of Asia. The powder from the seed kernels is a natural coagulant which can clarify even very turbid water, removing 90-99% of the bacteria in the process (Folkard and Sutherland, 1996; Ozumba, 2008).

Available literature from studies done in Europe, Asia and some neighbouring African countries show that the plant is rich in rhamnose sugar, glucosinolates and isothiocyanates (Fahey *et al.*, 2001; Benneth *et al.*, 2003; Fahey, 2005) and a number of vitamins and minerals (Barminas *et al.*, 1998; Fuglie, 1999; Sanchez-machado *et al.*, 2006). It has antibiotic activity (Das *et al.*, 1957; Ndiaye *et al.*, 2002; Haristoy *et al.*, 2005), and anti-cancer activities (Akhtar and Ahmad, 1995; Murakami, *et al.*, 1998). The *Moringa* seed contains water-soluble proteins which act as effective coagulants for water and waste water treatment (Santos *et al.*, 2005; Ghebremichael *et al.*, 2006; Bhuptawat *et al.*, 2007 and Akhtar *et al.*, 2007). The leaves and pods contain carbohydrates, fats, proteins and fiber. More importantly, they contain all the essential amino acids (Fuglie, 2001; Ozumba, 2008).

Tables 1-4 show the proximate analysis, mineral, vitamin and essential amino acid compositions of *Moringa oleifera* pods, fresh leaves and dried leaf powder -100g of edible portion (Fuglie, 1999, 2001).

Table 1: Proximate analysis of *Moringa oleifera* pods, fresh leaves and dried leaf powder per 100g of edible portion.

CONSTITUENTS	**PODS**	**LEAVES**	**LEAF POWDER**
Moisture (%)	86.9	75.0	7.5
Calories	26.0	92.0	205.0
Protein (g)	2.5	6.7	27.1
Fat (g)	0.1	1.7	2.3
Carbohydrate (g)	3.7	13.4	38.2
Fibre (g)	48.0	0.9	-

Source: Fuglie, 2001.

Table 2: Mineral composition of *Moringa oleifera* pods, fresh leaves and dried leaf powder per 100g of edible portion.

Mineral (mg/100g)	**Pods**	**Leaves**	**Leaf Powder**
Calcium , Ca	30.0	440.0	2,003.0
Magnesium, Mg	24.0	24.0	368.0
Phosphorus, P	110.0	70.0	204.0
Potassium , K	259.0	259.0	1,324.0
Copper, Cu	3.1	1.1	0.6
Zinc, Zn	-	-	3.3
Iron, Fe	53.0	7.0	28.2
Sulphur, S	137.0	137.0	870.0
Selenium Se	-	-	0.1
Oxalic acid	10.0	101.0	1,600.0

Source: Fuglie, 1999, 2001.

Table 3: Vitamin composition of *Moringa oleifera* parts pods, fresh leaves and dried leaf powder per 100g of edible portion.

Vitamins	**Pods**	**Leaves**	**Leaf Powder**
A-B Carotene (mg)	0.1	6.8	18.9
B-choline (mg)	423.0	423.0	-
B1-Thiamin (mg)	0.1	0.2	2.64
B2-riboflavin (mg)	0.1	0.1	20.5
B3-nicotinic acid (mg)	0.2	0.8	8.2
C-ascorbic acid (mg)	120.0	220.0	17.3
E-tocopherol acetate (mg)	-	-	113.0

Source: Fuglie, 1999, 2001.

Table 4: Essential amino acid composition of *Moringa oleifera* pods, fresh leaves and dried leaf powder per 100g of edible portion.

Amino Acids (mg/100g)	**Pods**	**Leaves**	**Leaf Powder**
Arginine	90.0	402.0	1,325.0
Histidine	27.5	141.0	613.0
Lysine	37.5	288.0	1,325.0
Tryptophan	20.0	127.0	425.0
Phenylalanine	108.0	429.0	1,388.0
Methionine	35.0	134.0	350.0
Threonine	98.0	328.0	1,188.0
Leucine	163.0	623.0	1,950.0
Isoleucine	110.0	422.0	825.0
Valine	135.0	476.0	1,063.0

Source: Fuglie, 2001.

Indeed *Moringa* has been accredited with multiple uses, some of which have been compiled by Fuglie (2001) and Ozumba (2008) and listed as follows:

- *Moringa*'s leaves, seeds, flowers, bark, and roots are used for a variety of medicinal purposes.
- Alley cropping: With rapid growth, long taproot, and large production of high protein biomass, *Moringa* plant is well suited for use in alley cropping.
- Fencing/Environmental uses: *Moringa* trees are used as living support for fencing around houses, gardens and yards. It can be used as shade as well as ornamentally, to improve environmental aesthetics.
- Biogas: The leaves provide excellent material for the production of biogas.
- Green manure/ fertilizer: It is cultivated intensively and then ploughed back into the soil to act as a natural fertilizer for other crops. The seed press cake remaining after oil extraction can be used as fertilizer.
- Gum, Honey, Bark: Gum from the tree trunk is used in calico printing, in making medicines and as a bland tasting condiment. Flowers are good source of nectar for honey-producing bees just as seed powder can be used to clarify honey without boiling. The bark fibre is used for rope and mat production. Also, the bark and gum contain tannin used in tanning of hides and skins.
- Livestock feed/ animal nutrition: The high bioavailability of the leaves and stems make *Moringa* an excellent feed for ruminants and rabbits. The leaves can be used for fish and snail feeding.
- Oil: The seed kernels contain about 40% edible oil similar in quality to olive oil. It is used for cooking, soap making, illumination, as cosmetics, as medicine and as a lubricant for fine watches and other precision instruments.
- Pulp: *Moringa* wood is soft and spongy, thus not good for firewood. However, the wood pulp is highly suitable for the manufacture of writing paper and newsprint. A blue dye is produced from the wood in Senegal and Jamaica.

2:6. **Pharmacological properties of *Moringa***

Every part of *Moringa* plant is used in traditional medicine in Africa, Asia and the Americas (Morton, 1991, Fuglie, 2001 and Ozumba, 2008). *Moringa* preparations have been cited in scientific literature as having antibiotic, antitrypanosomal, antiulcer, anti-inflamatory, hypoglycaemic and hypocholesterolemic effects (Fuglie, 1999; Ghasi *et al*, 2000). Various parts of the plant act as cardiac and circulatory stimulants, possess anti-tumour, diuretic and anti-oxidant activities and are thus employed in the treatment of many ailments in indigenous medical practice (Anwar *et al*, 2006).

Some of the medicinal uses of various parts of *Moringa oleifera* as compiled by Fuglie (2001); Sampson (2005) and Ozumba (2008) are outlined here:

Leaves

- *Moringa* leaves have stabilizing effect on blood pressure and is used to treat anxiety in India.
- In Senegal and India, an infusion of leaf juice is used to control glucose levels in diabetes.
- Leaf juice applied as drops is used against conjunctivitis.
- Leaves are anthelminthic. Example in Malaysia, a leaf poultice is applied to the abdomen to expel intestinal worms.
- In India, leaves are used to treat fever, scurvy, bronchitis, eye and ear infections, and catarrh.
- Leaves are used to treat malaria and jaundice.
- In Nigeria, leaf poultice is effective against inflammations and migraine headache.
- Eating leaves in Philippines is believed to increase a woman's milk production and also used for treatment of anemia.
- Leaf juice is taken as emetic
- Eating leaves is recommended in cases of gonorrhea on account of its diuretic action.
- In Nigeria, leaf sap is used as a purgative.
- When the leaves are mixed with honey and followed by a drink of coconut milk, it has remedial effect for diarrhea, dysentery and colitis.

- In Bahamas and Jamaica, men drink leaf decoction to build up blood and relieve dryness.
- Leaves are used to treat malnutrition.

Flowers

- Treatment of malnutrition
- Flowers are traditionally used as a tonic, diuretic, and abortifacient.
- Anthelminthic.
- Flowers are used to cure inflammations, muscle diseases, tumours and enlargement of the spleen.
- In Haiti, an infusion made from flowers is taken against colds.
- Flowers contain an antibiotic, pterygospermin, which is highly effective against cholera and at high concentration is a fungicide.
- Juice pressed from the flowers alleviates sore throat and catarrh.

Pods

- Treatment of malnutrition.
- Pods are anthelminthic.
- They are used in infections of liver and spleen, and in treating joint pains.

Roots

- Used as carminative and as a laxative in India.
- Roots are useful against intermittent fever and are chewed to relieve cold symptoms.
- They are used as abortifacient, diuretic and as cardiac and circulatory tonic.
- In Senegal and India, roots are pounded and mixed with salt to make a poultice for treating rheumatism and articular pains. The poultice is also used to relieve lower back or kidney pains.
- Roots are used to treat epilepsy, nervous debility and hysteria.
- Used as a purgative.

- In India and China, Nicaragua and Nigeria, a root poultice is used to treat inflammations especially pedal odema and arthritis.
- A decoction of roots is used to cleanse sores and ulcers.
- Used to treat scurvy in India.
- A snuff made from roots is inhaled to relieve earache and toothache.
- Root ash is used in the treatment of splenosis.
- A juice made from a combination of fresh roots, bark and leaves is inserted into nostrils to arouse a patient from coma or stupor.
- Root juice mixed with milk is useful against hiccups, asthma, gout, lumbago, rheumatism, enlarged spleen or liver, internal and deep-seated inflammations and calculous infections.

Bark (root and stem)

- In India, stem and root bark are aphrodisiac and anthelminthic.
- In Senegal, they are used to treat sores and skin infections. Also effective in treating boils.
- Bark is useful in treating scurvy.
- In India, they are taken as appetizers and digestives.
- Stem bark is used to cure eye diseases.
- In India, a paste made from ground bark is applied to relieve the pain caused by rheumatism, scorpion bites and sprains.
- Root bark has antiviral and analgesic properties. The juice is used as painkiller in toothache and earache.
- In Senegal, a decoction of root bark, roots, leaves and flowers is used to treat epilepsy, hysteria and intestinal spasms.
- In Philippines, the roots when chewed and applied to a snake bite, will keep the poison from spreading.
- In India, root bark is used to prevent the enlargement of the spleen and formation of tuberculous glands of the neck, to destroy tumours and to heal ulcers.

Gum

- Gum is used to relieve headaches and earache.
- In India and Senegal, gum is used in treating fevers, dysentery and asthma.
- In India, gum is used to treat syphilis and rheumatism.
- Gum is considered diuretic.
- It is used as astringent and skin tonics (rubefacient).
- Gum is used for dental caries and intestinal complaints in India.

Seeds

- Seeds are used against fevers.
- It is used for abdominal tumours.
- In Aruba, a paste of crushed seeds is spread on warts.

Seed oil

- In India, seed oil is applied externally to relieve pain and swelling in case of gout or rheumatism, and to treat skin diseases.
- Oil is used to treat hysteria and scurvy.
- It is applied to treat prostrate and bladder troubles.
- Oil is a tonic and a purgative.

Unfortunately, many of these reports of efficacy in human beings are not supported by placebo controlled, randomized clinical trials nor have they been published in high visibility journals (Fahey, 2005). In many cases, published *in vitro* (cultured bacterial cells) and *in vivo* (animal) trials do provide a degree of mechanistic support for some of the claims that have sprung from the traditional medicine lore. For example, Jaiswal *et al* (2009) reported that the aqueous extract of the leaves of *Moringa oleifera* drastically reduced the level of glucose in rats. Also, Ghasi *et al* (2000), concluded that the leaves of *Moringa oleifera* have definite hypocholesterolemic activity in rats and that there is valid pharmacological basis for employing them for this purpose in India.

2:7:0. **Phytochemicals**

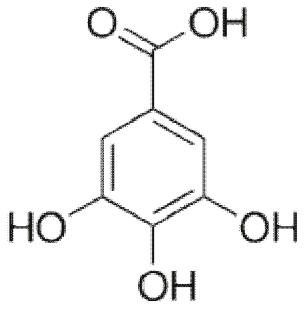
Phytochemicals are the chemicals produced by plants, which may have an impact on health, flavour, texture, smell, or colour of plants, but are not required by humans as essential nutrients (Rao, 2007). Phytochemicals have been used as drugs for millennia. For example, Hippocrates prescribed willow tree leaves to abate fever. Salicin, has anti-inflammatory and pain-relieving properties and was originally extracted from the bark of the white willow tree. It was later synthetically produced and became the staple over-the-counter drug called aspirin. There is evidence from laboratory studies that phytochemicals in fruits and vegetables may reduce the risk of cancer (Liu, 2004), possibly due to dietary fibers, polyphenol antioxidants and anti-inflammatory effects. Specific phytochemicals, such as fermentable dietary fibers, are allowed limited health claims by the United States of America Food and Drug Administration (FDA). The commonest phytochemicals include tannins, saponins, flavonoids, alkaloids and glycosides.

2:7:1. **Tannins**

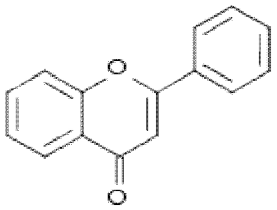
Tannins are polyphenolic compounds that either bind and precipitate or shrink proteins and various other organic compounds including amino acids and alkaloids. The astringency from the tannins is what causes the dry and puckery feeling in the mouth following the consumption of unripe fruit or red wine (Akubugwo *et al.*, 2007). Likewise, the destruction or modification of tannins with time plays an important role in the ripening of fruit and the aging of wine. They have molecular weights ranging from 500 to over 3,000 (Kadam *et al.*, 1990). Tannins are incompatible with alkalis, gelatin, heavy metals, iron, lime water, metallic salts, strong oxidizing agents and zinc sulfate, since they form complexes and precipitate in aqueous solution. They are glycosides of gallic or protocatechic acid and are responsible for the astringent taste of foods and drinks (Akubugwo *et al.*, 2007). They are anti-oxidants (Seeram *et al.*, 2005) and also have antimicrobial actions (Kolodziej and Kiderlen, 2005).

Tannins are divided into two groups namely the hydrolysable and the non-hydrolysable or condensed tannins (proanthocyanidins).

- The hydrolysable tannins contain polyhydric alcohol and usually glucose is esterified with either gallic acid to form gallotannins or ellagic acid to form ellagitannins (Wikipedia, free encyclopedia, 2010).They are mixtures of polygalloyl glucoses and/or poly-galloyl quinic acid derivatives containing in between 3 up to 12 gallic acid residues per molecule. Hydrolysable tannins are hydrolysed by weak acids or weak bases to produce carbohydrate and phenolic acids (Wikipedia, free encyclopedia, 2010).
- Condensed tannins are made up of phenol of flavone type and are called flavolans because they are polymers of flavans e.g. Flavan-3-ol. This is different from hydrolysable tannin by not having sugar residues. On heating with hydrochloric acid, condensed tannins yield phlobaphenes like phloroglucinol. In short, they are polymers of 2 to 50 (or more) flavonoid units that are joined by carbon-carbon bonds, which are not susceptible to being cleaved by hydrolysis. While hydrolysable tannins and most condensed tannins are water soluble, some very large condensed tannins are insoluble (Wikipedia, free encyclopedia, 2010).



Gallic Acid



Flavone

Figure 1: Hydrolysable and condensed tannins
Source: http://en.wikipedia.org/wiki/file:gallic_acid.svg
(Wikipedia, free encyclopedia, 2010)

Toxicity of tannins: Tannic acid slows down the absorption of iron and other trace minerals when used continuously or in high quantities. It also reduces the effectiveness of digestive enzymes such as chymotrypsin and pepsin (Hoon and Hoof, 2006). In sensitive individuals, a large intake of tannins may cause bowel irritation, kidney irritation, liver damage, irritation of the stomach and gastrointestinal pain (Tanimura *et al.*, 2005). With the exception of tea, long-term and/or excessive use of herbs containing high concentrations of tannins is not recommended. A correlation has been made between esophogeal or nasal cancer in humans and regular consumption of certain herbs with high tannin concentrations (Elvin-lewis *et al.*, 1977).

Application of tannins: Tannin has so many applications because of its properties.

- Tannin has anti-bacterial, anti-enzymatic and astringent properties, therefore, it can be used to treat burns, bed sores and minor ulceration (Harborne, 2006).
- It has constringing action upon mucous tissues such as tongue and inside of mouth.
- Ingestion of tannic acid causes constipation and is used to treat diarrhea in the absence of fever or inflammation.
- Anti-oxidant and anti-mutagenic properties of tannic acid are beneficial.

2:7:2 **Saponins**

Saponins are glucosides with foaming characteristics. Saponins consist of a polycyclic aglycones attached to one or more sugar side chains. The aglycone part, which is also called sapogenin, is either steroid (C27) or a triterpene (C30). The foaming ability of saponins is caused by the combination of a hydrophobic (fat-soluble) sapogenin and a hydrophilic (water-soluble) sugar part (Hostettmann and Marston, 1995; Asl and Hossein, 2008). Saponins have a bitter acid taste and are known to cause haemolysis of blood. Some saponins are toxic to cold-blooded organisms and insects at particular concentrations and are called sapotoxins.

Structurally, saponins are composed of one or more hydrophilic glycoside moieties combined with lipophilic triterpene derivatives and are therefore employed for washing and as emulsifying agents. Historically, saponins are plant-derived, but they have also been isolated from marine organisms.

In plants, saponin serves as anti-feedant, and protects it against microbes and fungi (Francis *et al.*, 2002). Some plant saponin (eg. from oat and spinach) may enhance nutrient absorption and aid in animal digestion. However, saponins are often bitter to taste and can reduce plant palatability or even imbue them with life threatening animal toxicity.

One research use of the saponin class of natural products involves their complexation with cholesterol to form pores in cell membrane bilayers, e.g., in red cell (erythrocyte) membranes, where complexation leads to red cell lysis (hemolysis) on intravenous injection (Francis *et al.*, 2002). In addition, the amphipathic nature of the class gives them activity as surfactants that can be used to enhance penetration of macromolecules such as proteins through cell membranes. Saponins have also been used as adjuvants in vaccines (Skene and Philip, 2006). Pharmacologically, saponins are used in the treatment of hypercholesterolemia, hyperglycemia, and as anti-oxidant, anti-cancer, anti-inflammatory and anti-bacterial (Francis *et al.*, 2002).

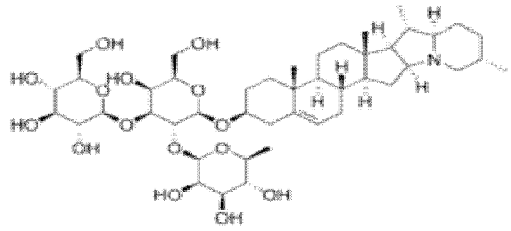


Figure 2: Chemical structure of the saponin: solanine.
Source: http://en.wikipedia.org/wiki/file:solanine.png

While such pharmacological claims require constant review, it appears that there are very limited United States of America, European Union etc agency-approved roles for saponins in human therapy. In their use as

adjuvants in the production of vaccines, toxicity associated with sterol complexation remains a major issue for attention (Skene and Philip, 2006). Even in the case of digoxin, therapeutic benefit from the cardiotoxin is a result of careful administration of an appropriate dose (Skene and Philip, 2006). Very great care needs to be exercised in evaluating or acting on specific claims of therapeutic benefit from ingesting saponin-type and other natural products.

2:7:3. **Flavonoids.**

Flavonoids are water soluble polyphenolic molecules containing 15 carbon atoms. They can be visualized as two benzene rings which are joined together with a short three carbon chain. One of the carbons of the short chain is always connected to a carbon of one of the benzene rings, either directly or through an oxygen bridge, thereby forming a third middle ring, which can be five or six-membered. The flavonoids consist of 6 major subgroups: chalcone, flavone, flavonol, flavanone, anthocyanins and isoflavonoids (Galeotti *et al.*, 2008). Together with carotenes, flavanoids are also responsible for the colouring of fruits, vegetables and herbs. Green and black tea contains about 25% flavonoids (Galeotti *et al.*, 2008).

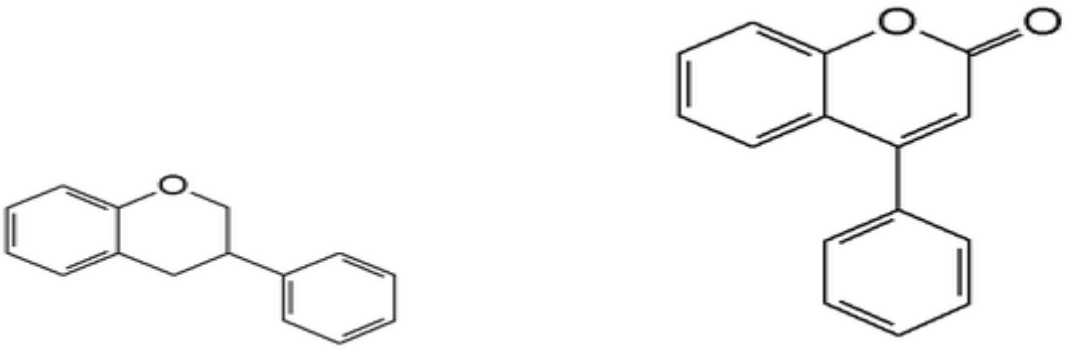


Figure 3: Isoflavan and Neoflavonoid structures.
Source: http://en.wikipedia.org/wiki/file:isoflavan.png

- Anthocyanins contribute to colour of petals, leaves and blueness of flowers.
- Flavanones act as co-pigments, intensifying the colour of yellow flavonol widespread in fruits and flowers.
- Flavonols when methylated contribute to the yellow colour of meadow pea.
- Chalcones and aurones contribute to the colour of petals in some plants.

The pharmacological uses of flavonoids (Cushnie and Lamb, 2005; Galeotti *et al.*, 2008):

- Lower cancer risk
- Reduce the risk of shock and heart disease
- Protect against age-related vision disorders such as cataracts and molecular degeneration.
- Alleviate inflammatory skin conditions such as eczema and bug bites.
- Relieve hay fever, sinusitics and asthma symptom.
- Reduce inflammation in the joints and muscles common to fibromyalgia, gout and rheumatoid arthritis.
- They minimize menopausal hot flushes.
- Attack viral infection.

2:7:4 **Alkaloids**

Alkaloids are complex nitrogenous compounds of heterocyclic structure such as derivatives of pyridine, quinoline, isoquinoline and indole, found mainly in plants and possess marked physiological activities (Manske, 1965). Their chemical classification is based on the aromatic basic structures. In plants, alkaloids occur combined with acid as a salt. Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants, and animals and are part of the group of natural products called secondary metabolites. Many alkaloids can be purified from crude extracts by acid-base extraction (Hesse, 2005). Many are toxic to other organisms. They often have pharmacological effects and are used as medications, as recreational drugs, or in entheogenic rituals. Examples are the local

anesthetic and stimulant cocaine, the stimulant caffeine, nicotine, the analgesic morphine, or the antimalarial drug quinine. Most alkaloids have a bitter taste (Hesse, 2005).

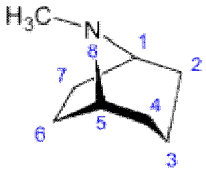
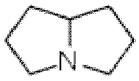
There are three main types of alkaloids:

- True alkaloid which are derived from amino acids and have heterocyclic ring with nitrogen.
- Proto alkaloids which are derived from amino acids but do not have heterocyclic ring with nitrogen. Example is colchicine.
- Pseudo alkaloids. They can be derived from terpenoids or purines and have heterocyclic ring with nitrogen.

The non-nitrogen containing ring or side chains are derived from terpene units and/or acetate while methionine is responsible for the addition of methyl group to nitrogen atom (Mercer and Godwin, 1993).

The most potent alkaloids act mainly on the central and autonomic nervous systems. Different levels of the nervous system may be depressed or stimulated by different alkaloids and those which stimulate the nervous system generally produce subsequent depression or even paralysis when used in large doses (Manske, 1965). Therefore, they can act as narcotics, sedatives, analgesics, anti-pyretics, anti-spasmodics and as stimulants, tonics and convulsants. They are used to stimulate or regulate the function of smooth muscles and operate through the nerve centres by which these muscles are controlled. Thus they can stimulate the bowel or the uterus or increase the secretion of bile or bronchi and acts as laxatives, emetics, expectorants and anti-asthmatic. The toxic action of alkaloids against lower form of animal life is made up of their application as anthelatics, anti-parasitic and locally as fish poison. The antibiotic effect is used in the specific treatment of certain infections (Manske, 1965; Hesse, 2005).

Table 5: True alkaloids

Class	Major groups	Main synthesis steps	Examples
Pyrrolidine derivatives 		Ornithine or arginine → putrescine → N-methylputrescine → N-methyl-Δ^1-pyrroline	Hygrine, hygroline, stachydrine
Tropane derivatives 	Atropine group Substitution in positions 3, 6 or 7	Ornithine or arginine → putrescine → N-methylputrescine → N-methyl-Δ^1-pyrroline	Atropine, scopolamine, hyoscyamine
	Cocaine group Substitution in positions 2 and 3		Cocaine, ecgonine
	Non-esters		Retronecine, heliotridine, laburnine
	Complex esters of monocarboxylic acids	In plants: ornithine or arginine → putrescine → homospermidine → retronecine	Indicine, lindelophin, sarracine
Pyrrolizidine derivatives 	Macrocyclic diesters		Platyphylline, trichodesmine
	1-aminopyrrolizidines (lolines)	In fungi: L-proline + L-homoserine → *N*-(3-amino-3-carboxypropyl)proline → norloline	Loline, *N*-formylloline, *N*-acetylloline

Source:http://en.wikipedia.org/wiki/file: alkaloid.

