BIOCHEMICAL RESPONSE OF WEANLING WISTAR RATS ADMINISTERED CRUDE EXTRACTS OF *Moringa oleifera* (Lam) LEAF

BY

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ABSTRACT

Leaf meal contains phytochemicals known to influence performance and health status when included in animal diets. *Moringa oleifera* (MO) crude extracts are rich in phytochemicals which could be harnessed for boosting animal health. There is dearth of information on response of rats to MO phytochemical fractions. Hence, effects of phytochemical fractions from MO Leaves (MOL) on biochemical response of Weanling Wistar Rats (WWR) were investigated.

The MOL were harvested from plants grown in the field for 20 weeks, air-dried and milled (45.6μm). Crude Extract of MOL (CEMOL) was obtained with methanol using soxhlet apparatus. The CEMOL was further partitioned into Crude Alkaloid Extract (CAE), Crude Saponin Extract (CSE) and Crude Tannin Extract (CTE) by column chromatography. The CEMOL, CAE, CSE and CTE were administered to WWR (n=130, 41.27±0.02 g) daily by oral drenching at 1.0, 1.5 and 2.0 mg/mL in a completely randomised design for three weeks. Distilled water served as control. Daily Feed Intake (DFI) and Weight Gain (WG) were measured. Blood (5 mL) was sampled and analysed for White Blood Cells (WBC), Total cholesterol (TC) and Immunoglobulins D, E, G and M content (mg/dL) using standard procedures. Data were analysed using descriptive statistics and ANOVA at α0.05.

Yield of CAE, CSE and CTE in CEMOL were 25%, 16% and 17%, respectively. Response of WWR to the different levels of treatments differed significantly. The DFI ranged from 22.0±7.0 g (2.0 mg/mL CAE) to 39.3±1.2 g (1.0 mg/mL CAE). The WG of WWR reduced with increasing levels of treatments. The WWR on 2.0 mg/mL CSE had the lowest WG (7.6±4.7 g) which was not significantly different from 8.1±7.0 g (2.0 mg/mL CAE), 10.6±0.1 g (1.5 mg/mL CSE) and 11.7±2.8 g (2.0 mg/mL CTE), while WWR on control treatment had the highest WG (38.5±0.8 g). The WBC of WWR treated with 1.5 mg/mL CTE (8.1±0.2x103/μL), 1.0 mg/mL CEMOL (8.9±0.1x103/μL), 1.0 mg/mL CTE (8.2±0.2x103/μL) and 2.0 mg/mL CTE (8.2±0.1x103/μL) and control (8.16±0.1x103/μL) were similar, but significantly lower than those treated with 2.0 mg/mL of CEMOL (12.3±0.1x103/μL), CAE (12.6±0.1x103/μL) and CSE (13.3±0.4x103/μL). The TC of WWR differed significantly and decreased with increasing level of treatments except for CTE where the reverse was the case. The TC of WWR ranged from 34.0±4.4 mg/dL (2.0

mg/dL CAE) to 72.3±6.5 mg/dL (1.0 mg/dL CAE). Immunoglobulins D, E, G and M contents ranged from 199.10±1.00 (1.0 mg/mL CTE) to 340.6±5.0 (2.0 mg/mL CAE), 0.40±0.01 (1.0 mg/mL CTE) to 0.70±0.01 (2.0 mg/mL CAE), 648.10±0.00 (2.0 mg/mL CTE) to 1027.80±0.01 (1.0 mg/mL CAE) and 110.90±10.01 (1.0 mg/mL CTE) to 197.30±3.00 (1.0 mg/mL CAE), respectively.

Crude extract of moringa leaves; crude alkaloid extract and crude saponin extracts at 2.0 mg/mL reduced weight and lowered cholesterol in rats. Immunoglobulins were improved by crude alkaloid extract at 1.0 mg/mL.

Keywords: *Moringa oleifera*, Alkaloids, Saponins, Tannins, Immunoglobulins.

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DEDICATION

_This work is dedicate to God, the giver of life, memory of my late mother Mrs Owoade Odukoya and only junior sister I ever had Late Mrs Oluwatoyin Olubukola Adeogun (Nee Odukoya).

CERTIFICATION

I certify that this work was carried out by Ajibola Adegboyega **ODUKOYA** in the Department of Animal Science, Faculty of Agriculture, University of Ibadan, under my supervision.

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CHAPTER ONE

1.0 INTRODUCTION

Performance and intestinal health of livestock have been improved for decades with the use of Antibiotic Growth Promoters (AGP) feed additives, anti-fungal agents, probiotics and prebiotics with preference to consumer demands. However, multiple complications from the continued use of antibiotics in livestock feeds have been noted in traces of animal products. These complications have threatened public health and have resulted in the ban of AGP in animal feeds in some countries. Similar observations were documented by Ao *et al*. (2011). However, Grashorn (2010) reported that in response to these ban of AGP and growing consumer concerns, the use of phytogenic feed additives would be a suitable alternative.