Table 6: Protoalkaloids

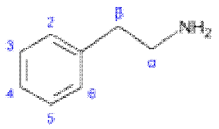
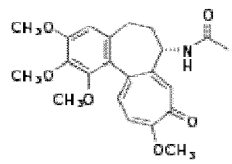
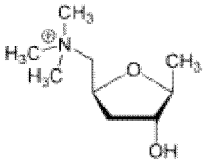
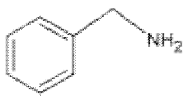
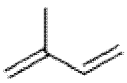
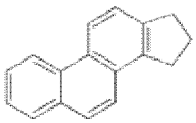
β-Phenylethylamine derivatives 	Tyrosine or phenylalanine → dioxyphenilalanine → dopamine → adrenaline and mescaline tyrosine → tyramine phenylalanine → 1-phenylpropane-1,2-dione → cathinone → ephedrine and pseudoephedrine	Tyramine, ephedrine, pseudoephedrine, mescaline, cathinone, catecholamines (adrenaline, noradrenaline, dopamine)
Colchicine alkaloids 	Tyrosine or phenylalanine → dopamine → autumnaline → colchicine	Colchicine, colchamine
Muscarine 	Glutamic acid→ 3-ketoglutamic acid → muscarine (with pyruvic acid)	Muscarine, allomuscarine, epimuscarine, epiallomuscarine
		Capsaicin, dihydrocapsaicin, nordihydrocapsaicin
Benzylamine 	Phenylalanine with valine, leucine or isoleucine	

Table 7: Polyamines alkaloids

Putrescine derivatives 		Paucine
Spermidine derivatives 		Lunarine, codonocarpine
		Verbascenine, aphelandrine
Spermine derivatives 	ornithine → putrescine→ spermidine → spermine	

Source:http://Wikipedia.org/wiki/file:alkaloid

Table 8: Pseudoalkaloids

Diterpenes 	Licoctonine type	Mevalonic acid → izopentenilpyrophosfate → geranyl pyrophosphate	Aconitine, delphinine
Steroids 		Cholesterol, arginine	Solasodine, solanidine, veralkamine

Source:http://en.wikipedia.org/wiki/file:alkaloid

2:7:5. **Glycosides**.

These are compounds containing a carbohydrate residue (glycone) and a non-carbohydrate residue (aglycone) in the same molecule. Glycosides can be linked by an O- (an O-glycoside), N- (a glycosylamine), S-(a thioglycoside) or C- (a C-glycoside) glycosidic bond (Lindhorst, 2007). Some of the groups belonging to glycosides are flavanol, phenol, isothiocyanate lactone etc. Glycosides are distinquished by their products on hydrolysis. In plants, they are found together with the hydrolysing enzymes. Generally, they are irritating to the gastric mucosa which leads to nausea and to their use as emetics and expectorants.

Cyanogenic glycosides: They are present in a number of food plants and seeds. In this case, the aglycone (non-sugar moiety) contains a cyanide group. In many plants, these glycosides are stored in the vacuole but if the plant is attacked, they are released and become activated by enzymes in the cytoplasm. These remove the sugar part of the molecule and release toxic hydrogen cyanide (Brito-Arias, 2007). Storing them in inactive forms in the cytoplasm prevents them from damaging the plant under normal conditions. Cassava contains cyanogenic glycosides and therefore has to be washed and ground under running water prior to consumption. Sorghum (*Sorghum bicolor*) expresses cyanogenic glycosides in its roots and thus is resistant to pests such as rootworms (*Diabrotica spp*.) that plague its cousin maize *(Zea mays L.*). It was once thought that cyanogenic glycosides might have anti-cancer properties, but this idea was disproved. A recent study may also show that increasing CO_2 levels, caused by anthropogenic emissions, may result in much higher levels of cyanogenic glycoside production in Sorghum and Cassava plants, making them highly toxic and inconsumable. A doubling of CO_2 concentration was found to double the concentration of cyanogenic glycosides in the leaves (Brito-Arias, 2007).

Cyanide is one of the most potent, rapidly acting, poisons known. They inhibit the oxidative processes of cells causing them to die very quickly. An adult man can withstand 50-60ppm of cyanide for an hour without serious consequences, because the body rapidly detoxifies it. Acute cyanide toxicity can cause headache, tightness in the throat and chest, and muscle weakness.

However, the effects of chronic exposure to cyanide are less known (Lindhorst, 2007).

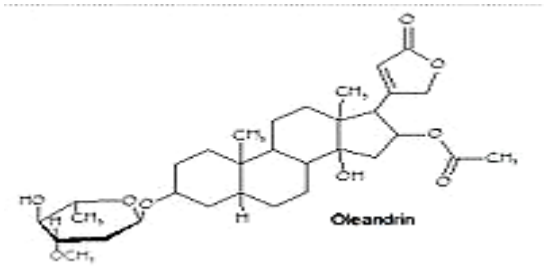


Figure 4: Chemical structure of glycoside: Oleandrin.
Source:http://en.wikipedia.org/wiki/file:glycoside.jpg.

2:7:6. **Phytate**

Phytic acid (known as inositol hexakisphosphate (IP6), or phytate when in salt form) is the principal storage form of phosphorus in many plant tissues, especially bran and seeds. Inositol penta- (IP5), tetra- (IP4), and triphosphate (IP3) are also called phytates. It is found in most grains, seeds and beans. The structure of phytic acid gives it the ability to bind minerals, proteins and starch, thus resulting in lower absorption of these substances. It has a strong binding affinity with important minerals such as calcium, magnesium, iron, and zinc. When a mineral binds to phytic acid, it becomes insoluble, precipitates and will be inabsorbable in the intestines. This process can therefore contribute to mineral deficiencies in people whose diets rely on these foods for their mineral intake, such as those in developing countries (Hurrell, 2003). It also acts as an acid, chelating the vitamin niacin, which is basis, causing the condition known as pellagra (Anderson, 2005). However, on the contrary, one study correlated decreased osteoporosis risk with phytic acid consumption (López-González *et al.*, 2008). Following from these opposing findings, phytic acid is an anti-nutrient, despite its possible therapeutic effects.

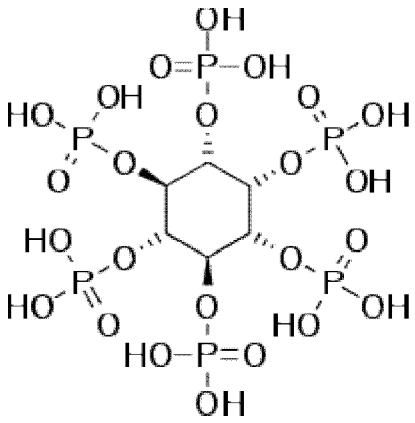


Figure5: Phytic acid

Source: http://en.wikipedia.org/wiki/file:phytic-acid.svg.

Therapeutic effect of phytic acid: Phytic acid may be considered a phytonutrient, providing an antioxidant effect. Phytic acid's mineral binding properties may also prevent colon cancer by reducing oxidative stress in the lumen of the intestinal tract (Vucenik and Shamsuddin, 2003). Researchers now believe that phytic acid, found in the fiber of legumes and grains, is the major ingredient responsible for preventing colon cancer and other cancers (Jenab and Thompson, 2000). Phytic acid protects against Parkinson's disease *in vitro* (Xu *et al.*, 2008). The compound significantly decreased apoptotic cell death induced by 1-methyl-4-phenylpyridinium in a cell culture model. It is also known that, at least in rodents, phytic acid crosses the blood-brain barrier (Grases *et al.*, 2001),thus, there is a strong possibility that neuroprotection occurs *in vivo* as well. Ironically it has also been shown that phytic acid is a required cofactor for YopJ, a toxin from *Yersinia pestis* (Mittal *et al.*, 2010). It is also a required cofactor for the related toxin AvrA from *Salmonella typhimurium* (Mittal *et al.*, 2010).

Phytic acid's chelating effect may serve to prevent, inhibit, or even cure some cancers by depriving those cells of the minerals (especially iron) they need to reproduce (Xu *et al.*, 2008). The deprivation of essential minerals like iron would, much like other broad treatments for cancers, also have

negative effects on non-cancerous cells. It is unknown whether this would affect other cells in the body that require iron (such as red blood cells) or whether the deprivation of minerals is more localized to the internal colon region. Phytic acid is one of few chelating therapies used for uranium removal (Cebrian *et al.*, 2007). As a food additive, phytic acid is used as a preservative with E number E391. Therefore, the health benefits of phytic acid include anti-oxidant, anti-cancer, hypocholesterolemic and hypolipidemic.

2:7:7. **Oxalic acid.**

Oxalic acid occurs in a large number of plant species where it usually occurs as crystals of the insoluble calcium salts (Turner, 1980). Oxalate (ethanedioate) is the dianion with formula $C_2O_4^{2-}$ also written $(COO)_2^{2-.}$ Many metal ions form insoluble precipitates with oxalate, a prominent example being calcium oxalate, the primary constituent of the most common kind of kidney stones.

The affinity of divalent metal ions is sometimes reflected in their tendency to form insoluble precipitates. Thus in the body, oxalic acid also combines with metals ions such as $Ca^{2+,}$ $Fe^{2+,}$ and Mg^{2+} to form crystals of the corresponding oxalates which are then excreted in urea. Those with kidney disorders, gout, rheumatoid arthritis, or certain forms of chronic vulvar pain (vulvodynia) are typically advised to avoid foods high in oxalic acid. The calcium oxalate crystals or precipitate (kidney stones) can obstruct the kidney tubules. An estimated 80% of kidney stones are formed from calcium oxalate (Coe *et al.*, 2005). In studies with rats, calcium supplements given along with foods high in oxalic acid can cause calcium oxalate to precipitate out in the gut and reduce the levels of oxalate absorbed by the body-by 97% in some cases-(Morozumi *et al.*, 2006).

It arises biosynthetically via the incomplete oxidation of carbohydrates. Oxalic acid can also be produced by the metabolism of ethylene glycol ("antifreeze"), glyoxylic acid or ascorbic acid (vitamin C). Some *Aspergillus* species produce oxalic acid, which reacts with blood or tissue calcium to

precipitate calcium oxalate (Pabuccuoglu, 2005). There is some preliminary evidence that the administration of probiotics can affect oxalic acid excretion rates (Lieske *et al.*, 2005). Although unusual, consumption of oxalates (for example, the grazing of animals on oxalate-containing plants such as greasewood or human consumption of Sorrel) may result in kidney disease or even death due to oxalate poisoning. The presence of *Oxalobacter formigenes* in the gut flora can prevent this.

Phytochemical constituents isolated from Moringa oleifera Lam.

Mehnaz (2008) compiled the literature on phytochemical constituents isolated from different parts of *Moringa oleifera.*

Roots : 4-(α-L-rhamnopyranosyloxy)- benzylglucosinolate and benzylglucosinolate (Bennett *et al.*, 2003)

Stem: 4-hydroxymellein, vanillin, β-sitosterone, octacosanic acid and β-sitosterol (Saluja *et al.*, 1978).

Bark: 4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate (Bennett *et al.*, 2003).

Whole gum exudates: L-arabinose, D-galactose, D-glucuronic acid, L-rhamnose, D-mannose, D-xylose and leucoanthocyanin (Khare *et al.*, 1997).

Leaves: Glycoside niazirin, niazirinin and three mustard oil glycosides, 4-[4'-O-acetyl- α -L-rhamnosyloxy) benzyl] isothiocyanate, niaziminin A and B (Faizi *et al.*, 1994; Faizi *et al.*, 1995).

Mature flowers: D-mannose, D-glucose, protein, ascorbic acid, polysaccharide (Pramanik and Islam, 1998).

Whole pods: Nitriles, isothiocyanate, thiocarbanates, 0-[2'-hydroxy-3'-(2''-heptenyloxy)]-propylundecanoate, 0-ethyl-4-[(α -1-rhamnosyloxy)-benzyl] carbamate, methyl-p-hydroxybenzoate and ?-sitosterol (Faizi *et al.*, 1995).

Mature seeds: Crude protein, Crude fat, carbohydrate, methionine, cysteine, 4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate, benzylglucosinolate, moringyne, mono-palmitic and di-oleic triglyceride (Bennett *et al.*, 2003).

Seed oil: Vitamin A, beta carotene, precursor of Vitamin A (Dahot and Memon, 1985; Memon *et al.*, 1985).

CHAPTER THREE

3:0 MATERIALS AND METHODS

3:1 Sample collection, identification and treatment:

The mature dry *Moringa oleifera* pods, fresh leaves, stems, roots and flowers were collected from family gardens in Ifite, Awka, Anambra state, Nigeria. The plant was identified by Prof. J.C. Okafor, taxonomist/consultant, Enugu and Moringa Development Association, Unizik, Awka. The pods were split open and the seeds removed, and then pooled together to form the bulk sample. The seeds, the leaves, and the flowers were dried under room temperature for two days and separately ground into fine powder using manual grinder, while the roots and stems were cut into pieces first using knives and dried separately, before grinding into fine powder. The milled samples were kept in screw-capped containers and stored in a deep freezer and analyzed within seven days.

3:2 Proximate analysis:

The methods of the Association of Official Analytical Chemists (AOAC, 1999) were used for the determination of moisture, crude protein, crude lipids, ash and dry matter of the seeds, leaves, flowers, roots and the stems of *Moringa oleifera*.

3:2:1 Determination of moisture and dry matter content

This procedure measures the water content of samples.

Equipments/Instruments

Mettler weighing balance,

Dessicator,

Oven,

Silica dish

Procedure: The silica dishes were washed, dried, heated and cooled in a dessicator, and then weighed. Finely ground *Moringa oleifera* leaves, seeds,

flowers, stems and roots (2.0g each) were weighed accurately into them. The clean dishes with the samples were weighed and then transferred into an oven and heated at 80°C for two hours until a constant weight is achieved. After two hours, the dishes were brought out and allowed to cool at room temperature in an activated gel dessicator and then re-weighed and recorded. The difference in weight in percentage (%) was calculated thus:

$$\% \text{ Moisture} = \frac{(a + b) - (a + c)}{\text{Weight of sample}} \times \frac{100}{1}$$

Where:

a + b = weight of sample + dish before drying

a + c = weight of sample + dish after drying

The dry matter (%) was derived from the formular:

Dry matter (%) = 100% - moisture (%)

3:2:2. **Determination of Crude proteins**

Equipments/Instruments:

-Kjeldahl digestion flask

-Distillation apparatus

Chemicals and Reagents:

All reagents are of analytical grade and are manufactured by BDH Chemicals (UK) except where otherwise stated. All solutions were made with distilled water and analytical grade reagents.

-40% NaOH (40g NaOH in 100ml distilled water).

-2% Boric acid (2g Boric acid in 100ml distilled water)

-0.1N HCl (0.83ml HCl and made up to 100ml)

-Copper sulphate

-Sodium sulphate

-Conc. Sulphuric acid

-Kjeldahl catalyst (Mixed 10g of anhydrous sodium sulphate and 1g of copper sulphate)

-Methyl red indicator (0.1g methyl red in 18.6ml 0.2M NaOH and made up to 250ml).

Principle:

The total protein content in a sample is quantified from its nitrogen content. First, the sample is digested in a boiling concentrated sulphuric acid containing copper as catalyst. During the digestion, the protein is oxidized and the ammonia formed remains in solution as ammonium sulphate. The concentration of ammonia liberated by alkaline distillation with NaOH is determined by distilling into boric acid solution. Back-titration is done on the solution using a standard acid (0.1N HCl).

Chemistry of reaction.

$2NH_3 + H_2SO_4 \rightarrow (NH_4)_2\ SO_4$

$(NH_4)_2\ SO_4 + 2NaOH \rightarrow Na_2SO_4 + 2NH_3\uparrow + 2H_2O$

$NH_3\uparrow + H_2O \rightarrow NH_4OH$

With boric acid, we have:

$H_3BO_3 + NH_3\uparrow \rightarrow NH_4^+H_2BO_3$

When we titrate with HCl, we have:

$NH_4OH + HCl \rightarrow NH_4Cl + H_2O$

Digestion:

One gramme (1.0g) each of the *Moringa oleifera* leaves, seeds, flowers, stems and roots powder were respectively weighed out and introduced separately into the bottom of a 100ml kjeldahl flask. Concentrated sulphuric acid, H_2SO_4, (20ml) was added and mixed gently by swirling under tap water. Anhydrous sodium sulphate (10g) and copper sulphate (1g) were

mixed together, and 3.0g of the mix (kjeldahl catalyst) was introduced into the separate digestion flasks. Anti-bumping chips were added into the mixture to prevent splashing during digestion. The entire mixture was boiled gently in the different kjeldahl flasks in a fume cupboard until charred particles disappeared and a clear green solution was obtained. Each digest was made up to 100ml with distilled water.

Distillation:

Ten milliliters (10ml) of 2% boric acid was measured into a 250ml beaker, and a methyl red indicator added. From the digest, 10ml was collected and placed in the distillation flask. The distillation apparatus was set up, and 30ml of 40% NaOH was added to the distillation flask slowly from a syringe. The distillation apparatus was heated for 25 min. The receiver beaker containing 2% boric acid was removed and titration done on the distillate with 0.1N HCl until the end point. The distillation procedure was done for the different parts of the plant.

Calculation:

$$\%\text{Nitrogen} = \frac{1.4 \times \text{titre value} \times \text{Dilution factor}}{\text{Weight.of sample (mg)}} \times 100$$

$$\%\text{ Crude protein} = \%\text{ Nitrogen} \times 6.25$$

3:2:3 Determination of crude Lipid content

Equipments/Instruments:

-Soxhlet apparatus consisting of Reflux condenser, extractor, flat bottomed flask, thimble, water inlet and outlet.

Chemical and Reagent:

-Petroleum ether, 60°C - 80°C (BDH Chemicals, UK).

Principle:

The soxhlet extraction method is used to determine the lipid content of a sample. It is mainly by the gravimetric method of AOAC (1999). The free lipid content (neutral fats-triglycerides) and the free fatty acids are determined by extracting the dried sample with a light petroleum fraction in a continuous extraction apparatus. The free lipid constituents can be extracted by less polar solvents (example light petroleum fractions and diethyl ether) while bound lipid components require more polar solvents (eg. alcohols). The solvent is distilled off and the extract is dried off in a dessicator and weighed.

Procedure:

The clean flat bottomed flask was weighed and the petroleum ether (60°C - 80°C) was added into it (half-filled). The flask was connected to the extractor (containing the thimble) and then to the reflux condenser with water inlet and outlet. A known weight (5g) of the *Moringa* leaves, seeds, flowers, stems and roots was separately put in the thimble (already dried in an oven). Anti-bumping granule was added into the solvent system and cotton wool used to cover the mouth of the apparatus to prevent loss of the solvent. The set up was heated, and as the solvent boiled, it evaporated and passed through the extractor to the condenser where the vapour cooled and dropped into the thimble in the extractor to extract the oil. When the solvent reached the highest mark, it flushed back to the flask by an automatic siphoning device. The extraction was done continuously for three hours for the leaves, seeds, flowers, stems and roots. The extractant was distilled off and the flask re-weighed after cooling in the dessicator.

Calculation:

$$\% \text{ lipid} = \frac{\text{weight of lipid}}{\text{Weight of sample}} \times \frac{100}{1}$$

$$\text{ie.} = \frac{\text{weight of flask and lipid - weight of flask}}{\text{weight of sample}} \times \frac{100}{1}$$

3.2.4 **Determination of Ash content**

The total ash is the inorganic residue left after the organic matter has been burnt. It measures the mineral content of samples.

Equipments/Instruments:

-Muffle furnace

-Crucibles.

-Dessicator

-Weighing balance

Procedure:

The crucibles were thoroughly washed, cleaned, and placed in an oven for two hours and cooled in a dessicator. The empty crucibles were transferred to the muffle furnace to burn off all organic matter and to stabilize the weight of the crucibles at 550°C temperature, and then cooled in a dessicator. Two grammes (2.0g) each of the defatted *Moringa oleifera* leaves, seeds, flowers, stems, and roots were respectively weighed into the labelled crucibles. These were placed in a murfle furnace and incinerated (ashed) at 550°C for 3hours. At the end of the ashing period, the samples were removed into a dessicator to cool to room temperature and re-weighed.

Calculation:

$$\%Ash = \frac{\text{weight of crucible and ash} - \text{weight of crucible}}{\text{Weight of sample}} \times 100$$

3:2:5 **Determination of Total carbohydrate**

This measures the carbohydrate content and in most cases includes the fibre content of the sample. The %Carbohydrate of *Moringa oleifera* leaves, seeds, flowers, stems, and roots were conveniently determined by the difference method (Amadi *et al*, 2004).

Total CHO = 100 – (%lipid + %protein + %ash + %moisture).

3:2:6 **Determination of Energy value of sample**

The energy value of the samples was calculated following the method of Onyeike *et al,* 2000. This was done by multiplying the values of proteins, lipids and carbohydrates with the factors 4, 9 and 4 respectively. The sum of these values is expressed in kcal/100g sample (Amadi *et al*, 2004). The energy value of *Moringa oleifera* leaves, seeds, flowers, stems, and roots were separately estimated.

3:3 **Amino acid analysis**

The amino acid profile of *Moringa oleifera* plant materials were determined using the methods described by Speckman *et al,* 1958. The Technicon Sequential Multisample Amino acid Analyzer (TSM) was used.

General description:

The Technicon Sequential Multisample Amino acid Analyzer (TSM) is an automated instrument designed to separate, detect and quantify amino acids. The system can separate and analyze free acidic, neutral and basic amino acids from a protein hydrolysate in one and one-quarter hours and from physiological fluids and or tissue extracts in five and one-half hours.

After loading samples, the operator starts the instrument which will then provide accurate, reproducible results on a peak corresponding to the magnitude of their respective concentrations.

Principle:

The operational principle of an amino acid analyzer is based on the separation of amino acid on ion exchange resin columns i.e. elution chromatography. The separated amino acids are reacted with ninhydrin and the absorbance of the resulting fractions measured on a colorimeter. The resultant absorbances are plotted on a strip of chart recorder. The area under each peak gives an estimate of the quantity of each amino acid present. The analyzer must be standardized prior to determining the unknown content of a sample.

Procedure:

The dried and milled leaves, seeds, flowers, roots and stems of *Moringa oleifera* were separately defatted, hydrolysed, evaporated in a rotary evaporator as detailed hereunder and then loaded into the Technicon Sequential Multi-Sample Amino Acid Analyzer (TSM).

(i) **Defatting of sample**:

Five grammes (5.0g) of the dried *Moringa oleifera* samples *(*leaves, seeds, flowers, stems and roots) were separately weighed into extraction thimble and the fat was extracted with chloroform/methanol (2:1) mixture using soxhlet extraction apparatus as described by AOAC (2006). The extraction lasted for 15 hours.

(ii) **Nitrogen determination:**

Two hundred milligrammes (200mg) of ground *Moringa oleifera* plant parts were separately weighed, wrapped in whatman filter paper (No.1) and put in a Kjeldahl digestion flask. Concentrated sulphuric acid (10ml) was added to each digestion flask. A catalyst mixture (0.5g) containing sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and selenium oxide (SeO_2) in the ratio of 10:5:1 was added into the flasks to facilitate digestion. Anti-bumping granules (4 pieces) were added.

The flasks were then put on a kjeldahl digestion apparatus for 3 hours until the liquid turned light green. The digested samples were cooled and diluted with distilled water to 100ml in standard volumetric flask. Aliquot (10ml) of the diluted solution with 10ml of 45% sodium hydroxide were put into the Markham distillation apparatus and distilled into 10ml 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70ml of distillate was collected. The distillation was done for each sample.

The distillate was then titrated with standardized 0.01N hydrochloric acid to grey coloured end point. The %Nitrogen in the original sample was calculated using the formula:

$$\%\text{Nitrogen} = \frac{(a-b) \times 0.01 \times 14 \times v}{wc} \times \frac{100}{1}$$

where

a = Titre value of the digested sample

b = Titre value of blank sample

v = Volume after dilution (100ml)

w = Weight of dried sample (mg)

c = Aliquot of the sample used (10ml)

14mg = Nitrogen constant in mg

100 = Conversion factor to percentage

(iii) **Hydrolysis of the sample**

Fifty milligrammes (50mg) of the defatted *Moringa oleifera* leaves, seeds, flowers, stems and roots were separately weighed into an ampoule. 6N HCl (7ml) was added and oxygen was expelled by passing nitrogen into the ampoule. This was to avoid possible oxidation of some amino acids such as methionine and cystine during hydrolysis. The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105°C ± 5°C for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humins. The filtrate was then evaporated to dryness at 40°C under vacuum in a rotary evaporator. The residue was dissolved with 5ml of acetate buffer (pH 2.0) and stored in plastic specimen bottles which were kept in a deep freezer.

(iv) **Loading of the hydrolysate into the TSM Analyzer**

The amount of hydrolysate loaded into the TSM Analyzer (Plate 7) was between 5-10microlitre. This was dispensed into the cartridge of the analyzer. The TSM Analyzer is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate. The period of the analysis lasted for 76 minutes. As the amino acids were separated on ion exchange resin during elution, a chromatogram was produced with corresponding peaks. The amino acid values of samples were calculated from the chromatogram peaks.

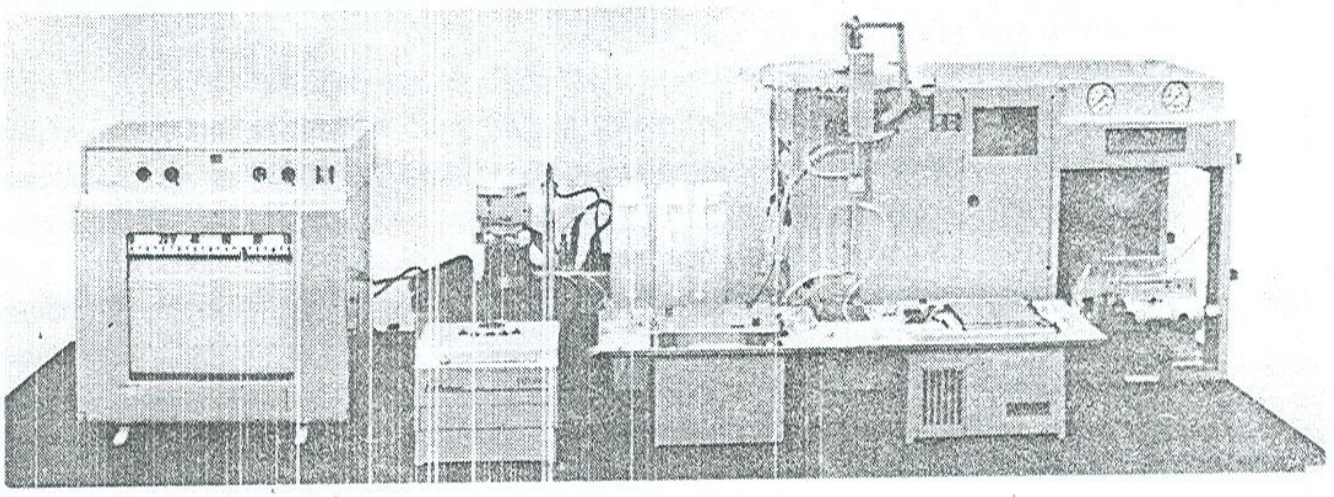


Plate 7: Technicon sequential Multi-Sample Amino Acid Analyzer (TSM)

Method of calculating amino acid values from the chromatogram peaks.

The net height (NH) of each peak produced by the chart recorder of TSM (each representing an amino acid) was measured. The half-height (NH/2) of the peak on the chart was found and the width of the peak at the half-height was accurately measured (width at NH/2) and recorded. Approximate area of each peak was then obtained by multiplying the height with the width at the half-height. Since internal standard was used, the norleucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formula:

$$NE = \frac{\text{Area of norleucine peak (NH} \times \text{W)}}{\text{Area of each amino acid}}$$

Reference to the NE above, a constant Sstd was calculated for each amino acid in the standard mixture

$$Sstd = NEstd \times mol.weight \times MAAstd$$

Where:

NEstd = Norleucine equivalent in the standard

Mol.weight = Molecular weight of amino acid

MAAstd = micromole of standard (0.025).

Finally, the amount of each amino acid present in the sample was calculated in g/16N or g/100g protein using the following formula:

Concentration (g/100g protein) = NH × Width at NH/2 × Sstd × C

Where:

$$C = \frac{\text{Dilution} \times 16}{\text{Sample wt. (g)} \times \%N \times 10 \times \text{vol.loaded}} \div NH \times W(nleu)$$

W (nleu) = width of norleucine

NH = net height

%N = % Nitrogen in the sample

Vol. loaded = Basic column = 10 µl

Acid/Neutral column = 5 µl.

3:4 **Determination of mineral composition**

The mineral content is obtained by first putting the sample in a muffle furnace at 550°C for 2-3 hours to remove the organic matter. The inorganic material (ash) left is introduced into the highly sophisticated atomic absorption spectrophotometer (AAS), which will read and quantify the mineral content (AOAC, 1999).

Equipments/Instruments

-Atomic absorption spectrophotometer (Techcomp AA6000)

-Oven (Gallenkamp).

-Computer system and spectrum analyst software

-Centrifuge

Chemicals and Reagents:

All reagents are of analytical grade and are manufactured by BDH chemicals Ltd (UK) except where otherwise stated.

-Conc. Sulphuric acid

-70% Nitric acid

-Aqua regia solution (1.2l distilled water + 400ml conc. HCl + 133ml 70% HNO_3 and made up to 2litres).

Procedure:

Moringa oleifera leaves, seeds, flowers, stems and roots (0.48-0.52g) were separately weighed into the ceramic crucibles and put in the muffle furnace at a temperature of 550°C for 2 hours. After the dry ash digestion, the crucibles were allowed to cool in the oven, and then poured into already labelled 50ml centrifuge tubes. The crucibles were rinsed with 5ml distilled water, and rinsed again with 5ml of aqua regia into the centrifuge tubes. The rinsing (with aqua regia) was done two times more to make a total volume of 20ml. The samples were properly mixed and centrifuged for ten minutes at 300rpm. The supernatants were decanted into clean vials for macro- and micro- nutrients determination using atomic absorption spectrophotometer (AAS).

The AAS was set to working condition. The hollow cathode lamps used were warmed up before use. Each mineral estimated has its own hollow cathode lamp. For the standard reading, blank solution was aspirated and its reading set to zero by pressing "Auto zero" button. The standard solutions were measured starting from the lowest concentration. The calibration curve was obtained by clicking on the "check working curve." The suitable working curve to be used was checked and selected.

For the sample reading, blank sample was aspirated and the reading set to zero by pressing "Auto zero" button. The *Moringa* samples were aspirated and measured. Finally the distilled water was aspirated for 5 minutes to clean the burner head. The measurement results were saved and printed out.

Calculation:

$$\text{Actual conc. (\%)} = \frac{a \times b \times c \times d}{\text{Wt. factor}}$$

Where

a = conc. (ppm)

b = vol. factor

c = dilution factor

d = correction factor

3.5 Determination of the vitamin content

Equipments/Instruments:

-High performance liquid chromatography (HPLC/W1/08).

-Analytical weighing balance

Chemicals and Reagents:

All reagents are of analytical grade and are manufactured by BDH chemicals Ltd (UK) except where otherwise stated.

-Methanol

-Glacial acetic acid

-HPLC solvent

-Sodium hexane sulphonic acid salt.

-Vitamin Standards: ascorbic acid (6.0mg), nicotineamide (4.0mg), pyridoxine hydrochloride (3.6mg), thiamine monohydrate (3.6mg) and riboflavin (2.6mg).

Hexane sulphonic buffer preparation: 1.8822g of sodium hexane sulphonic acid salt was weighed into a volumetric flask and dissolved with 1.5l distilled water. Glacial acetic acid (20ml) was then added and more distilled water was added to fill the flask and the resultant solution was mixed thoroughly.

Principle:

In solid or liquid biological sample, accurate method of analysis requires a procedure that extracts the different vitamins from complex matrices without loss. Hexane sulphonic acid acts as deproteinating agent while the glacial acetic acid keeps the medium acidic. The vitamins, being water soluble, dissolve readily in distilled water.

Procedure:

The chromatographic analysis was carried out isocratically using: wavelength (275nm), column (reversed phase C18,5pm ODS2. 4.6 × 250mm), flow rate (1.5ml/min.), and mobile phase (300ml methanol in 700ml buffer made of 30% methanol and 70% buffer). Each standard vitamin was weighed accordingly and put in 10ml volumetric flask. Five milliliters (5ml) of mixed buffer was added and stirred for about 2 minutes to ensure proper mixing and made up the volume with buffer to act as stock. Five milliliters (5ml) was taken from the stock solution and further diluted up to 10ml with buffer in another 10ml volumetric flask. Another separate 2.5ml was taken from the stock and made up to mark in 10ml volumetric flask. Both samples were stored at 4°C. Later the samples were filtered and each calibrant was injected twice in the HPLC (HPLC/W1/08).

For the sample preparation, each milled *Moringa* plant part (2.5g) was homogenised in a 10ml volumetric flask by adding 5ml of the buffer and agitated with a mechanical agitator for 5 minutes. This ensured proper mixing and was further diluted with buffer and made up to mark. The solution was filtered and injected into the HPLC (AOAC, 1999).

Calculation:

$$\text{Concentration, mg/l} = \frac{\text{mm/ml (from calibr.curve) x 1000 x dil.factor}}{\text{Weight of sample}}$$

3:6 S**creening for phytochemicals and anti-nutrients**

Equipments/Instruments:

-Spectrophotometer

-Centrifuge

-Soxhlet extractor

-Distillation apparatus

-Magnetic stirrer

-Water bath

-Picrate paper

Chemicals and Reagents:

All reagents are of analytical grade and are manufactured by BDH chemicals Ltd (UK) except where otherwise stated.

-HCl

-H_2S0_4

-Ammonium hydroxide

-Phenolphthalein

-Glacial acetic acid

-90% and70% ethanol

-5% CaCl

-K_2MnO_4

.Saturated Na_2CO_3 solution

-Tannic acid

-Folin-Denis reagent

-Acetone

-Methanol

-2.5% NaOH

-5% KI

-Silver nitrate

-Trichloroacetic acid

-Ferric chloride solution

-Ferric hydroxide

-Nitric acid

-Olive oil

-Bromine water

-5% and 10% Lead acetate

-Carbon tetrachloride

-Meyer's reagent (1.4g mercuric chloride in 60ml distilled water and 4.5g potassium iodide in 20ml distilled water, the two solutions were mixed and made up to 100ml with distilled water) .

Extraction methods:

Ethanol and water extractions were done on milled *Moringa oleifera* leaves, seeds, flowers, stems and roots respectively.

Ethanol extraction: Five grammes (5.0g) of each powdered sample was extracted with 90% ethanol in 250ml conical flask with constant stirring for one hour. The suspension in the conical flask was covered with a foil paper to prevent contamination of the solution and left standing overnight at room temperature (30°C). The mixture was filtered after 24 hours and the filtrate collected in a sample container for the phytochemical screening.

Water extraction: Five grammes (5.0g) of the powdered plant sample was extracted with 250ml distilled water, with constant stirring, covered and left for 24hours. The mixture was also filtered and the filtrate collected in a sample container for the phytochemical screening.

A. Qualitative screening for phytochemicals

Standard qualitative tests were conducted using AOAC (1984) to establish the presence of anti-nutrient chemicals in the different parts of *Moringa oleifera.*

3:6:1 Test for saponins

One milliliter (1.0ml) of extract was boiled with 5.0ml of distilled water for 5 minutes and was decanted while still hot. The filtrate was used for the following tests.

- **Frothing test**: The filtrate (1.0ml) was diluted with 4.0ml of distilled water, stirred vigorously and observed on standing for stable froth.
- **Emulsion test**: Two drops of olive oil was added to 1.0ml of filtrate. The solution was stirred and observed for the formation of emulsion.

3:6:2 Test for flavonoids

- To 1.0ml of extract was added 1.0ml of 10% $FeCl_3$. The formation of greenish-brown or black precipitate colour was taken as positive for a phenolic nucleus.
- One milliliter (1.0ml) of extract was added 1.0ml of 10% Lead acetate. The formation of yellow precipitate was taken as positive for flavonoid.
- One milliliter (1.0ml) of extract was added 1.0ml of dilute NaOH. Precipitation showed the presence of flavonoids.

3:6:3 Test for tannins

- **Bromine water test**: To 1.0ml of extract was added an equal volume of bromine water. The formation of a greenish to red precipitate was taken as positive for the presence of condensed tannins.

- **Hydrochloric acid test**: To 5.0ml of extract was added 2.0ml of 1% HCl. Deposition of a red precipitate was taken as evidence for the presence of phlobotannins.
- **Lead acetate test**: To 2.0ml of extract was added 3 drops of lead acetate solution. A dark blue to black precipitate was positive for phlobotannins.

3:6:4 Test for alkaloids

- To 1.0ml of extract was shaken with 5.0ml of 2% HCl on a steam bath and was filtered. 1.0ml of the filtrate was treated with Meyer's reagent and observed for cream coloured precipitate.

3:6:5 Test for anthracine glycoside

- A mixture of 5.0ml diluted sulphuric acid and 5.0ml ferric chloride was added to 1.0ml of extract. The resultant mixture was boiled for 5 minutes, cooled and filtered into a 50ml separatory funnel. The filtrate was shaken with an equal volume of carbon tetrachloride. The lower organic phase was carefully separated into a test tube and 5.0ml of dilute ammonium solution added to it with gentle shaking. Pink colouration in ammonium layer indicated the presence of anthracine glycoside.

3:6:6 Test for cyanogenic glycoside

- One gramme (1.0g) of powdered *Moringa* part was covered with sufficient water in a stoppered flask into which a sodium picric acid paper was suspended by trapping it with a cork. The flask was placed in a water bath for one hour. A change from the yellow colour of the paper to brick red colour was a positive result for cyanogenic glycosides

B. Quantitative determination of anti-nutrients

This is to establish the concentrations of the anti-nutrients by quantitative techniques using standard methods.

3:6:7 Determination of saponin

The method of analysis was according to Association of Official

Analytical Chemists, AOAC, (1984)

Principle: Saponin is an anti-nutrient that causes haemolysis of blood. It is a gravimetric method that employs the use of soxhlet extractor and two different organic solvents. The first solvent extracts lipids and interfering pigments while the second solvent extracts saponin proper.

Procedure: Five grammes (5.0g) of dry and milled *Moringa oleifera* leaves, seeds, flowers, stems and roots powder were separately weighed into a thimble, and then transferred into the soxhlet extractor chamber fitted with a condenser and a round bottomed flask. Enough acetone (half-filled flask) to cause a reflux was poured into the flask, and the sample was exhaustively extracted of its lipid and interfering pigments for 3 hours by heating the flask on a hot plate. The solvent was then distilled off.

For the second extraction, a pre-weighed round bottomed flask was fitted unto a soxhlet apparatus, bearing the thimble containing the sample, and enough methanol solvent poured into the flask. The saponin was exhaustively extracted for 3 hours by heating the flask on a hot plate. The solvent was distilled off and the flask re-weighed. The difference between the final and initial weights of the flask is the weight of saponin extracted.

Calculation:

$$\% \text{ saponin} = \frac{\text{wt. of saponin}}{\text{wt. of sample}} \times \frac{100}{1}$$

3:6:8 Determination of tannin

The method of analysis of AOAC (1984) was used for tannin determination. One gramme (1.0g) of dry and milled *Moringa oleifera* leaves, seeds, flowers, stems and roots were separately weighed into a conical flask and 100ml of distilled water added. This was boiled gently on a hot plate for one hour and filtered through a whatman filter paper into a 100ml volumetric flask. The paper was washed with distilled water and extract diluted to volume, and then cooled. This was the extract volume.

Note: The sample does not keep overnight but prepared shortly before colour development.

Colour development: Fifty milliliters (50ml) of distilled water and 10ml of diluted extract were pipetted into 100ml conical flask. Folin-Denis reagent (5.0ml) and 10ml of saturated Na_2CO_3 solution were added into the conical flask. This was diluted to volume with distilled water. After thorough mixing, the solution was allowed to stand for 30 minutes in a water bath (25°C). The optical density was measured at 700nm and the absorbance compared on a standard tannic acid curve.

For the preparation of tannic acid standard curve, 2.0g of tannic acid was dissolved with distilled water and diluted to the 200ml mark (1mg/ml). Varying concentrations (0.1-1.0mg/ml) of standard tannic acid solutions were pipetted into 10 different 100ml conical flasks. 5.0ml of Folin-Denis reagent and 10ml of saturated Na_2CO_3 solution were pipetted into the test tubes, and made up to the 100ml mark with distilled water. The solution was allowed to stand for 30 minutes in a water bath (25°C), and the optical density measured at 700nm with the aid of a spectrophotometer. A plot of absorbance versus tannic acid concentration was made, with the line passing through the origin.

Calculation:

$$\text{Tannic acid (mg/100g)} = \frac{\text{C (mg) x extract volume x 100}}{\text{Aliquot volume (ml) x wt of sample (g)}}$$

Where C (mg) = concentration of tannic acid read off the graph.

3:6:9. Determination of cyanogenic glycosides

The method of analysis is that of AOAC (1984). One gramme (1.0g) of dry and milled *Moringa oleifera* leaves, seeds, flowers, stems and roots were separately weighed into a 250ml round bottomed flask, and 200ml of distilled water added and allowed to stand for 2 hours (for autolysis to occur). Full distillation was carried out and 150-170ml of distillate was collected in a 250ml conical flask containing 20ml of 2.5% NaOH. Silicon oil or tannic acid (anti-foaming agent) was added before distillation.

To 100ml of the distillate containing cyanogenic glycoside was added 8ml of 6N NH_4OH and 2ml of 5% KI. This was mixed and titrated with 0.02N

silver nitrate (AgNO3) using a micro-burette against a black background. The end point was indicated by permanent turbidity.

Calculation:

Cyanogenic glycoside = $\frac{\text{Titre value(ml) x 1.08g x extract vol.(ml) x 100}}{\text{Aliquot vol. (ml) x sample wt. (g)}}$

3:6:10. **Determination of oxalates**

Five grammes (5.0g) of dry *Moringa* samples were extracted 3 times by warming (40-50°C) and stirring with magnetic stirrer for one hour in 20ml of 0.3N HCl. The combined extract was diluted to 100ml with distilled water and used for total oxalate estimation. This is the method of Munro and Bassir (1969) which is a modified method of Dye (1956). The extract (5.0ml) was made alkaline with 1.0ml of 5N ammonium hydroxide, and 2 drops of phenolphthalein indicator. This was made acidic by drop-wise addition of glacial acetic acid. One milliliter (1.0ml) of 5% $CaCl_2$ was then added and the mixture was allowed to stand for 3 hours. It was then centrifuged at 3000 rpm for 15 minutes. The supernatant was discarded and the precipitates washed 3 times with hot water with thorough mixing and centrifuging each time. 3N H_2SO_4 (2.0ml) was added to each tube and the precipitate dissolved by warming in a water bath (70-80°C). The content of each tube was titrated with freshly prepared 0.01N K_2MnO_4. Titration was carried out at ordinary temperature until the first pink colour appeared throughout the solution. It was allowed to stand until the solution was colourless. The solution was then warmed to 70-80°C and titration was continued until the formation of a pink colour which persisted for at least 30 seconds.

The oxalate in *Moringa* samples were calculated

Chemistry of reaction

$$C_2O_4^{2-} + 8H^+ + MnO_4^{2-} \leftrightarrow 2CO_2 + 4H_2O + Mn^{++}$$

Ratio of reacting ions = 1:1

From $M_1V_1 = M_2V_2$

Where:

M_1 = molarity of KMnO4

M_2 = molarity of extract (oxalate)

V_1 = volume of extract (oxalate)

V_2 = volume of $KMnO_4$ (titre value)

Molecular weight of $CaCO_3$ = 128.08

Weight of oxalate in titre = M_2 × molecular wt. = xg

Weight of oxalate in titre (2ml) = $\frac{x}{1000} \times 2g = Y$

100ml of oxalate extract will contain = $\frac{Y}{5} \times 100g = W$

% oxalate composition g/100g = $\frac{W}{5} \times \frac{100}{1}$

3:6:11. **Determination of phytate**

The extraction of phytate was by the method of Griffith and Thomas, 1981. Dry and milled *Moringa oleifera* leaves, seeds, flowers, stems and roots were defatted separately and 2.0g of each sample was extracted by shaking in 50ml of 0.18M trichloroacetic acid at room temperature for one hour. The suspension was centrifuged and an aliquot (10ml) was added to 4.0ml of 0.036M Ferric chloride solution and placed in boiling water. The precipitated ferric phytate was collected by centrifugation after 45 minutes. This was washed twice with 30ml of trichloroacetic acid and once with 50ml of distilled. The precipitate obtained was suspended in 3.0ml of 1.5M NaOH, diluted to 30ml with water and the resulting ferric hydroxide coagulated by heating. This was then centrifuged, washed with water, and dissolved in 40ml of 3.2M HNO_3 and made up to 100ml with distilled water. The iron content was determined spectrophometrically. The phytate content

can be calculated from the iron content, assuming a constant4:6 (iron: phosphorus) molecular ratio in the precipitate.

Calculation:

Atomic weight of iron (Fe) = 55.85

Atomic weight of phosphorus (P) = 31

Mol. weight of phytic acid (PA) , $C_6P_6O_{24}H_{18}$ = 660.8

Fe in sample = x µg (from std. curve).

Converting µg to µmole = $\frac{x}{55.85}$ = Y µmoles Fe

Using molecular ratio of iron/phosphorus = 4:6

Phosphorus = 6/4. Y = µmole P = Z

Thus, PA = $\frac{660.8}{185.82}$. Z µmoles

NOTE: 185.82 is 6 x molecular weight of P.

µmole PA x MW of PA = µg PA

Thus, phytate content = $\frac{185.82}{660.8}$ x µg PA

= µg phytate.

NB: Fe standard curve was used for this estimation.

3:7:0 **Dietary evaluation and hepatoxicity studies**

Twenty five (25) young wistar albino rats were used for the experiment. They were divided into five groups, each group containing five rats of average weight (53.11g). The two control groups were fed with casilan diet and commercially prepared rat pellets respectively while the three test groups were fed with compounded diets containing given rations of the leaves, the seeds and the flowers respectively. The components of the compounded diets are shown in Table 9. The feeding trial was done at 10% protein level. The trials lasted for 21 days. All the animals were kept in well

ventilated cages. Given amount of compounded feeds were provided in rats cages and the rats allowed to feed *ad libitum*. Water was also given *ad libitum*. At the beginning of each day, the amount of feeds consumed is determined by weighing left over rations and subtracting from the total amount of feeds initially provided. The weights of the rats were measured every three days until the end of the feeding trials and the growth pattern determined. The rats were anaesthetized with chloroform and blood samples collected by cardiac puncture. After clotting, blood samples were centrifuged at 1000 rpm and sera collected. The sera were used to determine the levels of alanine and aspartate transaminases, alkaline phosphatase and bilirubin, using the different kits purchased from Randox laboratories Ltd. Ardimore, UK.

Table 9: The composition of the compounded diets (g/kg diet)

Constituents	Moringa Leaf diet	Moringa Seed diet	Moringa Flower diet	Casilan diet
Corn starch	508	513	615	759
Oil	100	100	100	100
[a]Vit./Mineral mix	30	30	30	30
[b]Casilan	-	-	-	111
Moringa part	362	357	385	-

[a]Vit./Mineral mix.(Real agro-mix): Each 2.5kg contains:

vit.A=8,000,000 I.U;

vit.D$_3$=1,600,000 I.U;

vit.E=5,000,000 I.U;

vit.K=2,000.0mg;

thiamine, B_1=1,500.0mg;

riboflavin, B_2=4,000.0mg;

pyridoxine, B_6= 1,500.0mg;

niacin =16,500.0mg;

vit.B_{12} =10.0mg;

pantothenic acid =5,000.0mg;

folic acid=500.0mg;

biotin =20.0mg;

choline chloride =200.0g;

anti-oxidant =125.0g;

manganese =80.0g;

zinc =50.0g;

iron =20.0g;

copper =5.0g;

iodine =1.2g;

selenium =200.0mg;

cobalt =200.0mg.

The commercial rat pellets used was bought from Top feeds Ltd, Asaba, Delta state. It contains:

crude protein=18.00%;

fat=6.00%;

crude fibre=5.00kcal/kg;

calcium=1.00mg;

available phosphorus=0.40mg;

lysine=0.85mg;

methionine=0.35mg,

salt(min)=0.30mg;

metabolisable energy(min)=2900kcal/kg.

[b]Casilan 90(Calcium caseinate) was produced by H.J.Heinz Co. Ltd., Hayes, Middx. UB4 8AL, England. The nutritional profile is:

Energy (Kcal) = 370.0 per 100g powder

Protein (g) = 90.0 per 100g powder

Carbohydrate (g) = 0.3 per 100g powder

Fat (g) = 1.0 per 100g powder

Fibre (g) = Trace

Sodium (g) = 0.03 per 100g powder

Calcium (mg) =1400 per 100g powder.

The % crude protein content of the feeds were 10.58 ± 0.15, 10.03 ± 0.14, 9.41 ± 2.37, 9.04 ± 0.07 and 12.01 ± 0.24 for the seed, leaves, flower, casilan, and commercial rat diets respectively.

3:8:0 Enzyme analysis and bilirubin determination

3:8:1 Alanine aminotransferase or Glutamate-pyruvate transaminase (GPT)

Principle: Alanine transaminase (ALT) activity was measured *in vitro* according to the method of Reitman and Frankel, 1957. The enzyme is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine. The brown colour is measured colorimetrically at 546nm.

$$\alpha\text{-oxoglutarate} + \text{L-alanine} \xrightarrow{GPT} \text{L-glutamate} + \text{pyruvate}$$

Equipments/Instruments

-Spectrophotometer

Weighing balance

-Water bath

-Sterile syringes

Chemicals and Reagents:

-2,4-dinitrophenylhydrazine,R2.(2,4-DNPH)

-Solution buffer, R1, (phosphate buffer, L-alanine and α-oxoglutarate)

-Sodium hydroxide

Procedure:

The test tubes were well labelled as reagent blank and samples and 0.1ml of the sample was added into the sample test tubes only while 0.5ml of Reagent1 was added to all the test tubes. 0.1ml of distilled water was added to the reagent blank only. They were mixed and incubated for exactly 30 minutes at 37°C. Then, 0.5ml of Solution R2 was added to all the test tubes.They were mixed and allowed to stand for exactly 20 minutes at 20-25°C**.** Five **(**5.0) ml of sodium hydroxide was added to all the test tubes. They were mixed and the absorbance of the sample read against the reagent blank after 5 minutes.

Calculation

The enzyme activity (U/I) of ALT in the sera were obtained from the reference table in the ALT kit manual.

3:8:2 **Aspartate aminotransferase (AST)**

Principle: Aspartate aminotransferase, otherwise called Glutamate-oxaloacetate transaminase (GOT) is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine. The brown colour is measured colorimetrically at 546nm. This method, Reitman and Frankel, 1957 is for the quantitative *in vitro* determination of Aspartate aminotransferase in serum.

α-oxoglutarate + L-aspartate $\xrightarrow{GOT}$ L-glutamate + Oxaloacetate

Equipments/Instruments: As in ALT.

Chemicals and Reagents:

-2,4-dinitrophenylhydrazine,R2.(2,4-DNPH)

- Solution Buffer, R1, (phosphate buffer, L-aspartate and α-oxoglutarate)

-Sodium hydroxide

-Distilled water

Procedure:

The test tubes were well labelled as reagent blank and sample (test and control). 0.1ml of the sample was pipetted into the sample test tubes only, while 0.5ml of Reagent1 was pipetted into all the test tubes. Then 0.1ml of distilled water was pipetted into the reagent blank only. They were mixed and incubated for exactly 30 minutes at 37°C, before 0.5ml of Reagent2 (containing 2, 4-DNPH) was added to all the test tubes. They were mixed and allowed to stand for exactly 20 minutes at 20-25°C. Five (5.0) ml of sodium hydroxide was added to all the test tubes and mixed. The absorbance of the sample was read against the sample blank after 5 minutes at 546nm wavelenght. .