Plants contain secondary metabolites like carotenoids, tannins, saponins, and alkaloids that functions in processes that enhance growth, health of animals and aesthetics of animal products. These secondary metabolites promote productivity, animal health; ensure healthy and safe animal products (Wallace *et al*., 2010; Ao *et al*., 2011). More interest have been developed in the use of additives from herbs in human and animal nutrition with focus on optimizing the use of feed additives According to Wenk (2007), herbs, spices and their extracts have been exploited thousands of years ago, exhibiting distinctive aroma and various medicinal properties.

However, it is importnt to further research into the positive impact of these herbs and spices on performance and health of animals to justify their use. The author further suggested the use of dried form of herbs and spices in diets of livestock in order to maximize their specific functions. However, large volumes of these plant materials would be needed when added for the purpose of feed dilution of finished feed (Wenk, 2007).

Indresh (2007) noted that extracts of different aromatic plants are sold solely or in combination with purified active components in the market. The efficiency of the extraction process and potency of the extracts are dependent on the extraction solvent, plant variety, growing conditions and age of harvest. Bio-active components of herbs and spices are dependent on the dosage of herbs and spices used. However, herbs and spices do not only

function in enhancing appetite and digestion process, but also play crucial role in physiological functions and ensure good health and productivity of animals.

Different extracts of herbs and spices exhibit different patterns in digestion processes, other bio-compounds improved the activity of digestive enzymes of the gastric mucosa. Platel and Srinivasan (2001) attributed more functions to bio-compounds present in herbs and spices, noting that they accelerate digestion and shorten the time of passage of feed through the digestive tract, and was in line with Suresh and Srinivasan (2007) findings.

Maenner *et al*. (2011) established that herb extracts promote animal growth by changing the intestinal micro biota, and increasing the rate of nutrient digestibility and absorption. Extracts of herbs enhanced nitrogen absorption and functions in improvement of the immune response. It was concluded that herbs also increase the resistance of stressed animals, while increasing the absorption of essential nutrients under stress conditions.

Moringa oleifera (Lam.) is one of the plants of interest for phytogenic feed additive. It is a multipurpose tree with great potential for animal feed and human consumption. Its leaves are rich in nutrients and secondary metabolites such as vitamins, minerals, carotenoids, polyphenols and flavonoids (Gopalakrishnanb *et al*., 2016). Moringa leaf meal contains secondary metabolites, which might add value to animal products (Zanu *et al*., 2012).

1.1 PROBLEM STATEMENT

Leaf meals contain phytochemicals known to influence performance and health status when included in animal diets. Moringa leaf meal contains a cocktail of phytochemicals whose individual and synergetic effects have not been ascertained. There is need to provide baseline information on the concentration, modes of action, application and potentials of different bioactive compounds in Moringa that can effectively be utilized to promote growth and good health in humans and livestock. There is lack of information on individual impact of phytochemical fractions on farm animal. Hence, the effects of individual bioactive extracts of Moringa leaf on biochemical and haematological responses of Weanling Wistar Rats (WWR) need to be investigated.

1.2 JUSTIFICATION

Feed safety cannot be overemphasized when assessing modern feed industrial practices as an established connection exists between the production of healthy and safe animal products and provision of high quality feed. In view of the current importance and novelty of investigations on the biological effects of *Moringa oleifera* phytochemicals in animal feeds, there is need to provide baseline information on the concentration, modes of action, application and potentials of different bioactive compounds namely: flavonoids, alkaloids, saponins, cyanogenes, phytic acid and tannins in moringa that can effectively be utilized to promote growth and manage various ailments (ethno medicine) in humans and livestock. Hence, the effects of individual bioactive extracts from *Moringa oleifera* leaf on biochemical responses of Weanling Wistar Rats (WWR) were investigated.

1.3 GENERAL OBJECTIVE

This study was design to evaluate the effects of Moorings extracts and its fractions on the growth performance and gastrointestinal health of Wistar rats.