Calculation

The enzyme activity (U/I) of AST in the sera were obtained from the reference table in the AST kit manual.

3:8:3 Alkaline phosphatase

Principle: This is the quantitative *in vitro* determination of alkaline phosphatase (ALP) in serum and plasma. It is an optimized standard method according to the recommendations of the Deutsche Gesellschaft fur Klinische Chemie (1972). The absorbance was read at 405nm.

p-nitrophenylphosphate + H_2O $\xrightarrow{ALP}$ phosphate + p-nitrophenol

Equipments/Instruments.: As in ALT.

Chemicals and Reagents:

-Substrate, R1b, (p-nitrophenylphosphate)
-Buffer, R1a (Diaethanolamine buffer and magnesium chloride)
-Distilled water.

Procedure:

The test tubes were well labelled. One vial of substrate R1b was reconstituted with the appropriate volume of Buffer R1a (10ml for the 10 x10 ml kit AP 307) and 0.05ml of the sample was pipetted into a cuvette Three (3.0) ml of reagent (30°C) was added to the cuvette. It was mixed and the initial absorbance read at wavelength of 405nm, and timer started simultaneously.The absorbance was also read after1, 2 and 3 minutes.

Calculation:

The alkaline phosphatase activity was calculated using the formula:

U/l = 3300 x ΔA 405 nm/min MACRO.

3:8:4 **Total and Direct bilirubin.**

Principle: This is the quantitative *in vitro* determination of total and direct bilirubin in serum or plasma. The colorimetric method is based on that described by Jendrassik and Grof, 1938. Conjugated (direct) bilirubin reacts with diazotised sulphanilic acid in alkaline medium to form a blue coloured complex. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazotised sulphanilic acid.

Equipment/Instruments: As in ALP.

Chemicals and Reagents:

-Reagent1, R1, sulphanilic acid (contains 0.17N HCl).
-Reagent2, R2, Sodium nitrite.
-Reagent3, R3, Caffeine (contains sodium benzoate)
-Reagent4, R4, Tartarate (contains i.9N NaOH)
-0.9% NaCl solution
-Distilled water

Procedure for total bilirubin:

Test tubes were well labelled as sample blank and samples and 200µl of Reagent1 was pipetted into sample blank and sample. One drop (50µl) of Reagent2 was added to sample only. 1000µl of Reagent3 and Sample (200µl) were added to all. They were mixed and incubated for 10 minutes at 20-25°C. Then, 1000µl of Reagent4 was added to all. They were mixed and incubated for a further 5-30 minutes at 20-25°C. The absorbance of the sample was read against the sample blank at 578nm (A_{TB}).

Calculation:

Total bilirubin (µmol/l) = 185 x A_{TB} (578nm).

Where A_{TB} is the absorbance value.

Procedure for direct bilirubin:

Test tubes were well labelled as sample blank and samples and 200µl of Reagent1 was pipetted into sample blank and sample.One drop (50µl) of Reagent2 was added to sample only. 2000µl of 0.9% NaCl and 200µl of sample were added to all test tubes. They were mixed and incubated for 10 minutes at 20-25°C. The absorbance of the sample was read against the sample blank at 546nm (A_{DB}).

Calculation:

Direct bilirubin (µmol/l) = 246 X A_{DB} (546nm).

Where A_{DB} is the absorbance value.

3:9 Statistical Analyses

The results obtained were subjected to Analysis of variance (ANOVA) and multiple comparison (MANOVA) using the SPSS (version 10.0) statistical software package. Values are taken to be significant at $p < 0.05$.

CHAPTER FOUR

4.0 RESULTS

4:1 Proximate composition

The results of the proximate composition of *Moringa oleifera* seeds, leaves, flowers, roots and stems are shown in Figure 6 (See Appendix I for details). The % crude protein content is highest in the seeds (28.02±0.01%), leaves (27.60±0.14%), and flowers (25.99±0.07%) in that order, but low in the roots (5.02±1.52%) and stems (3.59±0.96%). The seeds (33.78 ± 2.41%) and leaves (20.00 ± 2.31%) have high amount of %crude lipid compared to the flowers (9.44 ± 3.08%), roots (6.33 ± 1.64%) and stems (1.77 ± 0.98%). The calorific values of the different parts of *Moringa oleifera* plant as documented in Appendix I are shown in Figure 7. At 531.18 Kcal/100g, the seed has the highest calorific value followed by the leaf (426.12Kcal/100g), the flower (391.20Kcal/100g), and the roots (384.05Kcal/100g) while the stem has the least calorific value at 380.05 Kcal/100g. The energy content profile, %crude proteins and %crude lipids of the different parts of the plant are in the order:

Seeds> $leaves > flowers > roots > stems.$

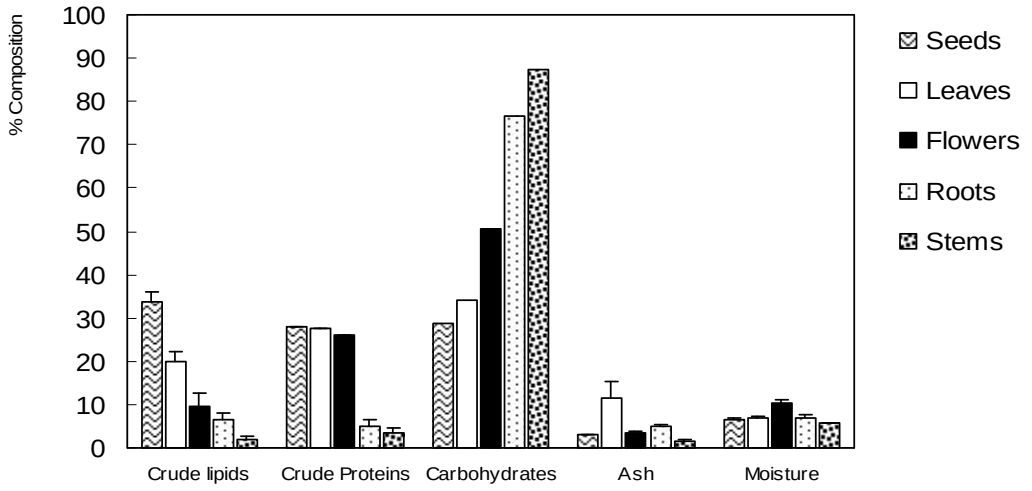


Figure 6: Proximate composition of the seeds, leaves, flowers, roots and stems of *Moringa oleifera*.

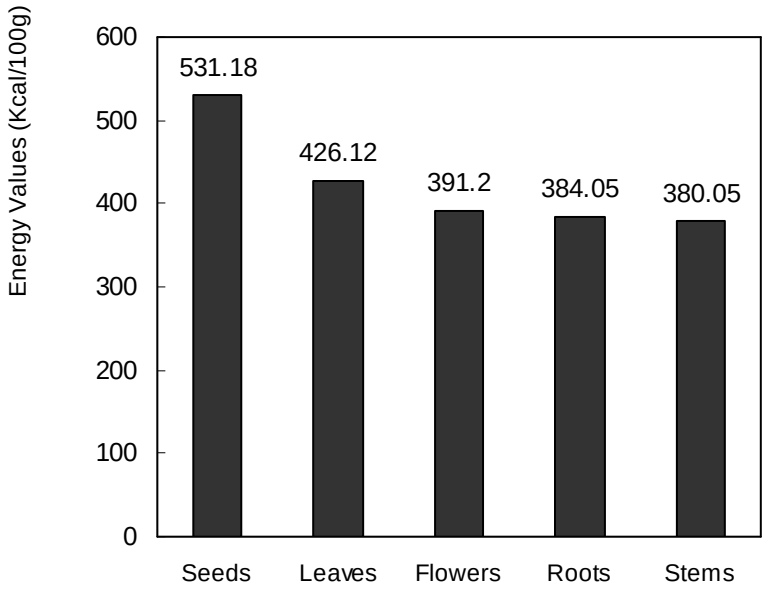


Figure 7: The calorific values of the different parts of *Moringa oleifera* plant.

4:2 **Amino acid composition**

Appendix II is the chromatograph for amino acid standards while appendices III (a) to III(e) are the chromatographs of the amino acid profile of *Moringa oleifera* flower, leaf, seed, root and stem respectively.

Figure 8 shows the amino acid composition in g/100g protein of the different samples as derived from the respective chromatographs. All the fractions consistently are composed of 17 out of the 20 naturally occurring amino acids lacking only in asparagine, glutamine and tryptophan. The ratio of essential amino acids (EAA) to non-essential amino acids (NEAA) in the sample specimens are shown in Figure 9 (See Appendix IV for details). It can be deduced from figure 9 that the content of essential amino acids and non-essential amino acids are almost equal in concentration in all circumstances; for example, the percentage of EAA in the leaves is 50.66 while the %NEAA is 49.33. For the seeds it is 48:52, 53:47 for the flower, 50:50 for the stem and 43:57 for the root.

Figure 10 shows the proportion of branched chains, acidic, aromatic and basic amino acids in the different parts of the plant studied (See Appendix V for details). The most abundant EAA are leucine, a branched-chain amino acid (24.54g/100g protein or 9.93%), arginine, a basic amino acid (19.84g/100g protein or 8.01%) and phenylalanine, an aromatic amino acid (16.19g/100g protein or 5.67%) while the most abundant NEAA are the acidic amino acids glutamic acid (40.88g/100g protein or 16.69%) and aspartic acid (22.94g/100g protein or 11.97%). The proportion of total amino acids, essential and non-essential amino acids in the different parts of the plant are shown in Figure 11. For details, see Appendix IV. The proportion of total amino acids, EAA and NEAA in the whole plant follow the pattern as thus:

leaf > seed > flower > stem > root.

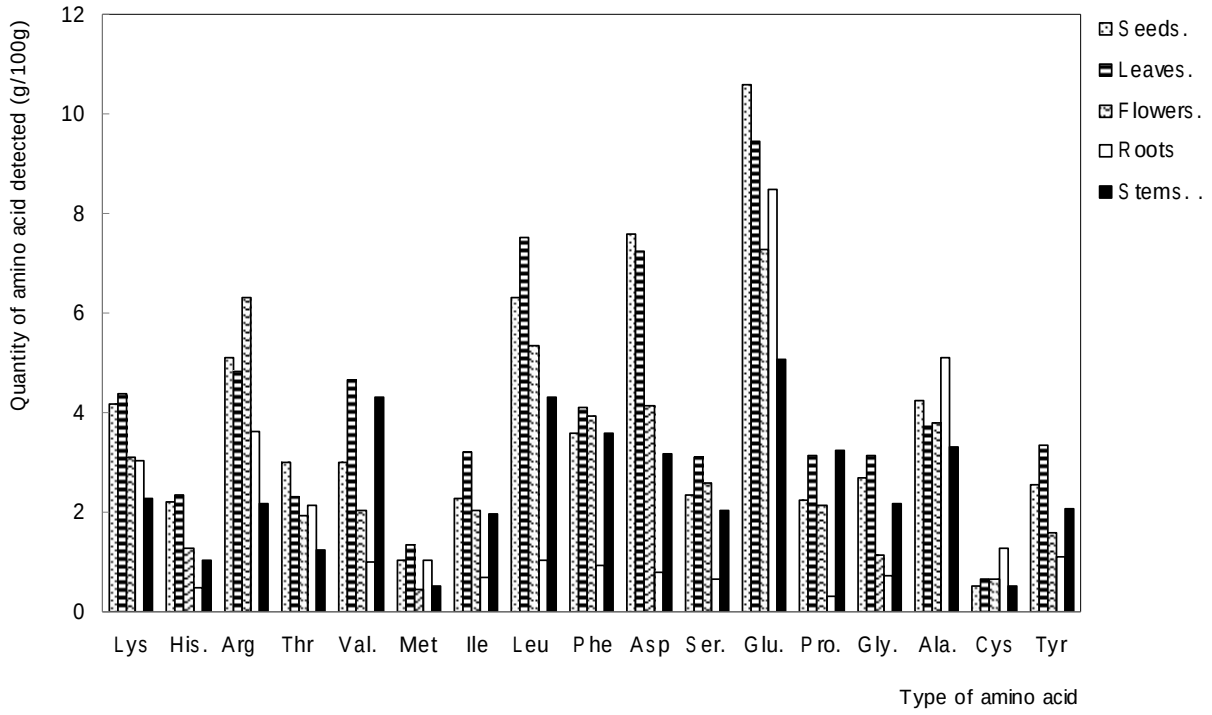


Figure 8: The amino acid profile of the different parts of *Moringa oleifera* plant.

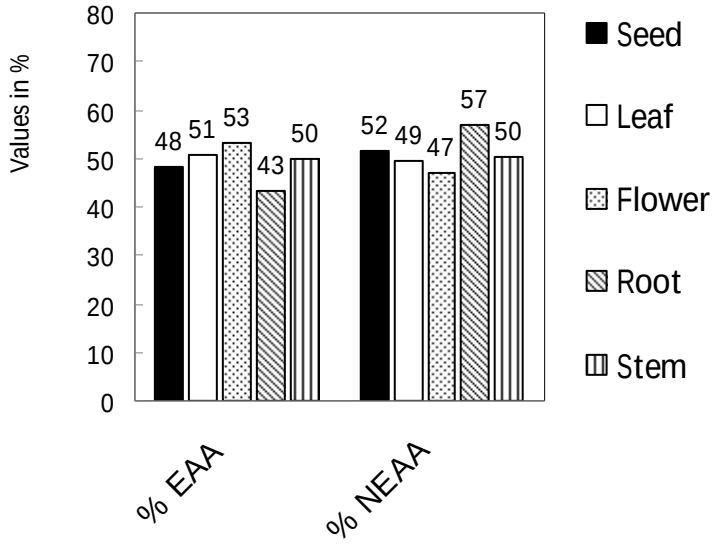


Figure 9: %EAA and %NEAA in all the parts of the plant studied.

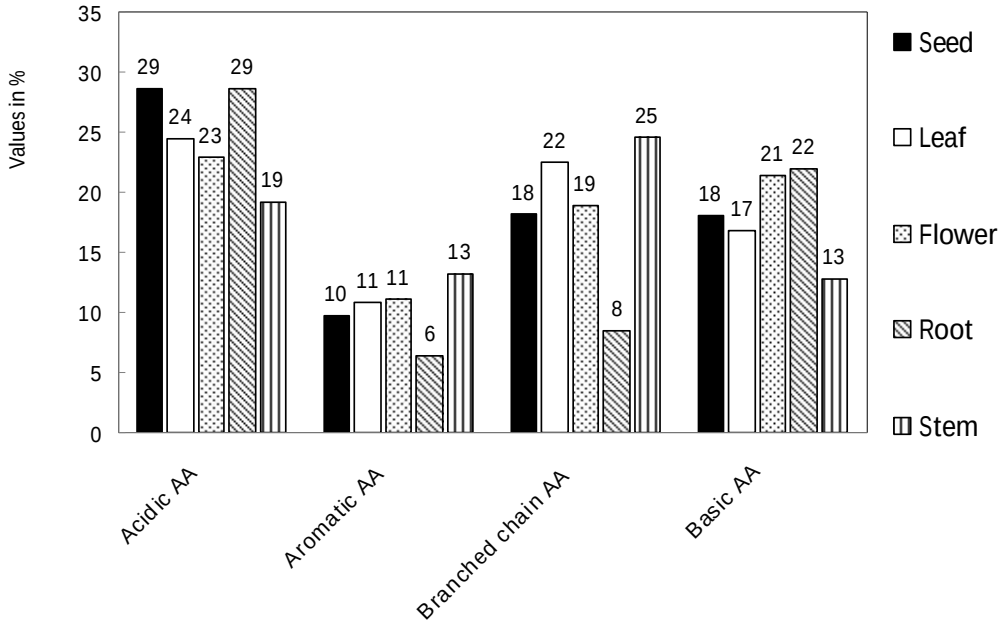


Figure 10: The proportion of acidic, aromatic, branched chain and basic amino acids in different parts of *Moringa oleifera.*

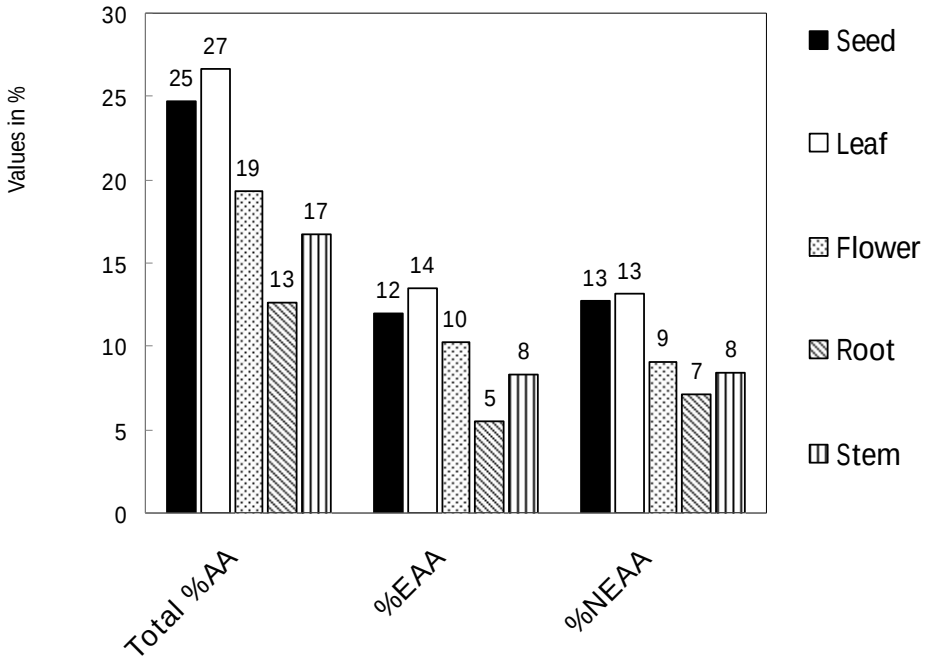


Figure 11: The proportion of total amino acids, essential and non-essential amino acids in the plant.

4:3 **Mineral contents**

The mineral composition of *Moringa oleifera* plant is shown in Fig. 12 while the details are presented in Appendix VI. The sodium (Na) content is highest in the roots (514mg/100g) followed by that in the stem (378.38mg/100g), in the seeds (129.03mg/100g) and in the flowers (120.94mg/100g) while the least value is found in the leaves (104.06mg/100g). The calcium (Ca) contents are 2.84mg/100g, 13.45mg/100g, 2.32mg/100g, 3.99mg/100g and 1.38mg/100g in the seeds, leaves, flowers, roots and stems respectively. Although, no potassium (K) was detected in the seeds, the other parts of the plant contain potassium. The leaves, flowers, roots and stem contain 20.81mg/100g, 3.02mg/100g, 15.40mg/100g and 32.40mg/100g of potassium respectively.

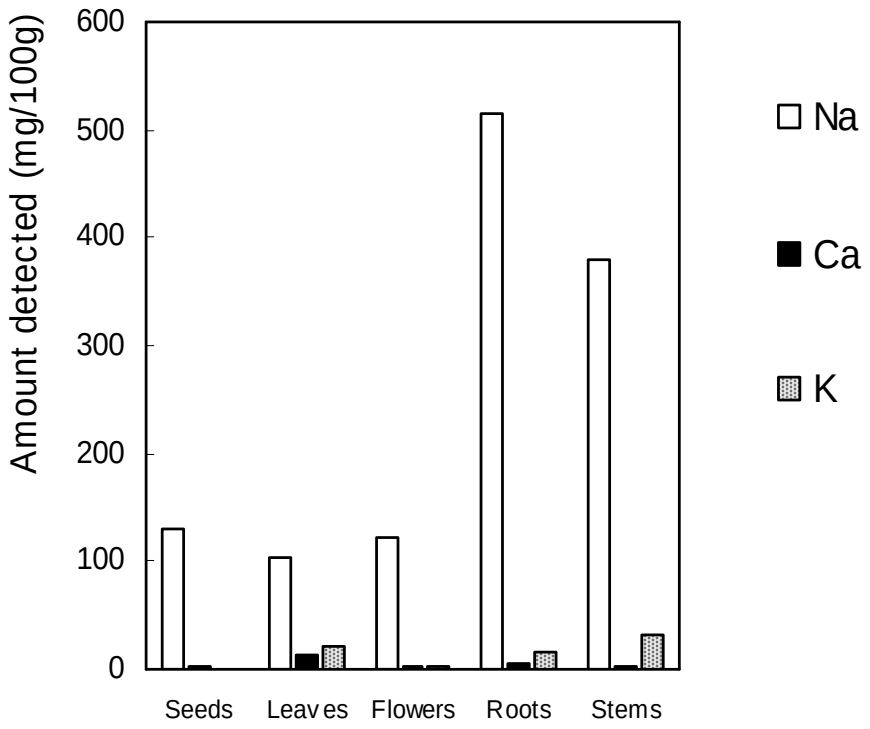


Figure 12: The mineral content of the seeds, leaves, flowers, roots and stems of *Moringa oleifera.*

4:4 **Vitamin composition**

The vitamin composition of *Moringa oleifera* seeds, leaves, flowers, roots and stems are shown in Figure 13 (See Appendix VII for details). Only the leaves contain all the water-soluble vitamins estimated in the following proportions: ascorbic acid (773.30mg/100g), thiamine (18.47mg/100g), riboflavin (14.82mg/100g), pyridoxine (57.29mg/100g) and niacin (50.35mg/100g). The concentration of ascorbic acid are higher in the leaves (773.30mg/100g) and flowers (459.21mg/100g) and lower in the seeds (94.74mg/100g), stems (71.44mg/100g) and roots (48.13mg/100g).

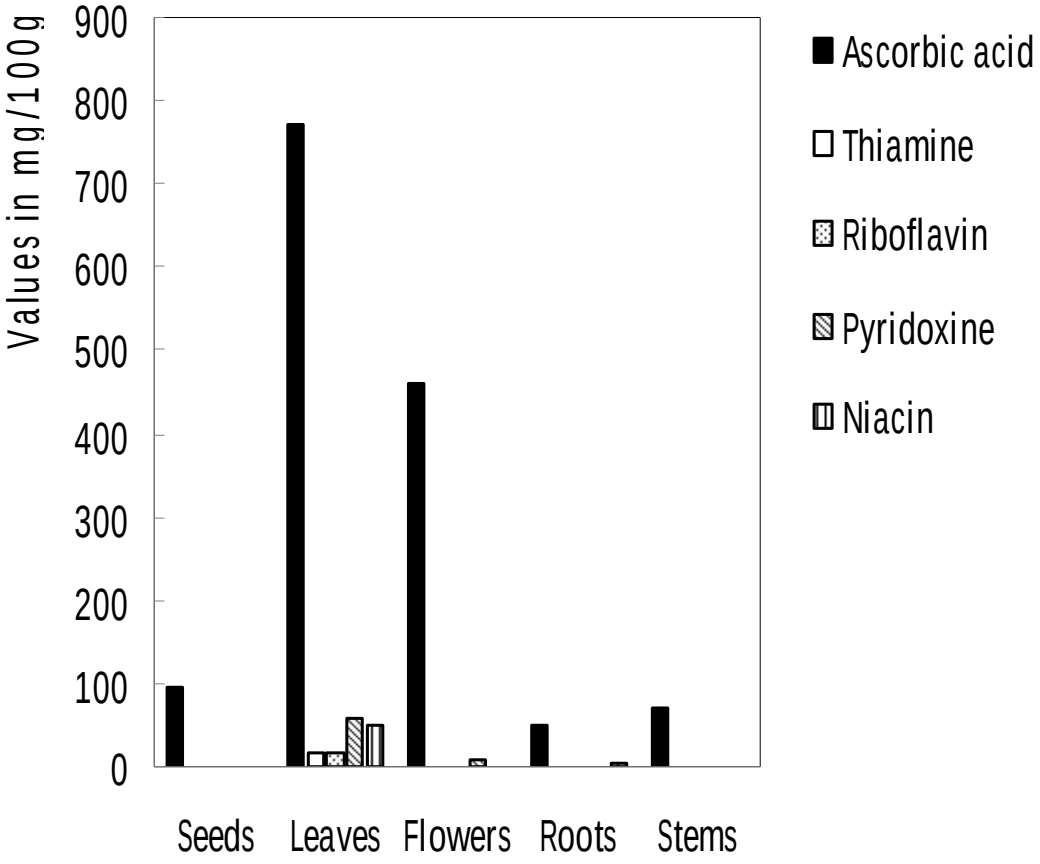


Figure 13: The vitamin composition of the seeds, leaves, flowers, roots and stems of *Moringa oleifera.*

4:5 **Anti-nutritional factors**

Table 10 is the summary of the qualitative test for the anti-nutrients and phytochemicals. All the anti-nutrients screened are present in the plant except anthracine glycosides. Tannins and cyanogenic glycosides were detected only in the leaves but when quantitative analysis was done, they were found in all the parts of the plant studied. This suggests that qualitative screening is not enough to confirm the presence of these anti-nutrients in plant samples. Figure 14 shows the quantitative determination of the anti-nutritional factors in the different parts of *Moringa oleifera* plant studied while the details are shown in Appendix VIII. The tannins are highest in the leaves (420mg/100g) followed by that in the stems (100mg/100g), in the flowers (60mg/100g) and in the roots (45mg/100g) and

lowest in the seeds (40mg/100g). Almost the same pattern of result was found with cyanogenic glycosides. It was highest in the leaves (32.40mg/100g) followed closely by that in the stems (31.40mg/100g), then in the seeds (4.59mg/100g), in the flowers (4.31mg/100g) and the roots (2.72mg/100g) in that order. On the other hand, the phytates were low in all the parts of the plant studied. We had 0.013mg/100g, 0.048mg/100g, 0.064mg/100g, 0.435mg/100g and 0.436mg/100g for the leaves, stems, roots, seeds and flowers respectively (See Appendix VIII). The levels of oxalates were similar in the stems (51.24mg/100g), in the seeds (51.24mg/100g) and in the flowers (51.23mg/100g) when compared to that in the leaves (7.20mg/100g) and in the roots (17.08mg/100g). Saponin value was highest in the flowers (15.23mg/100g) followed closely by that in the stems (12.10mg/100g), in the leaves (11.80mg/100g), in the seeds (9.40mg/100g) and lowest in the roots (4.20mg/100g).

Table 10: Summary of the phytochemical screening of the different parts of *Moringa oleifera*

Phytochemicals	SEEDS		LEAVES		FLOWERS		ROOTS		STEMS	
	E	W	E	W	E	W	E	W	E	W
Tannins	-	-	+	+	-	-	-	-	-	-
Cyanogenic glycosides	-	-	+	+	-	-	-	-	-	-
Anthracine glycosides	-	-	-	-	-	-	-	-	-	-
Saponins	+ +	+ +	+	+ +	+ +	+ +	+	+	+	+
Flavonoids	-	-	+	+ +	+ +	+	+++	+ +	+ +	+ +
Alkaloids	-	+	-	-	-	-	+ +	+ +	-	-

Where,

E = Ethanol extract,

W = Water extract.

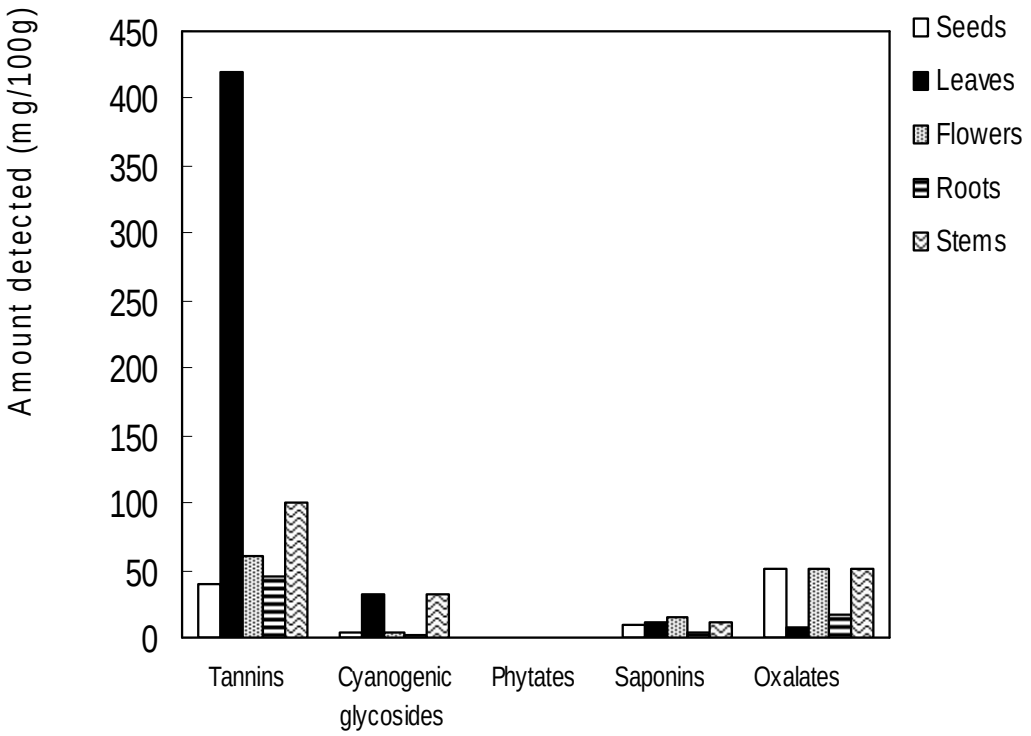


Figure 14: The levels of anti-nutritional factors in the seeds, leaves, flowers, roots and stems of *Moringa* oleifera.

4:6 **Dietary evaluation using animal experiment**

Appendix IX is the growth statistics of experimental rats fed with compounded and standard dietary regimens while Figure 15 shows the growth rate profile of rats fed with different compounded diets containing *Moringa* seed, leaf and flower rations respectively, and casilan diet and commercially prepared rat pellets for a period of 21 days. The rats fed with commercial rat pellets and *Moringa* leaf diet showed increase in weights from 51.90 ± 1.38 to 112.14 ± 7.36 and from 46.73 ± 3.81 to 66.59 ± 5.27 respectively after 21 days of feeding. The casilan, *Moringa* seed and flower diets decreased the growth rate of rats from 55.94 ± 2.48 to 42.89 ± 1.56; from 55.77 ± 2.61 to 36.87 ± 3.70 and from 55.20 ± 1.60 to 39.70 ± 1.15 respectively.

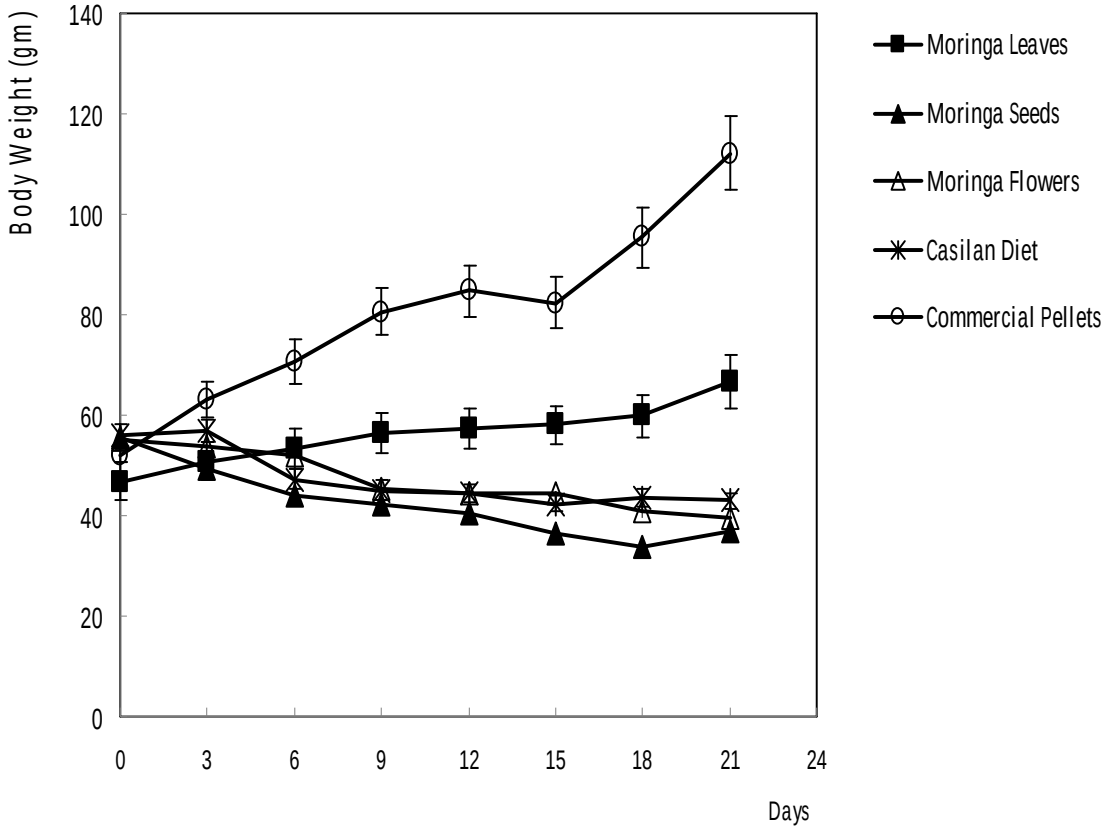


Figure 15: Growth rate profiles of rats fed with the assorted feed rations (each point is the mean of the growth rates ± S.E.M: where n=5).

4:7 **Liver assessment of dietary regimens**

Figure 16 shows the liver enzyme activities in rats fed with the different feeds (See Appendix X for details). The enzyme activity of aspartate transaminase (U/l) in test rations (*Moringa* leaf, seed, flower) and control rations (casilan and commercial rat pellets) are 9.0 ± 1.00, 16.5±6.50, 16.5±2.04, 21.4±3.77 and 23.8±1.34 respectively, and are within the normal values (14-59 U/l) for humans and by extrapolation to rats except the leaf ration. It should be noted that the aspartate transaminase (AST) activity of the liver of rats fed *Moringa* feed rations are low compared with those fed with commercial rat pellets and casilan.

For alanine transaminase (ALT), *Moringa* leaf and seed rations gave lower enzyme activity (6.67±1.34 and 8.00±0.00 U/l respectively) while the seed,

casilan and commercial rat pellets enzyme activity are 9.60±0.87, 10.00±0.79 and 11.20±0.44 respectively, this is within the normal range (9-72 U/l) for humans and by extrapolation rats. The enzyme activity of alkaline phosphatase was found to be within the human normal range (38-126 U/l) for all the feed rations except for the enzyme activity of commercial rat pellets (34.25±7.56).

Figure 17 shows the total and direct bilirubin concentrations in rats fed with the different feed rations. They are all above the normal values (3-22µmol/l) except for the commercial rat pellets which gave 21.20 ± 5.30µmol/l. Total bilirubin (34.50± 1.66µmol/l) and direct bilirubin (25.60± 1.33µmol/l) are highest in rats fed with rations containing *Moringa* seed and least in those fed with commercially prepared rat pellets (21.20±5.30 and 12.00±2.78µmol/l respectively).

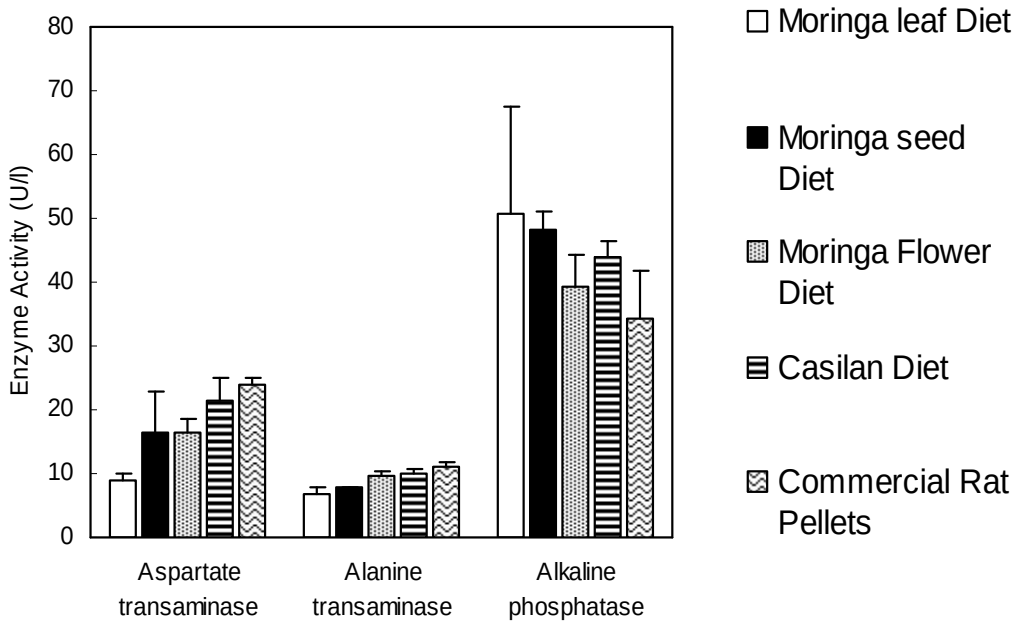


Figure 16: Liver enzyme activities in rats fed with the different feed rations.

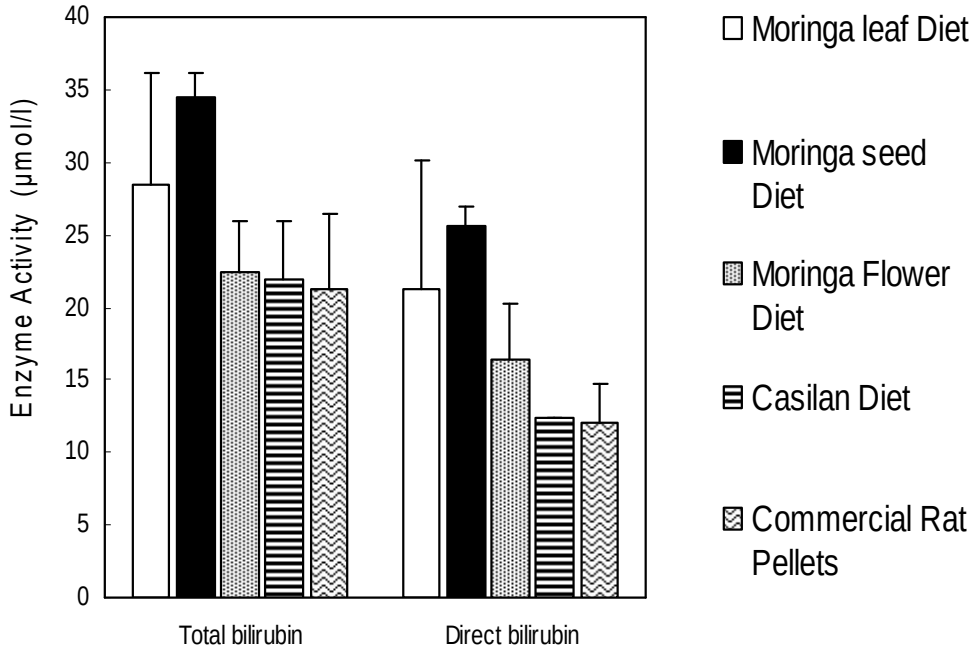


Figure 17: The concentrations of total and direct bilirubin in rats fed with the different feed rations.

4.8: **Statistical Analysis of Results**

Oneway analysis of variance (ANOVA) is a quick test to see if significant difference exists between the various parameters considered in the proximate analysis. The result in Table 11 shows that the mean difference is significant at 0.05 level ($p<0.05$) in all instances. However, further use of multiple comparison of analysis of variance (MANOVA) indicates that some parameters are significant between groups while others are not at 95% confidence level. For example, the lipid concentration in the seeds and leaves when compared to the lipid concentration in the stems, roots and flowers were significantly different ($p<0.05$), but when the lipid concentration in the flower was compared with those in the root and stem, the mean differences were not significant ($p>0.05$). Also the protein concentration in the leaf was not significant with those in the seed and flower ($p>0.05$), but significant with those in the root and stem ($p<0.05$). (See details in Appendix XI).

Table 11: Oneway ANOVA for proximate analysis of different parts of *Moringa oleifera*

	Sum of squares	df	Mean square	F	Sig.
Lipid				33.869	.000
B/twn grps.	1968.281	4	492.070		
Within grps	145.285	10	14.529		
Total	2113.566	14			
Protein				243.507	.000
B/twn grps.	1265.128	4	316.282		
Within grps	6.494	5	1.299		
Total	1271.623	9			
Ash				5.563	.013
B/twn grps.	182.429	4	45.607		
Within grps	81.987	10	8.199		
Total	264.416	14			
Moisture				15.305	.000
B/twn grps.	41.873	4	10.468		
Within grps	6.840	10	0.684		
Total	48.713	14			

Oneway ANOVA of the amino acids in the different parts of the *Moringa oleifera* in Table 12 indicates that there is no significance difference between the essential and non-essential amino acids in all parts of the plant, since the significance results are well over 0.05.($p>0.05$).

Table 12: Oneway ANOVA of the amino acids in the different parts of the *Moringa oleifera*

Plant parts	Sum of squares	df	Mean square	F	Sig.
SEED					
B/twn grps.	2.017	1	2.017	.307	.588
Within grps	98.669	15	6.578		
Total	100.685	16			
LEAVES					
B/twn grps.	.575	1	.575	.107	.748
Within grps	80.634	15	5.376		
Total	81.209	16			
FLOWER					
B/twn grps.	3.243E-04	1	3.243E-04	.000	.993
Within grps	61.793	15	4.120		
Total	61.793	16			
ROOT					
B/twn grps.	2.349	1	2.349	.505	.488
Within grps	69.715	15	4.648		
Total	72.064	16			
STEM					
B/twn grps.	.427	1	.427	.229	.639
Within grps	28.016	15	1.868		
Total	28.443	16			

Oneway ANOVA for the dietary evaluation in Table 13 indicates that with the exception of day 1, there is significant difference between the different groups considered. The details are shown in the multiple comparison ANOVA (Appendix XII).

Table 13: Oneway ANOVA for the dietary evaluation of the different feed rations

Days	Sum of squares	df	Mean square	F	Sig.
DAY 1					
B/twn grps.	340.182	4	85.045	2.532	.073
Within grps	671.832	20	33.592		
Total	1012.014	24			
DAY 3					
B/twn grps.	606.461	4	151.615	3.778	.019
Within grps	802.543	20	40.127		
Total	1409.004	24			
DAY 6					
B/twn grps.	2104.270	4	526.067	11.025	.000
Within grps	954.306	20	47.715		
Total	3058.575	24			
DAY 9					
B/twn grps.	5050.364	4	1262.591	26.444	.000
Within grps	954.905	20	47.745		
Total	6005.268	24			
DAY 12					
B/twn grps.	6563.067	4	1640.767	32.077	.000
Within grps	1023.012	20	51.151		
Total	7586.079	24			
DAY 15					
B/twn grps.	6724.213	4	1681.053	34.311	.000
Within grps	979.892	20	48.995		
Total	7704.105	24			
DAY 18					
B/twn grps.	12067.629	4	3016.907	48.954	.000
Within grps	1170.921	19	61.627		
Total	13238.549	23			
DAY 21					
B/twn grps.	19665.576	4	4916.394	57.669	.000
Within grps	1534.542	18	85.252		
Total	21200.118	22			

Oneway ANOVA for the enzyme activity and the concentrations of both direct and total bilirubin in rats fed with the different feed rations in Table 14 indicates that significant difference between group members exist only in the alanine transaminase activity group which has a significance of 0.03 ($p<0.05$). The details of the relationships that exist between the various factors are observable in the multiple comparison of ANOVA (See Appendix XIII).

Table 14: Oneway ANOVA for the enzyme activity and the concentrations of both direct and total bilirubin in rats fed with the different feed rations.

Protein	Sum of squares	df	Mean square	F	Sig.
Asp. enzy.					
B/twn grps.	490.510	4	122.627	2.612	.075
Within grps	751.300	16	46.956		
Total	1241.810	20			
Ala.enzyme	46.421	4	11.605	3.525	.030
B/twn grps.	52.673	16	3.292		
Within grps	99.094	20			
Total					
Alk.Pase.					
B/twn grps.	672.682	4	168.170	.299	.873
Within grps	6755.630	12	562.969		
Total	7428.312	16			
Total bil.					
B/twn grps.	331.600	4	82.900	.619	.659
Within grps	1338.800	10	133.880		
Total	1670.400	14			
Dir.bil.					
B/twn grps.	307.109	4	76.777	.967	.476
Within grps	635.159	8	79.395		
Total	942.268	12			

CHAPTER FIVE

DISCUSSION AND CONCLUSION

The nutritional properties of *Moringa oleifera* leaf are well known that there seems to be little doubt of the substantial health benefit to be realized by consumption of *Moringa* leaf powder in situations where starvation is imminent. Studies on the dietary constituents of the leaves credit it with essential amino acids, which is important in bridging the protein gap of poor countries like Nigeria. However, the dietary potentials of other parts of the plant are largely lacking in literature, and thus, the utmost importance of this work in evaluating *Moringa oleifera* for dietary or pharmacological purposes.

Moringa oleifera seeds (28.02 ± 0.01%), leaves (27.60 ± 0.14%) and flowers (25.99 ± 0.07%) in Figure 6 are rich in proteins since according to Pearson (1976), any plant food that provides more than 12% of its calorific value from protein is considered a good source of protein. It is not surprising therefore, that despite the high protein content, feed rations prepared with the mature raw seed and flower fractions of this plant did not support growth of rats after 21 days feeding. Only the leaves ration supported growth rate marginally. The presence of anti-nutrients might inhibit the bioavailability of proteins and minerals (Davidson *et al.*, 1975; Akubugwo *et al.*, 2007). Tannins for example lower the available proteins by antagonistic competition and can therefore elicit protein deficiency syndrome (Akubugwo *et al.*, 2007). Although, soaking, boiling or frying could have removed some of the anti-nutrients (Ekop and Eddy, 2005; Kidmose *et al.*, 2006), caution should be taken in the use of raw *Moringa* plant parts as food material until suitable processing method is developed to get rid of some anti-nutrients and perhaps some other adverse factors militating against the use of the plant parts as nutrients (Oliveira *et al.*, 1999). However, in Senegal, Ghana, India and other parts of the world, *Moringa oleifera* has been reported to be a nutritional supplement, apart from its medicinal and water treatment applications. *Moringa oleifera* leaves are reported to be used in treating malnutrition and promoting physical and mental well being with visibly effective results, particularly in children, pregnant and lactating

mothers (Fuglie, 1999, 2001; Namibiar *et al.*, 2003 and Ozumba *et al.*, 2009).

The percentage crude protein of the leaves of *Moringa oleifera* found in Awka, Anambra state is similar to those found in other parts of the world like India and Senegal. An example is that reported by Fuglie (1999, 2001) for *Moringa oleifera* dry leaf powder (27.10%) in Senegal. The crude protein (CP) content of leaves, soft twigs and stems of *Moringa oleifera* was 260, 70 and 60 g kg^{-1} respectively **(**Makkar and Becker, 1997). The protein content of the leaves is similar, but the value of that in the stem is higher than the protein in this study (3.59%). The crude protein content of the mature seeds is lower than what was reported by Oliveira *et al.*, 1999 (332.5 g/Kg). It should be noted that the % crude protein content of the seeds, leaves and flowers of *Moringa oleifera* grown in Awka are higher than that reported in some legumes such as *Canavalia ensiformis* seeds, 24.48±0.28% (Igwilo *et al.*, 2007a); *Gnetum africana* seeds, 17.50% (Ekop, 2007); *Amaranthus hybridus* leaves, 17.92% (Akubugwo *et al.*, 2007), and *Momordica balsamia* leaves, 11.29% (Hassan and Umar, 2006). The protein content of the seeds and leaves compared favourably with that of *Piper guineeses*, 29.78% (Akindahunsi and Salawa, 2005), while that of the flower compares favourably with the protein content of *Ipomoea batatas* leaves, 24.85% (Anita *et al.*, 2006). The implication of this is that these *Moringa oleifera* parts are good sources of proteins for man and animals.

The result of the percentage crude lipid in the leaves is higher than what was reported by Fuglie (2001) for dry leaf powder (2.3%), but lower than that reported by Oliveira *et al.*, 1999, for the seed (412.0 g /Kg). The seeds (33.78 ± 2.41%) and leaves (20.00 ± 2.31%) have higher amount of crude lipid compared to the flowers (9.44 ± 3.08%), roots (6.33 ± 1.64%) and stems (1.77 ± 0.98%). In particular, is the crude lipid content of the seed which is higher than that found in some vegetables consumed in West Africa, 8.3-27.0% (Ifon and Bassir, 1980; Akubugwo *et al.*, 2007; and Agbo, 2004). Therefore, the seed of *Moringa oleifera* is a very good source of lipid when compared to other parts of the plant and some vegetables consumed in West Africa.

The percentage carbohydrate in the leaves (33.93) compares favourably with that reported by Fuglie, 2001 (38.2), but higher than the amount reported by Oliveira *et al.,* 1999 for the seeds (212.2g/kg). The % moisture in the seeds (6.40 ±0.31), leaves (6.87 ± 0.50), flowers (10.43 ± 0.58), roots (6.93 ± 0.58) and stems (5.57 ± 0.35) compare favourably with that reported by Fuglie, 2001 for the dry leaf powder (7.5%). The leaf has the highest % ash (11.60 ± 3.65) compared to the other parts of the plant studied. However, the ash content, which is the index of the mineral content in biota, is low compared to that reported for *Talinum triangulare* leaves, 20.05%, (Ladan *et al.*, 1996) and in *Amaranthus hybridus*, 13.80%,(Akubugwo *et al.*, 2007). The implication, of these findings is that *Moringa oleifera plant* grown in Awka contains the necessary nutrients to sustain growth in humans and animals.

The calorific values of *Moringa oleifera* plant (380.05 - 531.18Kcal/100g) are higher than the values reported for some Nigerian vegetables, 248.8 - 307.1, (Anita *et al.*, 2006; Akubugwo *et al.*, 2007). Nevertheless, the calorific values agree with the general observation that vegetables have low energy values (Lintas, 1992). Therefore, dependency on the plant for sole source of calories is insufficient.

The proximate analysis results when subjected to one way analysis of variance (ANOVA) showed that the mean differences are significant at the 0.05 level ($p<0.05$). However, when multiple comparisons (MANOVA) are used, the protein values in the seed are not significant with that of the leaves and flowers ($p>0.05$) but significant with stem and root ($p<0.05$). The lipid concentration in the seeds and leaves are significant with other parts of the plant studied ($p<0.05$) while that in the flowers are not significant with lipid concentration in the roots and stems ($p>0.05$). The moisture content in the leaves, stems, roots and seeds are significant with that in the flowers only ($p<0.05$) but not significant with other parts of the plant studied ($p>0.05$). There is a significant positive correlation between the crude lipid and protein content of the plant with the energy values of the plant at 0.05. This is understandable since these are high energy rich molecules.

The amino acid content of the seeds, leaves, flowers, roots and stems of *Moringa oleifera* and the ratio of essential and non-essential amino acids in

Figures 8 and 9 are of great interest. The results indicate that the ratio of the essential amino acids (EAA) and non-essential amino acids (NEAA) are almost equal unlike in many plants where NEAA are always remarkably higher than EAA (Aremu *et al.*, 2006 and Akubugwo *et al.*, 2007). For example, the percentage of EAA in the leaves and flowers are 50.66% and 53.02% while the NEAA are 49.33% and 46.98% respectively. Statistical analyses indicate that there is no significant difference ($p>0.05$) between the essential and non-essential amino acids present in all the parts of the plant studied. It was also observed that the most abundant EAA is leucine, a branched-chain amino acid (24.54g/100g protein or 9.93%) while the most abundant NEAAs are the acidic amino acids, glutamic acid (40.88g/100g protein or 16.69%) and aspartic acid (22.94g/100g protein or 11.97%). Similar observations were made in other plants by Adeyeye (2004) and Akubugwo *et al* (2007). This suggests that *Moringa oleifera* plant cultivated in Awka, Nigeria, has essential amino acids in abundance.