1.4 SPECIFIC OBJECTIVES

1. To examine the alteration in chemical profile of Moringa plant leaf meal and leaf extracts at various ages of growth.
2. To identify extraction methods that optimises recovery of crude extract of *Moringa oleifera*.
3. To identify specific bioactive compounds in extract of Moringa leaf that are responsible for physiological responses in albino rats.
4. To test the inclusion level of bioactive compounds that will affect physiological responses in rats *in vivo.*

CHAPTER TWO

2.0 LITERATURE REVIEW

Interest in the use of herbal feed additives for livestock production is fast growing as a result of the increased incidence of microbial resistance to antibiotics and their effects on health. The need for alternatives growth promoters is being developed. The ban on use of certain antibiotics due to harmful residual effects, resistance and cost effectiveness has led to the development of a number of herbal feed additives, found to have beneficial effects on animal production. Herbs have properties that can improve digestibility, act as antioxidant, immune-stimulant, antimicrobial and anti-inflammatory agent. Research on standardisation of appropriate dosages of herb-based feed additives for specific functions need to be studied. This assertion justified the report on the use of garlic as an alternative growth promoter in livestock production, as it improved digestibility, growth rate and carcass traits. According to Yang *et al.* (2007), lemon grass and peppermint are good feed additives that enhanced production performance of dairyand beef cattle. Maenner *et al*. (2011) reported that menthol (*Mentha arvensis*) improved ileal digestibility of proteins and amino acids in weaned piglets.

2.1 INTERVENTION OF SPICES AND HERBS IN ANIMAL NUTRITION

2.1.1 SPICES AND HERBS AS APPETITE AND DIGESTIVE STIMULANTS

Measurement of the appetizing impact of spices and herbs is however subjected to the taste preferences of different animal species. The feed intake of pigs on diets supplemented with garlic or rosemary was significantly higher compared to diets supplemented with oregano or ginger. It was reported that weaned pigs consumed less feed when diets were replaced with thyme or oregano (Jugl-Chizzola *et al*., 2006). Platel and Srinivasan (2001) affirmed that digestive processes differ significantly as a result of the wide range of active compounds in different herbs and spices. The authors noted further that some herbs improved the production of bile acids and their excretion in bile for improved lipids digestion and absorption .

2.1.2 ANTI-INFLAMMATORY ACTION OF HERBS AND SPICES

Bio-compounds of black pepper, red pepper, cumin, cloves, curcuma, ginger, mint and cinnamon have showcased anti-inflammatory effect in animal studies. However, Manjunatha and Srinivasan (2006) have noted terpenoids and flavonoids as the major active molecules. These substances inhibit the metabolism of inflammatory prostaglandins, and could be found in other herbs and spices like chamomile, marigold, liquorice and anise.

2.1.3 IMMUNOSTIMULANT FUNCTION OF HERBS AND SPICES

According to Craig (2001), the immune systems of animals are nourished with essential bio-compounds of herbs and spices, and are not limited to flavonoids, vitamin C and carotenoids. The author noted further that herb containing molecules of immunostimulatory properties that can stimulate the interpheron synthesis and improve the activity of, macrophages, lymphocytes and NK cells

2.2 BENEFICIAL IMPACT OF HERBAL FEED ADDITIVES AND THEIR MODE OF ACTION

Herbs contain various bioactive compounds which interact with the environment and function as defense system against physiological stressors (Rhodes, 1996). However, apart from the toxic effect of some, many of these bioactive compounds have been reported to show beneficial impacts in animal metabolism, as well as food products. Some of these combounds belong to the classes of flavonoids, glucosinolates and isoprene derivatives with some acting as antibiotics or as antioxidants (Rhodes, 1996).

According to Dorman and Deans (2000), phytochemicals found in herbal plants possess anti-microbial activity and can favorably stimulate the eubiosis of the microflora, thereby selectively influencing microorganisms. Most herbal feed additives show their antibacterial effect by acting on the bacterial cell wall structure, denaturing and coagulating proteins.

2.2.1 ADVANTAGES OF HERBAL FEED ADDITIVES

According to Tamara *et al*. (2009), the major advantages and limitation of herbal extract utilisation in animal nutrition includes the presence of natural constituent of feeds, absence of residual effects, non-hazardous eco-friendly, and minimum problem of drug resistance.

2.2.2 LIMITATIONS OF HERBAL FEED ADDITIVES

The limitations in the use of herbal feed additives includes the difficulty in quantification and standardization as a result of the complex composition of bio-molecules; wide diversity in plant composition as a resut of location, soil type, weather conditions, altitude, harvesting and storage conditions, and season in which the plant is grown.

2.3 MORINGA PLANT

Sanchez *et al*. (2006) reported that *Moringa oleifera* was used as supplement in the feed of ruminants under tropical conditions and observed a positive performance enhancing effect. Foidl *et al*. (2001) noted in a study, the advantages of herbal mixtures showcasing the growth enhancing effects of a liquid extract of plants.