The proportion of aromatic amino acids in all the parts of the plant studied indicated that it is lower than branched chain, acidic or basic amino acids (Figure 10). This is quite interesting since aromatic amino acids (eg. Tyr and Phe) take part in secondary metabolism or could make better anti-oxidants (Awah *et al.*, 2010). In this study, seventeen (17) amino acids were found instead of the twenty (20) naturally occurring amino acids commonly found in proteins (Mc Donald *et al.*, 1995 and Akubugwo *et al.*, 2007). Glutamine and Asparagine, which are merely amide derivatives, were not detected perhaps because they are easily converted to their corresponding acids, glutamic and aspartic acids respectively (Salo-Vaananen and Koivistoinen, 1996). Also, tryptophan was not detected because of its complete destruction during acid hydrolysis (Wathelet, 1999 and Akubugwo *et al.*, 2007). This might explain the higher levels of glutamic acid and aspartic acid in the results of this study. The results showed that the amino acid contents of the leaves are higher than the values reported by Fuglie (2001) for the dry leaf powder. However, this study confirmed the earlier observations of Fuglie (2001), Oliveira *et al* (1999) and Makkar and Becker (1997) that *Moringa oleifera* contained all the essential amino acids needed for normal body functioning.

There was significant positive correlation in the amino acid components of the seeds, stems, roots, flower and leaves. This indicated that the distribution of amino acids in the seeds and other parts of the plant studied are highly similar, suggesting that the seeds might provide just exactly the same type of amino acid as the stems, roots, flowers and the leaves of *Moringa oleifera*. The amino acid content of the leaves are also comparable to those grown in other parts of the world (Fuglie 1999, 2001) while those of flowers, seeds, stems and roots are novel because they have not been reported in literature.

In Figure 12, the seeds, leaves, flowers, roots and stems of *Moringa oleifera* contained sodium (Na) and calcium (Ca) ions. Although, no potassium was detected in the seeds during the study, Fuglie (2001) reported that the pods contain 259 mg of potassium per 100g sample. The amount of calcium in this study is far lower than what was reported by Fuglie (2001) in the leaves of *Moringa oleifera* (440mg/100g). The implication of this is that *Moringa oleifera* grown in Awka, Nigeria, might not be a good source of calcium.

The vitamin composition (Figure 13) showed that it is only the leaves that contained all the water-soluble vitamins studied. However, apart from riboflavin (14.82mg/100g), the values are higher than what were reported by Fuglie (2001), Foild *et al*, (2001) and Ozumba (2008). Fuglie reported that the leaves of *Moringa oleifera* contain thiamine (2.64mg), riboflavin (20.5mg), nicotineamide (8.2mg), and ascorbic acid (17.3mg). Although, the pods contain 0.05mg/100g, 0.07mg/100g, 0.2mg/100g and 120mg/100g as thiamine, riboflavin, niacin and ascorbic acid respectively, this study detected only ascorbic acid (94.74mg/100g) in the seeds. The ascorbic acid result in the leaves has a significant correlation with that of flower at 95% confidence interval. The result may still suggest that the leaves of *Moringa oleifera* grown in Awka, Nigeria, might be a good source of anti-oxidants since vitamin C is a very good anti-oxidant.

The levels of tannins and oxalates are high in almost all the parts of *Moringa oleifera* plant studied (Figure 14). Makkar and Becker (1997) reported that tannins, saponins, and cyanogenic glycosides were detected in the stems of *Moringa oleifera* but the concentrations were negligible. The levels of phytates (mg/100g) are low in the plant, 0.013, 0.048, 0.064, 0.435 and

0.436 for the leaves, stems, roots, seeds and flowers respectively. This is lower than what was reported by Makkar and Becker (1997) for the leaves of *Moringa oleifera* (21g/Kg).The value is also lower than what was reported by Akubugwo *et al.*, (2007) for *Amaranthus hybridus* (1.32mg/100g). The low content of these anti-nutrients in the plant is a good omen for the use of those plant parts as nutrients. The levels of these anti-nutrients in the flowers and roots have significant positive correlation with that of stem while the seeds and flowers have significant positive correlation with the roots at 95% confidence level. The anti-nutrient values indicate that *Moringa oleifera*, apart from its high nutrient composition, is also medically and pharmacologically important. It is not therefore surprising that the plant is used in traditional medicine in Africa, Asia, and Americas (Morton, 1991; and Fuglie, 2001). Tannic acid is astringent and is known to be used in the treatment of bedsores and minor ulceration (Harborne, 2006; Akubugwo *et al.*, 2007). Saponins are used in the manufacture of shampoos, insecticides and various drug preparations and synthesis of steroid hormone (Okwu, 2003).

Phytic acid has complicated effect in the human system, particularly indigestion of food and flatulence (Maynard, 1997 and Akubugwo *et al.*, 2007). Tannins have antagonistic competition with proteins, thereby, lowering their bio-availability, thus, eliciting protein deficiency syndrome and kwarshiorkor. However, these anti-nutrients can easily be removed by soaking, boiling or frying (Ekop and Eddy, 2005; Kidmose *et al.*, 2006).

The growth rate profile following the administration of the different feeds (Figure 15) showed that the rats fed with *Moringa* leaf diet and commercial rat pellets increased in weights while those fed with casilan, *Moringa* seed and flower diets decreased the growth rate of rats through reduction in body weights. When the results were subjected to oneway analysis of variance, it was easily observed that with exception of day 1, there was significant difference between the different groups considered ($p<0.05$). However, when it was further subjected to multiple comparison of analysis of variance (MANOVA), it was observed that at 21 day feeding, the commercial rat pellets and *Moringa* leaf diet were significantly different when compared to the other feed rations ($p<0.05$). But when the result of the seed diet was

compared to that of the flower or casilan diet, there was no significant difference ($p>0.05$). From these results, the commercial rat pellets gave the best result followed by *Moringa* leaf diet. The results from the raw seed and flower diets showed that they did not support growth probably due to the presence of some anti-nutrients such as tannins and oxalates (Akubugwo *et al.*, 2007; Maynard, 1997). Furthermore, Hoon and Hoof (2006) suggested that non-digestibility of proteins may cause growth retardation. Oleivera *et al.*, (1999) reported that feeding rats with a diet containing the seed meal showed loss of appetite, impaired growth, lower net protein utilization and enlargement of stomach in comparison with rats fed on an egg-white diet. They went further to say that consumption of *Moringa oleifera* raw mature seeds should be viewed with some caution until suitable processing methods are developed to abolish the yet unknown adverse factors. Tannins can lower the available proteins by antagonistic competition and can therefore elicit protein deficiency syndrome, kwarshiorkor and marasmus. They can also prevent protein digestion by inhibiting the activation of pepsinogen and chymotrypsinogen to pepsin and chymotrypsin respectively (Hoon and Hoof, 2006). It is however, surprising that *Moringa* leaf that contained the highest tannin level is best suited for dietary purpose among the *Moringa* plant parts studied. This fact could be attributed to the ratio of tannin to protein present in the leaf for chelation and to the nature of the medium present. Phytic acid and oxalates have complicated effects in the human system including reacting tightly with divalent ions such as calcium, iron and zinc ions, thereby making them unavailable to the body (Akubugwo *et al.*, 2007). However, these anti-nutrients might be removed through soaking, boiling or even frying (Ekop and Eddy, 2005; Kidmose *et al.*, 2006; Igwilo *et al.*, 2007a; Igwilo *et al.*, 2007b).

The enzyme activity (Fig.11) and the bilirubin concentration (Fig.12) of rats fed with the different feeds, when subjected to Oneway analysis of variance, showed that significant difference between group members exist only in alanine transaminase activity ($p<0.05$). The implication of this is that there is significant difference in the alanine transaminase activity of rats fed with the different feed rations. Further analysis using multiple analysis of variance indicated that there is a significance difference ($p<0.05$) in the alanine

transaminase activity of rats fed with *Moringa* leaf diet and the control (commercial rat pellets) at 95% level of significance, ($p=0.025$). The alanine transaminase activity of the rats fed with commercial rat pellets is significantly greater than that of those fed with *Moringa* leaf diet. This indicated that although both feed rations supported growth, the alanine transaminase activity was higher in the rats fed with commercial rat pellets. It might suggest that *Moringa* leaf diet has a positive effect on the liver. This is so because alanine transaminase is an intracellular enzyme of the hepatocytes. Thus an increase is an indication of necrosis and is important in monitoring liver cytolysis. The transaminases are important in assessing liver damage due to drugs or any plant extract. This observation seems to be supportive of its use in treating liver disease in the northern part of Nigeria where *Moringa oleifera* leaf is consumed in large quantities.

In the bilirubin concentration, there is no significance difference in the total and direct bilirubin in rats fed with any of the feeds and the control ($p>0.05$), indicating that the different feed rations has no appreciable effect on the bilirubin level and by implication, no significant effect on the liver.

Conclusion

The proximate analysis showed that *Moringa oleifera* plant is rich in nutrients particularly the leaves, seeds and the flowers. The amino acid composition of the different parts of the plant showed that they are rich in both essential and non-essential amino acids, confirming the earlier reports by Makkar and Becker (1997), Oliveira *et al* (1999) and Fuglie (2001) that *Moringa oleifera* contains all the essential amino acids needed for normal body functioning. Analysis of variance indicated that the distribution of amino acids in the seeds and other parts of the plant are highly similar, suggesting that the seeds may provide just exactly the same type of amino acid as the stems, roots, flower and the leaves of *Moringa oleifera.*

However, when the raw *Moringa oleifera* feed supplements were used in animal feeding trials, only the leaves supported growth marginally while the seeds and flowers did not support growth at all. This could be attributed to

the presence of anti-nutrients which interfered with protein digestion, but truely could be removed through boiling or frying (Ekop and Eddy, 2005; Kidmose *et al*., 2006).

Suggestion for further research

Further research should focus at determining the effects of processing on the nutritional values of the different parts of the plant to ascertain the suitability as a complete supplement for humans and animals. It is also important to look at the long term toxicity studies of *Moringa oleifera* plant parts to ascertain its safety as food supplement.

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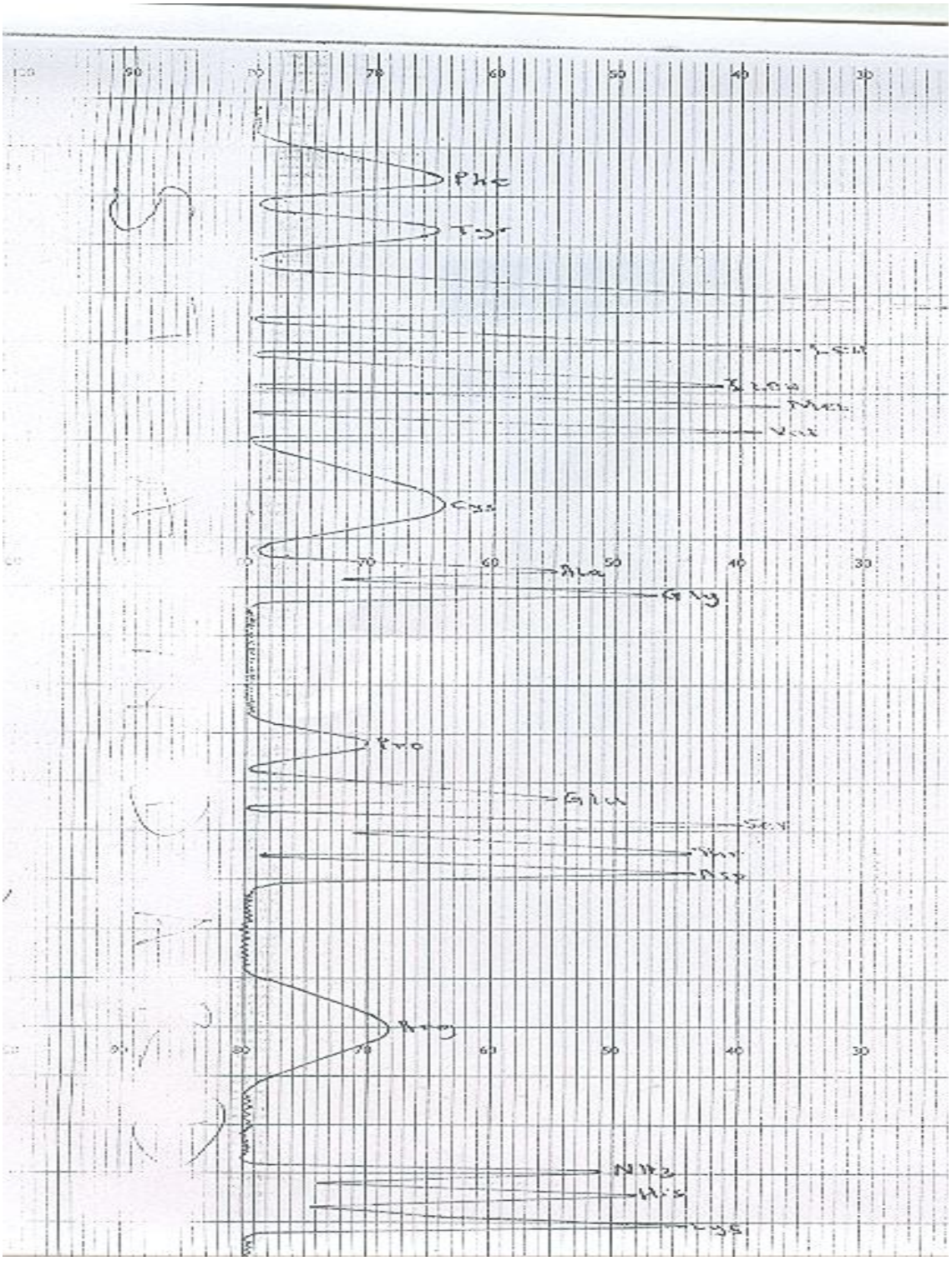
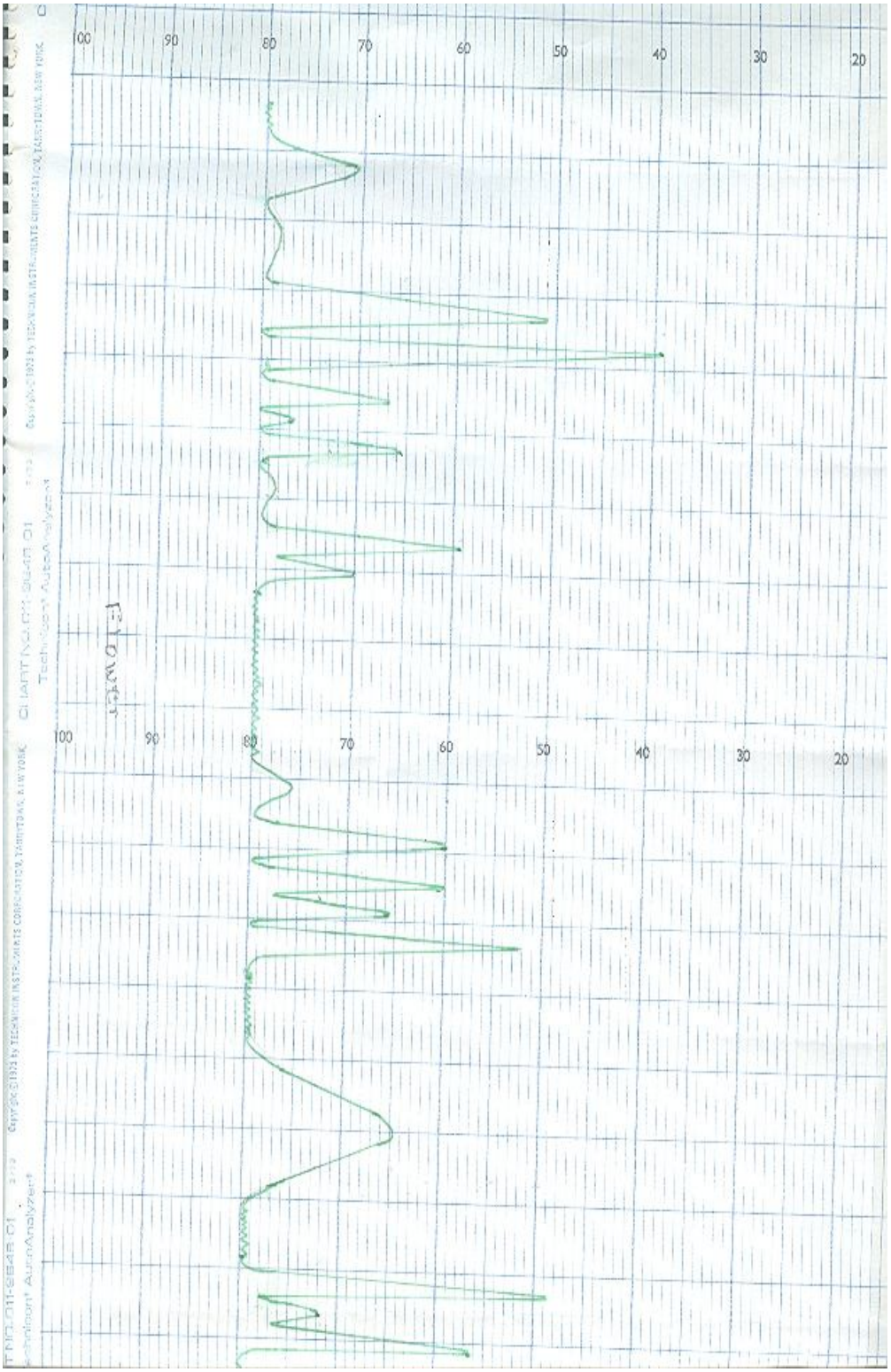
APPENDIX.

Parameter	Seeds %DW	Leaves	Flowers	Roots	Stems
Crude lipids (%)	**33.78±2.41** (35.67;36.67;29.0)	**20.00±2.31** (24;16;20)	**9.44±3.08** (12.0;13.0;3.33)	**6.33±1.64** (9.5;5.5;4.0)	**1.77±0.98** (3.7;1.1;0.5)
Crude Proteins (%)	**28.02±0.01** (28.00; 28.03)	**27.60±0.14** (27.74;27.46)	**25.99±0.07** (26.06;25.92)	**5.02±1.52** (3.5; 6.54)	**3.59±0.96** (2.63; 4.54)
Carbohydrates (%)	28.77	33.93	50.57	76.75	87.44
Ash (%)	**3.03±0.07** (3.1;2.9;3.1)	**11.60±3.65** (18.9;8.0;7.9)	**3.57±0.12** (3.5;3.4;3.8)	**4.97±0.53** (6.0;4.6;4.3)	**1.63±0.22** (1.2;1.9;1.8)
Moisture (%)	**6.40±0.31** (6.2;6.0;7.0)	**6.87±0.50** (7.8;6.7;6.1)	**10.43±0.58** (10.5;11.4;9.4)	**6.93±0.58** (6.7;8.0;6.0)	**5.57±0.35** (5.5;6.2;5.0)
Energy values (Kcal/100g)	531.18	426.12	391.20	384.05	380.05

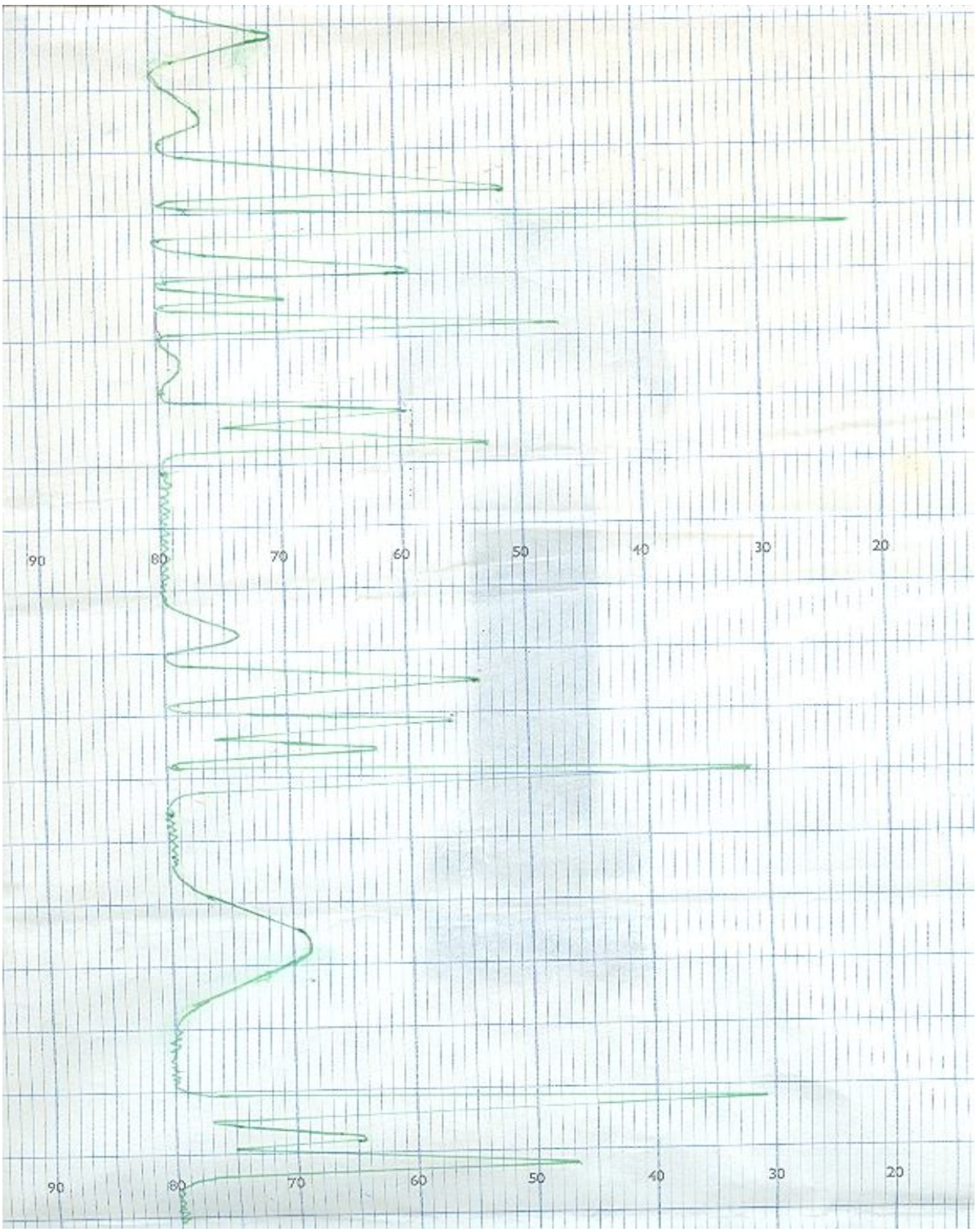
Appendix I: Proximate composition of the seeds, leaves, flowers, roots and stems of *Moringa oleifera* (Mean ± S.E.M).



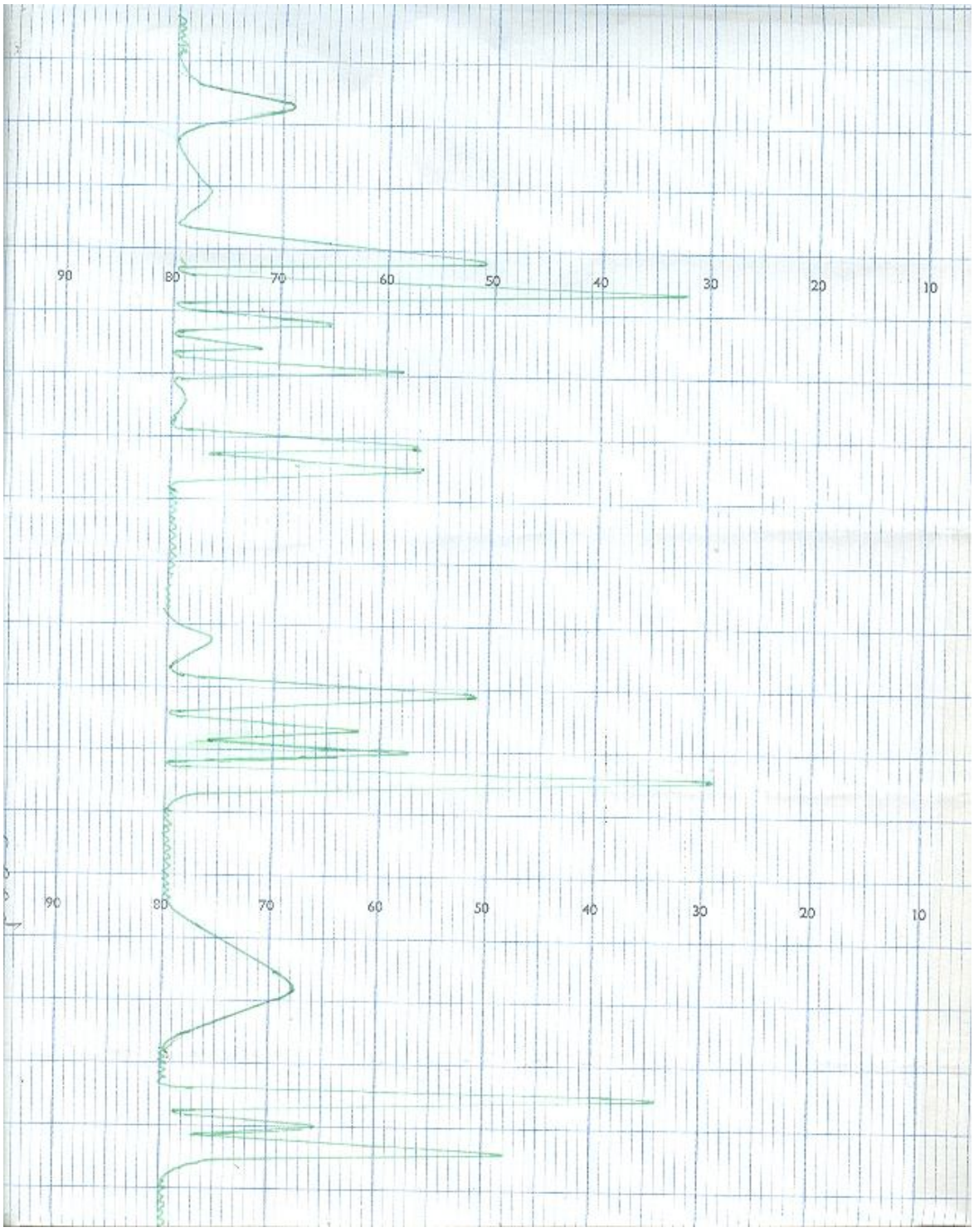
Appendix II: Chromatograph for the standard amino acids.



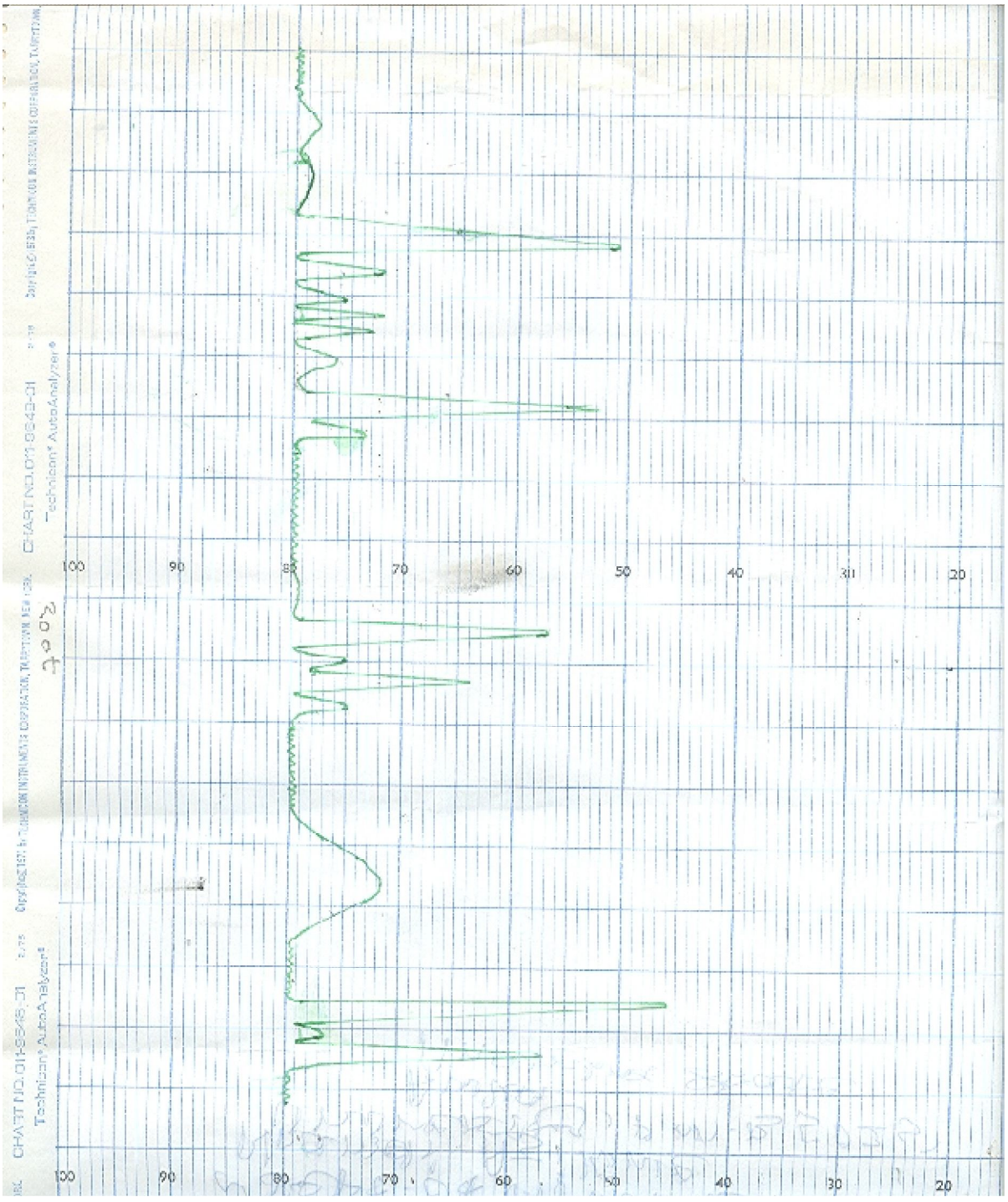
Appendix III(a): Chromatograph for *Moringa* flower.



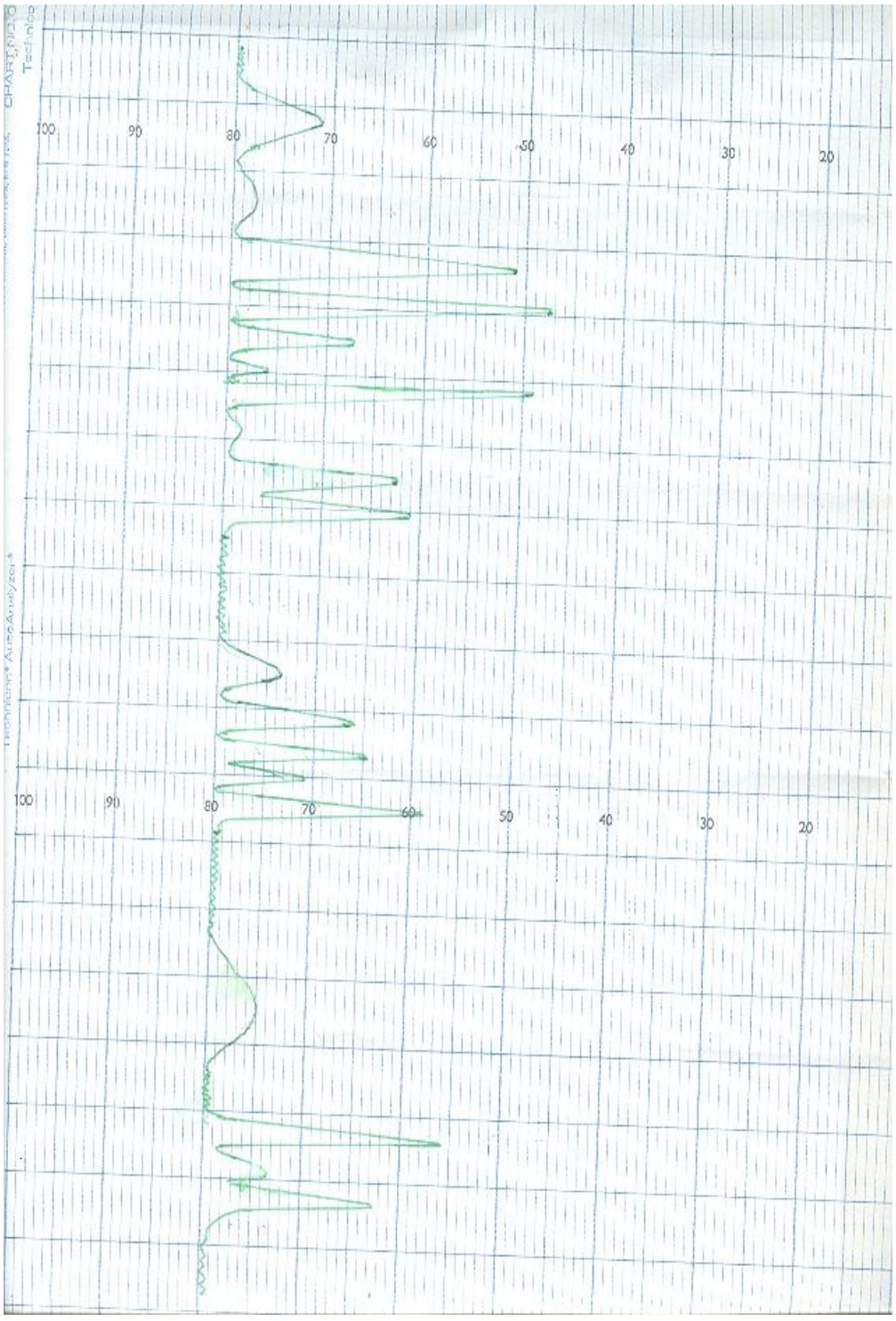
Appendix III (b): Chromatograph for *Moringa* leaf.



Appendix III(c): Chromatograph for *Moringa* Seed.



Appendix III (d): Chromatograph for *Moringa* Root.



Appendix III (e): Chromatograph for *Moringa* Stem.

	Total EAA (g/100g protein)	Total NEAA (g/100g protein)	Total AA (g/100g protein)	% EAA	% NEAA	% AA	%EAA in whole plant	%NEAA in whole plant.
Seed	30.70	32.81	63.51	48.34	51.66	24.69	11.94	12.76
Leaf	34.72	33.81	68.53	50.66	49.34	26.64	13.50	13.14
Flower	26.37	23.37	49.74	53.02	46.98	19.34	10.25	9.09
Root	14.02	18.42	32.44	43.22	56.78	12.61	5.45	7.16
Stem	21.42	21.58	43.00	49.81	50.19	16.72	8.33	8.39
Total			257.22					

Appendix IV: % of EAA and NEAA in all the parts of the plant and the proportion of %AA, %EAA and %NEAA in whole plant.

	Total AA (g/100g protein)	Total branched chain AA(Val,Leu,Ile)	% branched chain AA	Total acidic AA(asp,glu)	% acidic AA	Total aromatic AA(phe, tyr)	% aromatic AA
Seed	63.51	11.59	18.25	18.20	28.66	6.14	9.67
Leaf	68.53	15.38	22.44	16.71	24.38	7.44	10.86
Flower	49.74	9.40	18.90	11.41	22.94	5.54	11.14
Root	32.44	2.75	8.48	9.26	28.55	2.05	6.32
Stem	43.00	10.57	24.58	8.23	19.14	5.66	13.16

Appendix V (a): Proportion of branched chains, acidic and aromatic amino acids.

	Total AA (g/100g protein)	Total basic AA	% basic AA
Seed	63.51	11.46	18.04
Leaf	68.53	11.56	16.87
Flower	49.74	10.66	21.43
Root	32.44	7.14	22.01
Stem	43.00	5.49	12.77

Appendix V (b): Proportion of basic amino acid.

Mineral content	Seeds	Leaves	Flowers	Roots	Stems
Na (mg/100g)	129.03	104.06	120.93	514.80	378.38
Ca (mg/100g)	2.84	13.45	2.32	3.99	1.38
K (mg/100g)	-	20.81	3.02	15.4	32.4
Pb (mg/100g)	-	-	-	-	-
Ba (mg/100g)	-	-	-	-	-

Appendix VI: Mineral composition of the seeds, leaves, flowers, roots and the stems of *Moringa oleifera.*

Vitamins	Seeds	Leaves	Flowers	Roots	Stems
Ascorbic acid (mg/100g)	94.74	773.30	459.21	48.13	71.44
Thiamine (B1),mg/100g	-	18.47	-	-	-
Riboflavin (B2),mg/100g	-	14.82	-	-	-
Pyridoxine (B6), mg/100g	-	57.29	7.69	-	-
Niacin (B3), mg/100g	-	50.35	-	5.83	1.32

Appendix VII: Vitamin composition of the seeds, leaves, flowers, roots and the stems of *Moringa oleifera.*

Anti-nutrients	Seeds	Leaves	Flowers	Roots	Stems
Tannins (mg/100g)	40	420	60	45	100
Cyanogenic glycosides (mg/100g)	4.59	32.40	4.31	2.72	31.40
Phytates (mg/100g)	0.435	0.013	0.436	0.064	0.048
Saponins (mg/100g)	9.40	11.80	15.20	4.20	12.10

Appendix VIII: The levels of anti-nutritional factors in the seeds, leaves, flowers, roots and stems of *Moringa oleifera.*

Commercial pellets

Rats 1	2	3	4	5	Mean	± SEM
50.03	53.05	51.90	51.37	45.15	51.90	1.38
68.14	62.33	71.98	60.90	51.71	63.01	3.46
67.60	70.13	84.65	73.59	56.74	70.54	4.51
75.73	80.37	94.55	85.93	66.66	80.65	4.69
81.33	83.84	100.50	89.41	68.31	84.68	5.25
77.81	84.81	97.20	85.86	65.86	82.31	5.15
89.60	94.60	113.14	101.70	77.90	95.39	5.88
102.09	112.95	129.69	125.90	90.07	112.14	7.36

Casilan diet.

1	2	3	4	5	Mean	± SEM
48.45	59.09	60.34	50.70	61.34	55.94	2.48
43.34	53.73	54.61	47.14	55.60	56.88	2.24
40.36	49.97	50.77	43.75	51.60	47.29	2.07
38.71	47.79	48.30	41.32	48.52	45.03	1.91
38.61	47.60	47.42	41.33	47.90	44.57	1.80
38.15	45.31	44.69	38.34	44.82	42.26	1.53
38.83	46.37	44.46	-	44.50	43.54	1.63
38.29	45.01	43.70	-	44.56	42.89	1.56

Flower diet.

1	2	3	4	5	Mean	± SEM
50.21	57.63	52.10	56.40	59.89	55.25	1.66
48.73	57.11	51.78	54.03	58.50	54.03	1.65
49.80	54.59	52.50	51.98	55.58	52.89	0.95
44.81	46.40	46.96	42.59	46.70	45.49	0.76
43.01	46.40	45.14	41.61	46.01	44.43	0.85
43.61	46.80	45.67	39.31	45.86	44.25	1.25
39.64	44.15	41.25	36.39	42.61	40.81	1.24
38.68	42.98	40.01	35.46	41.54	39.73	1.20

Leaf diet.

1	2	3	4	5	Mean	± SEM
51.02	36.65	55.24	38.46	52.28	46.73	3.81
55.04	40.11	58.52	42.58	57.82	50.81	3.92
55.20	40.77	62.37	44.76	61.09	53.44	3.93
57.16	46.36	66.54	47.81	64.14	56.40	4.10
56.23	48.44	68.10	49.18	65.03	57.40	4.00
57.63	49.87	69.69	49.10	63.85	58.03	3.97
58.99	50.95	72.56	51.20	65.73	59.89	4.18
54.90	62.61	79.80	-	69.05	66.59	5.27

Seed diet

1	2	3	4	5	Mean	± SEM
51.90	57.79	52.54	51.45	65.17	55.77	2.61
46.66	50.55	48.32	45.21	56.41	49.44	1.96
41.32	45.41	42.49	40.96	49.61	43.95	1.61
40.26	43.73	40.25	37.61	49.09	42.19	1.98
37.85	42.45	39.47	36.92	46.21	40.58	1.69
33.75	37.74	36.32	33.65	41.50	36.59	1.45
29.40	36.56	32.79	31.30	38.64	33.74	1.70
33.55	38.57	35.55	34.28	42.41	36.87	1.52

DAYS	Grp. A Diet (Mean ±SEM)	Grp.B Diet (Mean±SEM)	Grp.C Diet (Mean±SEM)	Grp. D Diet (Mean±SEM)	Grp.E Diet (Mean±SEM)
1	46.73±3.81	55.77±2.61	55.20± 1.60	55.94±2.48	51.90±1.38
3	50.81±3.92	49.44±1.96	54.00± 1.58	56.88±2.24	63.01±3.46
6	53.44±3.93	43.95±1.61	52.00± 0.91	47.29±2.07	70.54±4.51
9	56.40±4.10	42.19±1.98	45.50± 0.73	45.03±1.91	80.65±4.69
12	57.40±4.00	40.58±1.69	44.50±0.87	44.57±1.80	84.68±5.25
15	58.03±3.97	36.59±1.45	44.24±0.73	42.26±1.53	82.31±5.15
18	59.89±4.18	33.74±1.70	40.80± 1.19	43.54±1.63	95.39±5.88
21	66.59±5.27	36.87±1.52	39.70± 1.15	42.89±1.56	112.14±7.36

Appendix IX: The growth rates of rats fed with the different diets.

	Moringa leaf Diet (Mean ±SEM)	Moringa seed Diet (Mean±SEM)	Moringa flower Diet (Mean±SEM)	Casilan Diet (Mean±SEM)	Comm. Rat pellets (Mean ±SEM)
Aspartate transaminase activity (U/l)	9.0±1.00	16.5±6.50	16.5±2.04	21.4±3.77	23.8±1.34
Alanine transaminase activity (U/l)	6.67±1.34	8.00±0.00	9.60±0.87	10.00±0.79	11.20±0.44
Alkaline phosphatise (U/l)	50.75±16.67	48.17±2.76	39.16±4.98	43.89±2.65	34.25±7.56
Total bilirubin (µmol/l)	28.50±7.62	34.50± 1.66	22.50± 3.51	22.00± 4.01	21.20±5.30
Direct bilirubin (µmol/l)	21.23±8.84	25.60± 1.33	16.35± 3.86	12.40± 0.01	12.00±2.78

Appendix X: The enzyme activity and bilirubin concentration of rats fed with the leaves, seeds and flower of *Moringa oleifera* diets and the control diet.

. Appendix XI: Multiple comparison of ANOVA for proximate analysis.

Dependent Variable	(I)VAR00001	(J)VAR00001	Mean Diff. (I-J)	Std. Error	Sig.
LIPID	Seed %DW	Leaves	13.7800*	3.1122	.009
		Flowers	24.3367*	3.1122	.000
		Roots	27.4467*	3.1122	.000
		Stems	32.0133*	3.1122	.000
	Leaves	Seed	-13.7800*	3.1122	.009
		Flowers	10.5567*	3.1122	.043
		Roots	13.6667*	3.1122	.009
		Stems	18.2333*	3.1122	.001
	Flowers	Seed	-24.3367*	3.1122	.000
		Leaves	-10.5567*	3.1122	.043
		Roots	3.1100	3.1122	.850
		Stems	7.6767	3.1122	.175
	Roots	Seed	-27.4467*	3.1122	.000
		Leaves	-13.6667*	3.1122	.009
		Flowers	-3.1100	3.1122	.850
		Stems	4.5667	3.1122	.603
	Stems	Seed	-32.0133*	3.1122	.000
		Leaves	-18.2333*	3.1122	.001
		Flowers	-7.6767	3.1122	.175
		Roots	-4.5667	3.1122	.603
PROTEIN	Seed %DW	Leaves	0.4150	1.1397	.995
		Flowers	2.0250	1.1397	.472
		Roots	22.9950*	1.1397	.000
		Stems	24.4300*	1.1397	.000
	Leaves	Seed	0.4150	1.1397	.995
		Flowers	1.6100	1.1397	.647
		Roots	22.5800*	1.1397	.000
		Stems	24.0150*	1.1397	.000
	Flowers	Seed	-2.0250	1.1397	.472
		Leaves	-1.6100	1.1397	.647
		Roots	20.9700*	1.1397	.000
		Stems	22.4050*	1.1397	.000
	Roots	Seed	-22.9950*	1.1397	.000
		Leaves	-22.5800*	1.1397	.000
		Flowers	-20.9700*	1.1397	.000
		Stems	1.4350	1.1397	.724

	Stems	Seed	-24.4300*	1.1397	.000
		Leaves	-24.0150*	1.1397	.000
		Flowers	-22.4050*	1.1397	.000
		Roots	-1.4350	1.1397	.724
ASH	Seeds %DW	Leaves	-8.5667*	2.3379	.028
		Flowers	-0.5333	2.3379	.999
		Roots	-1.9333	2.3379	.916
		Stems	1.4000	2.3379	.972
	Leaves	Seed	8.5667*	2.3379	.028
		Flowers	8.0333*	2.3379	.040
		Roots	6.6333	2.3379	.101
		Stems	9.9667*	2.3379	.011
	Flowers	Seed	0.5333	2.3379	.999
		Leaves	-8.0333*	2.3379	.040
		Roots	-1.4000	2.3379	.972
		Stems	1.9333	2.3379	.916
	Roots	Seed	1.9333	2.3379	.916
		Leaves	-6.6333	2.3379	.101
		Flowers	1.4000	2.3379	.972
		Stems	3.3333	2.3379	.627
	Stems	Seed	-1.4000	2.3379	.972
		Leaves	-9.9667*	2.3379	.011
		Flowers	-1.9333	2.3379	.916
		Roots	-3.3333	2.3379	.627
MOISTURE	Seeds %DW	Leaves	-0.4667	0.6753	.954
		Flowers	-4.0333*	0.6753	.001
		Roots	-0.5000	0.6753	.942
		Stems	0.8333	0.6753	.733
	Leaves	Seed	0.4667	0.6753	.954
		Flowers	-3.5667*	0.6753	.003
		Roots	-3.3333E-0	0.6753	1.000
		Stems	1.3000	0.6753	.364
	Flowers	Seed	4.0333*	0.6753	.001
		Leaves	3.5667*	0.6753	.003
		Roots	3.5333*	0.6753	.003
		Stems	4.8667*	0.6753	.000
	Roots	Seed	0.5000	0.6753	.942
		Leaves	3.3333E-O	0.6753	1.000
		Flowers	-3.5333*	0.6753	.003

		Stems	1.3333	0.6753	.343
	Stems	Seed	-0.8333	0.6753	.733
		Leaves	-1.3000	0.6753	.364
		Flowers	-4.8667*	0.6753	.000
		Roots	-1.3333	0.6753	.343

Dependable Variable	(I)VAR00001	(J)VAR00001	Lower Bound (95% Conf. Interval)	Upper Bound (95% Conf. Interval)
LIPID	Seed %DW	Leaves	3.5374	24.0226
		Flowers	14.0940	34.5793
		Roots	17.2040	37.6893
		Stems	21.7707	42.2560
	Leaves	Seed	-24.0226	-3.5374
		Flowers	0.3140	20.7993
		Roots	3.4240	23.9093
		Stems	7.9907	28.4760
	Flowers	Seed	-34.5793	-14.0940
		Leaves	-20.7993	-0.3140
		Roots	-7.1326	13.3526
		Stems	-2.5660	17.9193
	Roots	Seed	-37.6893	-17.2040
		Leaves	-23.9093	-3.4240
		Flowers	-13.3526	7.1326
		Stems	-5.6760	14.8093
	Stems	Seed	-42.2560	-21.7707
		Leaves	-28.4760	-7.9907
		Flowers	-17.9193	2.5660
		Roots	-14.8093	5.6760
PROTEIN	Seed %DW	Leaves	-4.1569	4.9869
		Flowers	-2.5469	6.5969
		Roots	18.4231	27.5669
		Stems	19.8581	29.0019
	Leaves	Seed	-4.9869	4.1569
		Flowers	-2.9619	6.1819
		Roots	18.0081	27.1519
		Stems	19.4431	28.5869
	Flowers	Seed	-6.5969	2.5469
		Leaves	-6.1819	2.9619

		Roots	16.3981	25.5419
		Stems	17.8331	26.9769
	Roots	Seed	-27.5669	-18.4231
		Leaves	-27.1519	-18.0081
		Flowers	-25.5419	-16.3981
		Stems	-3.1369	6.0069
	Stems	Seed	-29.0019	-19.8581
		Leaves	-28.5869	-19.4431
		Flowers	-26.9769	-17.8331
		Roots	-6.0069	3.1369
ASH	Seed %DW	Leaves	-16.2610	-0.8723
		Flowers	-8.2277	7.1610
		Roots	-9.6277	5.7610
		Stems	-6.2943	9.0943
	Leaves	Seeds	0.8723	16.2610
		Flowers	0.3390	15.7277
		Roots	-1.0610	14.3277
		Stems	2.2723	17.6610
	Flowers	Seed	-7.1610	8.2277
		Leaves	-15.7277	-0.3390
		Roots	-9.0943	6.2943
		Stems	-5.7610	9.6277
	Roots	Seed	-5.7610	9.6277
		Leaves	-14.3277	1.0610
		Flowers	-6.2943	9.0943
		Stems	-4.3610	11.0277
	Stems	Seed	-9.0943	6.2943
		Leaves	-17.6610	-2.2723
		Flowers	-9.6277	5.7610
		Roots	-11.0277	4.3610
MOISTURE	Seed %DW	Leaves	-2.6891	1.7558
		Flowers	-6.2558	-1.8109
		Roots	-2.7224	1.7224
		Stems	-1.3891	3.0558
	Leaves	Seeds	-1.7558	2.6891
		Flowers	-5.7891	-1.3442
		Roots	-2.2558	2.1891
		Stems	-0.9224	3.5224
	Flowers	Seed	1.8109	6.2558

		Leaves	1.3442	5.7891
		Roots	1.3109	5.7558
		Stems	2.6442	7.0891
	Roots	Seed	-1.7224	2.7224
		Leaves	-2.1891	2.2558
		Flowers	-5.7558	-1.3109
		Stems	-0.8891	3.5558
	Stems	Seed	-3.0558	1.3891
		Leaves	-3.5224	0.9224
		Flowers	-7.0891	-2.6442
		Roots	-3.5558	0.8891

*Mean difference is significant at the .05 level.

Appendix XII: Multiple comparison of ANOVA for Dietary evaluation.

Dependable Variable	(I)Feed Type	(J) Feed Type	Mean Diff. (I-J)	Std. Error	Sig.
DAY 1	Comm.pellets	Casilan	-5.6840	3.6656	.544
		Flower	-4.9460	3.6656	.665
		Leaf	3.5700	3.6656	.864
		Seed	-5.4700	3.6656	.579
	Casilan diet	C.pellets	5.6840	3.6656	.544
		Flower	0.7380	3.6656	1.000
		Leaf	9.2540	3.6656	.125
		Seed	0.2140	3.6656	1.000
	Flower diet	C.pellets	4.9460	3.6656	.665
		Casilan	-0.7380	3.6656	1.000
		Leaf	8.5160	3.6656	.179
		Seed	-0.5240	3.6656	1.000
	Leaf diet	C.pellets	-3.5700	3.6656	.864
		Casilan	-9.2540	3.6656	.125
		Flower	-8.5160	3.6656	.179
		Seed	-9.0400	3.6656	.139
	Seed diet	C.pellets	5.4700	3.6656	.579
		Casilan	-0.2140	3.6656	1.000
		Flower	0.5240	3.6656	1.000
		Leaf	9.0400	3.6656	.139
DAY 3	Comm.pellets	Casilan	12.1280*	4.0064	.047
		Flower	8.9820	4.0064	.205

		Leaf	12.1980*	4.0064	.045
		Seed	13.5820*	4.0064	.022
	Casilan diet	C.pellets	-12.128*	4.0064	.047
		Flower	-3.1460	4.0064	.932
		Leaf	7.000E-	4.0064	1.000
		Seed	1.4540	4.0064	.996
	Flower diet	C.pellets	-8.9820	4.0064	.205
		Casilan	3.1460	4.0064	.932
		Leaf	3.2160	4.0064	.927
		Seed	4.6000	4.0064	.779
	Leaf diet	C.pellets	-12.198*	4.0064	.045
		Casilan	-7.0000E	4.0064	1.000
		Flower	-3.2160	4.0064	.927
		Seed	1.3840	4.0064	.997
	Seed diet	C.pellets	-13.582*	4.0064	.022
		Casilan	-1.4540	4.0064	.996
		Flower	-4.6000	4.0064	.779
		Leaf	-1.3840	4.0064	.997
DAY 6	Comm.pellets	Casilan	23.2520*	4.3688	.000
		Flower	17.6520*	4.3688	.005
		Leaf	17.7040	4.3688	.005
		Seed	26.5840*	4.3688	.000
	Casilan diet	C.Pellets	-23.252*	4.3688	.000
		Flower	-5.6000	4.3688	.705
		Leaf	-5.5480	4.3688	.712
		Seed	3.3320	4.3688	.938
	Flower diet	C.Pellets	-17.652*	4.3688	.005
		Casilan	5.6000	4.3688	.705
		Leaf	5.200E-	4.3688	1.000
		Seed	8.9320	4.3688	.282
	Leaf diet	C.Pellets	-17.704*	4.3688	.005
		Casilan	5.5480	4.3688	.712
		Flower	-5.2000E	4.3688	1.000
		Seed	8.8800	4.3688	.287
	Seed diet	C.Pellets	-26.584*	4.3688	.000
		Casilan	-3.3320	4.3688	.938
		Flower	-8.9320	4.3688	.282
		Leaf	-8.8800	4.3688	.287
DAY 9	Comm.pellets	Casilan	35.7200*	4.3701	.000

		Flower	35.1560*	4.3701	.000
		Leaf	24.2460*	4.3701	.000
		Seed	38.4600*	4.3701	.000
	Casilan diet	C.pellets	-35.720*	4.3701	.000
		Flower	-0.5640	4.3701	1.000
		Leaf	-11.4740	4.3701	.103
		Seed	2.7400	4.3701	.969
	Flower diet	C.pellets	-35.156*	4.3701	.000
		Casilan	0.5640	4.3701	1.000
		Leaf	-10.9100	4.3701	.131
		Seed	3.3040	4.3701	.940
	Leaf diet	C.pellets	-24.246*	4.3701	.000
		Casilan	11.4740	4.3701	.103
		Flower	10.9100	4.3701	.131
		Seed	14.2140*	4.3701	.029
	Seed diet	C.pellets	-38.460*	4.3701	.000
		Casilan	-2.7400	4.3701	.969
		Flower	-3.3040	4.3701	.940
		Leaf	-14.214*	4.3701	.029
DAY 12	Comm.pellets	Casilan	40.106*	4.5233	000
		Flower	40.244*	4.5233	.000
		Leaf	27.282*	4.5233	.000
		Seed	44.098*	4.5233	.000
	Casilan diet	C.Pellets	-40.106*	4.5233	.000
		Flower	0.1380	4.5233	1.000
		Leaf	-12.8240	4.5233	.069
		Seed	3.9920	4.5233	.900
	Flower diet	C.Pellets	-40.244*	4.5233	.000
		Casilan	-0.1380	4.5233	1.000
		Leaf	-12.9620	4.5233	.065
		Seed	3.8540	4.5233	.911
	Leaf diet	C.Pellets	-27.282*	4.5233	.000
		Casilan	12.8240	4.5233	.069
		Flower	12.9620	4.5233	.065
		Seed	16.816*	4.5233	.011
	Seed diet	C.Pellets	-44.098*	4.5233	.000
		Casilan	-3.9920*	4.5233	.900
		Flower	-3.8540	4.5233	.911
		Leaf	-16.816*	4.5233	.011

DAY 15	Comm.pellets	Casilan	40.0460*	4.4269	.000
		Flower	38.0580*	4.4269	.000
		Leaf	24.2800*	4.4269	.000
		Seed	45.7160*	4.4269	.000
	Casilan diet	C.pellets	-40.046*	4.4269	.000
		Flower	-1.9880	4.4269	.991
		Leaf	-15.766*	4.4269	.015
		Seed	5.6700	4.4269	.705
	Flower diet	C.pellets	-38.058*	4.4269	.000
		Casilan	-1.9880	4.4269	.991
		Leaf	-13.778*	4.4269	.039
		Seed	7.6580	4.4269	.439
	Leaf diet	C.pellets	-24.280*	4.4269	.000
		Casilan	15.7660*	4.4269	.015
		Flower	13.7780*	4.4269	.039
		Seed	21.4360*	4.4269	.001
	Seed diet	C.pellets	-45.716*	4.4269	.000
		Casilan	-5.6700	4.4269	.705
		Flower	-7.6580	4.4269	.439
		Leaf	-21.436*	4.4269	.001
DAY 18	Comm.pellets	Casilan	51.8480*	5.2661	.000
		Flower	54.5800*	4.9650	.000
		Leaf	35.5020*	4.9650	.000
		Seed	61.6500*	4.9650	.000
	Casilan diet	C.pellets	-51.848*	5.2661	.000
		Flower	2.7320	5.2661	.984
		Leaf	-16.346*	5.2661	.041
		Seed	9.8020	5.2661	.370
	Flower diet	C.pellets	-54.580*	4.9650	.000
		Casilan	-2.7320	5.2661	.984
		Leaf	-19.078*	4.9650	.009
		Seed	7.0700	4.9650	.621
	Leaf diet	C.pellets	-35.502*	4.9650	.000
		Casilan	16.3460*	5.2661	.041
		Flower	19.0780*	4.9650	.009
		Seed	26.1480*	4.9650	.000
	Seed diet	C.pellets	-61.650*	4.9650	.000
		Casilan	-9.8020	5.2661	.370

		Flower	-7.0700	4.9650	.621
		Leaf	-26.148*	4.9650	.000
DAY 21	Comm.pellets	Casilan	69.2500*	6.1938	.000
		Flower	72.4060*	5.8396	.000
		Leaf	45.5500*	6.1938	.000
		Seed	75.2680*	5.8396	.000
	Casilan diet	C.pellets	-69.250*	6.1938	.000
		Flower	3.1560	6.1938	.985
		Leaf	-23.700*	6.5289	.014
		Seed	6.018	6.1938	.864
	Flower diet	C.pellets	-72.406*	5.8396	.000
		Casilan	-3.1560	6.1938	.985
		Leaf	-26.856*	6.1938	.003
		Seed	2.8620	5.8396	.987
	Leaf diet	C.pellets	-45.550*	6.1938	.000
		Casilan	23.7000*	6.5289	.014
		Flower	26.8560*	6.1938	.003
		Seed	29.7180*	6.1938	.001
	Seed diet	C.pellets	-75.268*	5.8396	.000
		Casilan	-6.0180	6.1938	.864
		Flower	-2.8620	5.8396	.987
		Leaf	-29.718*	6.1938	.001

Dependent Variable	(I)Feed Type	(J)Feed Type	Lower Bound (95% Conf. Inter.)	Upper Bound (95% Conf. Inter.)
DAY 1	Comm.pellets	Casilan	-16.6530	5.2850
		Flower	-15.9150	6.0230
		Leaf	-7.3990	14.5390
		Seed	-16.4390	5.4990
	Casilan diet	C.pellets	-5.2850	16.6530
		Flower	-10.2310	11.7070
		Leaf	-1.7150	20.2230
		Seed	-10.7550	11.1830
	Flower diet	C.pellets	-6.0230	15.9150
		Casilan	-11.7070	10.2310
		Leaf	-2.4530	19.4850
		Seed	-11.4930	10.4450
	Leaf diet	C.pellets	-14.5390	7.3990

		Casilan	-20.2230	1.7150
		Flower	-19.4850	2.4530
		Seed	-20.0090	1.9290
	Seed diet	C.pellets	-5.4990	16.4390
		Casilan	-11.1830	10.7550
		Flower	-10.4450	11.4930
		Leaf	-1.9290	20.0090
DAY 3	Comm.pellets	Casilan	0.1394	24.1166
		Flower	-3.0066	20.9706
		Leaf	0.2094	24.1866
		Seed	1.5934	25.5706
	Casilan diet	C.pellets	-24.1166	-0.1394
		Flower	-15.1346	8.8426
		Leaf	-11.9186	12.0586
		Seed	-10.5346	13.4426
	Flower diet	C.pellets	-20.9706	3.0066
		Casilan	-8.8426	15.1346
		Leaf	-8.7726	15.2046
		Seed	-7.3886	16.5886
	Leaf diet	C.pellets	-24.1866	-0.2094
		Casilan	-12.0586	11.9186
		Flower	-15.2046	8.7726
		Seed	-10.6046	13.3726
	Seed diet	C.pellets	-25.5706	-1.5934
		Casilan	-13.4426	10.5346
		Flower	-16.5886	7.3886
		Leaf	-13.3726	10.6046
DAY 6	Comm.pellets	Casilan	10.1789	36.3251
		Flower	4.5789	30.7251
		Leaf	4.6309	30.7771
		Seed	13.5109	39.6571
	Casilan diet	C.pellets	-36.3251	-10.1789
		Flower	-18.6731	7.4731
		Leaf	-18.6211	7.5251
		Seed	-9.7411	16.4051
	Flower diet	C.pellets	-30.7251	-4.5789
		Casilan	-7.4731	18.6731
		Leaf	-13.0211	13.1251
		Seed	-4.1411	22.0051

	Leaf diet	C.pellets	-30.7771	-4.6309
		Casilan	-7.5251	18.6211
		Flower	-13.1251	13.0211
		Seed	-4.1931	21.9531
	Seed diet	C.pellets	-39.6571	-13.5109
		Casilan	-16.4051	9.7411
		Flower	-22.0051	4.1411
		Leaf	-21.9531	4.1931
DAY 9	Comm.pellets	Casilan	22.6428	48.7972
		Flower	22.0788	48.2332
		Leaf	11.1688	37.3232
		Seed	25.3828	51.5372
	Casilan diet	C.pellets	-48.7972	-22.6428
		Flower	-13.6412	12.5132
		Leaf	-24.5512	1.6032
		Seed	-10.3372	15.8172
	Flower diet	C.pellets	-48.2332	-22.0788
		Casilan	12.5132	13.6412
		Leaf	-23.9872	2.1672
		Seed	-9.7732	16.3812
	Leaf diet	C.pellets	-37.3232	-11.1688
		Casilan	-1.6032	24.5512
		Flower	-2.1672	23.9872
		Seed	1.1368	27.2912
	Seed diet	C.pellets	-51.5372	-25.3828
		Casilan	-15.8172	10.3372
		Flower	-16.3812	9.7732
		Leaf	-27.2912	-1.1368
DAY 12	Comm.pellets	Casilan	26.5705	53.6415
		Flower	26.7085	53.7795
		Leaf	13.7465	40.8175
		Seed	30.5625	57.6335
	Casilan diet	C.pellets	-53.6415	-26.5705
		Flower	-13.3975	13.6735
		Leaf	-26.3595	0.7115
		Seed	-9.5435	17.5275
	Flower diet	C.pellets	-53.7795	-26.7085
		Casilan	-13.6735	13.3975
		Leaf	-26.4975	0.5735

		Seed	-9.6815	17.3895
	Leaf diet	C.pellets	-40.8175	-13.7465
		Casilan	-0.7115	26.3595
		Flower	-0.5735	26.4975
		Seed	3.2805	30.3515
	Seed diet	C.pellets	-57.6335	-30.5625
		Casilan	-17.5275	9.5435
		Flower	-17.3895	9.6815
		Leaf	-30.3515	-3.2805
DAY 15	Comm.pellets	Casilan	26.7988	53.2932
		Flower	24.8108	51.3052
		Leaf	11.0328	37.5272
		Seed	32.4688	58.9632
	Casilan diet	C.pellets	-53.2932	-26.7988
		Flower	-15.2352	11.2592
		Leaf	-29.0132	-2.5188
		Seed	-7.5772	18.9172
	Flower diet	C.pellets	-51.3052	-24.8108
		Casilan	-11.2592	15.2352
		Leaf	-27.0252	-0.5308
		Seed	-5.5892	20.9052
	Leaf diet	C.pellets	-37.5272	-11.0328
		Casilan	2.5188	29.0132
		Flower	0.5308	27.0252
		Seed	8.1888	34.6832
	Seed diet	C.pellets	-58.9632	-32.4688
		Casilan	-18.9172	7.5772
		Flower	-20.9052	5.5892
		Leaf	-34.6832	-8.1888
DAY 18	Comm.pellets	Casilan	36.0115	67.6845
		Flower	39.6492	69.5108
		Leaf	20.5712	50.4328
		Seed	46.7192	76.5808
	Casilan diet	C.pellets	-67.6845	-36.0115
		Flower	-13.1045	18.5685
		Leaf	-32.1825	-0.5095
		Seed	-6.0345	25.6385
	Flower diet	C.pellets	-69.5108	-39.6492
		Casilan	-18.5685	13.1045

		Leaf	-34.0088	-4.1472
		Seed	-7.8608	22.0008
	Leaf diet	C.pellets	-50.4328	-20.5712
		Casilan	0.5095	32.1825
		Flower	4.1472	34.0088
		Seed	11.2172	41.0788
	Seed diet	C.pellets	-76.5808	-46.7192
		Casilan	-25.6385	6.0345
		Flower	-22.0008	7.8608
		Leaf	-41.0788	-11.2172
DAY 21	Comm.pellets	Casilan	50.5209	87.9791
		Flower	54.7481	90.0693
		Leaf	26.8209	64.2791
		Seed	57.6101	92.9259
	Casilan diet	C.pellets	-87.9791	-50.5209
		Flower	-15.5731	21.8851
		Leaf	-43.4422	-3.9578
		Seed	-12.7111	24.7471
	Flower diet	C.pellets	-90.0639	-54.7481
		Casilan	-21.8851	15.5731
		Leaf	-45.5851	-8.1269
		Seed	-14.7959	20.5199
	Leaf diet	C.pellets	-64.2791	-26.8209
		Casilan	3.9578	43.4422
		Flower	8.1269	45.5851
		Seed	10.9889	48.4471
	Seed diet	C.pellets	-92.9259	-57.6101
		Casilan	-24.7471	12.7111
		Flower	-20.5199	14.7959
		Leaf	-48.4471	-10.988

*Mean difference is significant at the .05 level.

Appendix XIII: Multiple comparison of ANOVA for enzyme activity and concentration of total and direct bilirubin concentrations.

Dependent Variable	(I)Feed Type	(J)Feed Type	Mean Diff.(I-J)	Std. Error	Sig.
Aspartate transaminase activity	Leaf diet	Seed	-7.5000	5.5950	.671
		Flower	-7.2000	5.0043	.613
		Casilan	-12.4000	5.0043	.145
		Comm.pellets	-14.8000	5.0043	.061
	Seed diet	Leaf	7.5000	5.5950	.671
		Flower	0.3000	5.0043	1.000
		Casilan	-4.9000	5.0043	.861
		Comm.pellets	-7.3000	5.0043	.601
	Flower diet	Leaf	7.2000	5.0043	.613
		Seed	-0.3000	5.0043	1.000
		Casilan	-5.2000	4.3339	.751
		Comm.pellets	-7.6000	4.3339	.432
	Casilan diet	Leaf	12.4000	5.0043	.145
		Seed	4.9000	5.0043	.861
		Flower	5.2000	4.3339	.751
		Comm.pellets	-2.4000	4.3339	.980
	Comm. pellets	Leaf	14.8000	5.0043	.061
		Seed	7.3000	5.0043	.601
		Flower	7.6000	4.3339	.432
		Casilan	2.4000	4.3339	.980
Alanine transaminase activity	Leaf diet	Seed	-1.3300	1.4815	.894
		Flower	-2.9300	1.3251	.225
		Casilan	-3.3300	1.3251	.137
		Comm.pellets	-4.5300*	1.3251	.025
	Seed diet	Leaf	1.3300	1.4815	.894
		Flower	-1.6000	1.3251	.747
		Casilan	-2.0000	1.3251	.571
		Comm.pellets	-3.2000	1.3251	.162
	Flower diet	Leaf	2.9300	1.3251	.225
		Seed	1.6000	1.3251	.747
		Casilan	-0.4000	1.1475	.996
		Comm.pellets	-1.6000	1.1475	.640
	Casilan diet	Leaf	3.3300	1.3251	.137
		Seed	2.0000	1.3251	.571

		Flower	0.4000	1.1475	.996
		Comm.pellets	-1.2000	1.1475	.831
	Comm. pellets	Leaf	4.5300*	1.3251	.025
		Seed	3.2000	1.3251	.162
		Flower	1.6000	1.1475	.640
		Casilan	1.2000	1.1475	.831
Alkaline phosphatase	Leaf diet	Seed	2.5833	18.1218	1.000
		Flower	11.5900	18.1218	.965
		Casilan	6.8833	18.1218	.995
		Comm.pellets	16.5000	16.7775	.858
	Seed diet	Leaf	-2.5833	18.1218	1.000
		Flower	9.0067	19.3730	.989
		Casilan	4.3000	19.3730	.999
		Comm.pellets	13.9167	18.1218	.935
	Flower diet	Leaf	-11.5900	18.1218	.965
		Seed	-9.0067	19.3730	.989
		Casilan	-4.7067	19.3730	.999
		Comm.pellets	4.9100	18.1218	.999
	Casilan diet	Leaf	-6.8833	18.1218	.995
		Seed	-4.3000	19.3730	.999
		Flower	4.7067	19.3730	.999
		Comm.pellets	9.6167	18.1218	.982
	Comm. pellets	Leaf	-16.5000	16.7775	.858
		Seed	-13.9167	18.1218	.935
		Flower	-4.9100	18.1218	.999
		Casilan	-9.6167	18.1218	.982
Total bilirubin	Leaf diet	Seed	-6.0000	10.0205	.972
		Flower	6.0000	10.0205	.972
		Casilan	6.5000	10.0205	.963
		Comm.pellets	7.3000	7.7618	.875
	Seed diet	Leaf	6.0000	10.0205	.972
		Flower	12.0000	11.5707	.833
		Casilan	12.5000	11.5707	.813
		Comm.pellets	13.3000	9.6807	.656
	Flower diet	Leaf	-6.0000	10.0205	.972
		Seed	-12.0000	11.5707	.833
		Casilan	0.5000	11.5707	1.000
		Comm.pellets	1.3000	9.6807	1.000
	Casilan	Leaf	-6.5000	10.0205	.963

	diet	Seed Flower Comm.pellets	-12.5000 -0.5000 0.8000	11.5707 11.5707 9.6807	.813 1.000 1.000
	Comm. pellets	Leaf Seed Flower Casilan	-7.3000 -13.3000 -1.3000 -0.8000	7.7618 9.6807 9.6807 9.6807	.875 .656 1.000 1.000
Direct bilirubin	Leaf diet	Seed Flower Casilan Comm.pellets	-4.3667 4.8833 8.8333 8.2083	8.1340 8.1340 8.1340 6.8054	.981 .971 .809 .749
	Seed diet	Leaf Flower Casilan Comm.pellets	4.3667 9.2500 13.2000 12.5750	8.1340 8.9104 8.9104 7.7166	9.81 .832 .600 .520
	Flower diet	Leaf Seed Casilan Comm.pellets	-4.8833 -9.2500 3.9500 3.3250	8.1340 8.9104 8.9104 7.7166	.971 .832 .990 .991
	Casilan diet	Leaf Seed Flower Comm.pellets	-8.8333 -13.2000 -3.9500 -0.6250	8.1340 8.9104 8.9104 7.7166	.809 .600 .990 1.000
	Comm. pellets	Leaf Seed Flower Casilan	-8.2083 -12.5750 -3.3250 0.6250	6.8054 7.7166 7.7166 7.7166	.749 .520 .991 1.00

Dependent Variable	(I)Feed type	(J)Feed type	Lower Bound (95% Conf. Interval)	Upper Bound (95% Conf. Interval)
Aspartate transaminase activity	Leaf diet	Seed Flower Casilan Comm.pellets	-24.6414 -22.5318 -27.7318 -30.1318	9.6414 8.1318 2.9318 0.5318
	Seed diet	Leaf Flower Casilan	-9.6414 -15.0318 -20.2318	24.6414 15.6318 10.4318

		Comm.pellets	-22.6318	8.0318
	Flower diet	Leaf	-8.1318	22.5318
		Seed	-15.6318	15.0318
		Casilan	-18.4777	8.0777
		Comm.pellets	-20.8777	5.6777
	Casilan diet	Leaf	-2.9318	27.7318
		Seed	-10.4318	20.2318
		Flower	-8.0777	18.4777
		Comm.pellets	-15.6777	10.8777
	Comm.pellets	Leaf	-0.5318	30.1318
		Seed	-8.0318	22.6318
		Flower	-5.6777	20.8777
		Casilan	-10.8777	15.6777
Alanine transaminase activity	Leaf diet	Seed	-5.8688	3.2088
		Flower	-6.9896	1.1296
		Casilan	-7.3896	0.7296
		Comm.pellets	-8.5896	-0.4704
	Seed diet	Leaf	-3.2088	5.8688
		Flower	-5.6596	2.4596
		Casilan	-6.0596	2.0596
		Comm.pellets	-7.2596	0.8596
	Flower diet	Leaf	-1.1296	6.9896
		Seed	-2.4596	5.6596
		Casilan	-3.9157	3.1157
		Comm.pellets	-5.1157	1.9157
	Casilan diet	Leaf	-0.7296	7.3896
		Seed	-2.0596	6.0596
		Flower	-3.1157	3.9157
		Comm.pellets	-4.7157	2.3157
	Comm.pellets	Leaf	0.4704	8.5896
		Seed	-0.8596	7.2596
		Flower	-1.9157	5.1157
		Casilan	-2.3157	4.7157
Alkaline phosphatase	Leaf diet	Seed	-55.1794	60.3460
		Flower	-46.1727	69.3527
		Casilan	-50.8794	64.6460
		Comm.pellets	-36.9779	69.9779
	Seed diet	Leaf	-60.3460	55.1794
		Flower	-52.7443	70.7576

		Casilan	-57.4509	66.0509
		Comm.pellets	-43.8460	71.6794
	Flower diet	Leaf	-69.3527	46.1727
		Seed	-70.7576	52.7443
		Casilan	-66.4576	57.0443
		Comm.pellets	-52.8527	62.6727
	Casilan diet	Leaf	-64.6460	50.8794
		Seed	-66.0509	57.4509
		Flower	-57.0443	66.4576
		Comm.pellets	-48.1460	67.3794
	Comm.pellets	Leaf	-69.9779	36.9779
		Seed	-71.6794	43.8460
		Flower	-62.6727	52.8527
		Casilan	-67.3794	48.1460
Total bilirubin	Leaf diet	Seed	-38.9788	26.9788
		Flower	-26.9788	38.9788
		Casilan	-26.4788	39.4788
		Comm.pellets	-18.2452	32.8452
	Seed diet	Leaf	-26.9788	38.9788
		Flower	-26.0806	50.0806
		Casilan	-25.5806	50.5806
		Comm.pellets	-18.5606	45.1605
	Flower diet	Leaf	-38.9788	26.9788
		Seed	-50.0806	26.0806
		Casilan	-37.5806	38.5806
		Comm.pellets	-30.5605	33.1605
	Casilan diet	Leaf	-39.4788	26.4788
		Seed	-50.5806	25.5806
		Flower	-38.5806	37.5806
		Comm.pellets	-31.0605	32.6605
	Comm.pellets	Leaf	-32.8452	18.2452
		Seed	-45.1605	18.5605
		Flower	-33.1605	30.5605
		Casilan	-32.6605	31.0605
Direct bilirubin	Leaf diet	Seed	-32.4682	23.7348
		Flower	-23.2182	32.9848
		Casilan	-19.2682	36.9348
		Comm.pellets	-15.3031	31.7197
	Seed diet	Leaf	-23.7348	32.4682

		Flower	-21.5337	40.0337
		Casilan	-17.5837	43.9837
		Comm.pellets	-14.0844	39.2344
	Flower diet	Leaf	-32.9848	23.2182
		Seed	-40.0337	21.5337
		Casilan	-26.8337	34.7337
		Comm.pellets	-23.3344	29.9844
	Casilan diet	Leaf	-36.9348	19.2682
		Seed	-43.9837	17.5837
		Flower	-34.7337	26.8337
		Comm.pellets	-27.2844	26.0344
	Comm.pellets	Leaf	-31.7197	15.3031
		Seed	-39.2344	14.0844
		Flower	-29.9844	23.3344
		Casilan	-26.0344	27.2844

*Mean difference is significant at the .05 level.