Moringa plant species are numerous and majority of these species have not been fully exploited. Out of all these species, *oleifera* is the most popular and cultivated in most part of the tropical countries and for years been identified as a traditional medicinal and industrial plant. It is a perennial plant with low quality wood. It is common in countries of Bangladesh, India, Afghanistan, Pakistan, Caribbean, Latin America, tropical Asia, Ghana and even Northern part of Nigeria. All part of Moringa plant are edible and medicinal. It is believed that Moringa species are still very much underutilized despite its great potential in animal nutrition and medicine, but is gradually gaining popularity due to its special economic potential. It has been reported that various part of moringa plant can be used for treatment of various sickness including but not limited to hepato- protective, circulatory stimulants, antioxidant, lowering cholesterol. Moringa *oleifera* being the most common specie of the monogenetic family, few other species like *Moringa stenopetala, Moringa peregrine* and *Moringa concanensis* have been discovered to be very useful and with almost the same potential like *Moringa oleifera* (Nikkon *et al*. 2003).

2.3.1 TAXONOMIC CLASSIFICATION

Kingdom : Plantae

Subkingdom : Tracheobionta

Super division : Spermatophyta

Division : Magnoliophyta

Class : Eudicots

Subclass : Rosids

Order : Brassicales

Family : Moringaceae

Genus : *Moringa*

Source: (Olson, 1999)

Plate 1.1 ***Moringa oleifera*** **plant**.

2.3.2 MORINGA FAMILY

Moringa families consist of 33 species, four are accepted, four are substitute and twenty five are unassessed.

2.3.3 NUTRITIONAL VALUE OF *Moringa oleifera* PLANT

Herbs in human and livestock diets are crucial sources of biologically active substances like fibre, vitamins and antioxidants. Vegetables and some plant species also contribute proteins, minerals and some other nutrients as well as moisture and energy that are of immense importance to human and livestock (Adenipekun and Oyetunji, 2010). *Moringa oleifera* leaves are high in nutrients and phytochemicals which makes it a plant of interest with the claim of increased productivity of animals placed on its leaf meal diet, because a well-nourished animal will develop stronger immunity to resists diseases. There are also claims that its leaf meal can be used for infants and breast feeding mothers (Anwar *et al*. 2007). There has been a heightened interest in the use of Moringa even in countries where they are not naturally cultivated. Moringa has been notably used in humans and livestock for first hand or temporary chemoprophylaxis treatments and also in the treatments of diseases and parasites in livestock (Anwar *et al*., 2007).

Moringa leaves are more easily incorporated into the diets of humans and animals. Various studies had been conducted on moringa leaves as reported by Abbas and Ahmed (2012). All reporting a positive effect on animal performances. Moringa leaves on dry matter basis contain; 5.9 % moisture, 38.6 % carbohydrate, 17.1% fat and 27.2 % protein. *Moringa oleifera* seeds contained; 90% moisture, 7.54% fiber, 6.53% ash, 31.65% protein and 34.80% ether extract, on dry matter basis. There have been reports of higher contents of essential and Sulphur amino acids in the leaves and kernels, respectively of moringa compared to amino acid, but the kernel is devoid of other essential amino acids (Makkar and Becker, 1997).

A comprehensive analysis of plant leaves kernel of moringa suggested a high crude protein content and oil, making it more suitable for use as a potential source of protein in animal diet while the high grade of fatty acid present in the oils makes them characteristically

comparable to fatty acids in olive oil. In another study, Brisibe *et al*. (2009) reported that nutritional variations of the leaves could be observed due to genetic, climatical factors and methods of cultivation, thereby resulting in the discrepancies in the results reported by different researchers. In a study conducted to assess the nutritional profile of moringa plant leaves (Makker and Becker, 1997), the authors observed variations in the reported values, attributing the variations to the ages of plants use, the environmental or edaphic factors prevailing where they were grown.

Table 2.2 shows the analysis of result for *Moringa oleifera* pods, fresh and dried leaf powder.

Table 2.1: Nutritional value of *Moringa oleifera* leaves and pods

Nutrients	Pod	Leaves	Leave powder
Moisture (%)	86.9	75.0	7.5
Metabolizable energy (Kcal)	26	92	205
Crude protein (g)	2.5	6.7	27.1
Crude fat (g)	0.1	1.7	2.3
Carbohydrate (g)	3.7	13.4	38.2
Fibre (g)	4.8	0.9	19.2
Minerals (mg/g)			
Calcium	30	440	2,003
Magnesium	24	24	368
Phosphorus	110	70	204
Potassium	259	259	1,324
Copper	3.1	1.1	0.57
Iron	5.3	7	28.2
Sulphur	137	137	870
Oxalic acid (mg)	10	101	1.6
Vitamin A (mg)	0.11	6.8	16.3
Vitamin B (mg)	423	423	-
Vitamin B1 (mg)	0.05	0.21	2.64
Vitamin B2 (mg)	0.07	0.05	20.5
Vitamin B3 (mg)	0.2	0.8	8.2
Vitamin C (mg)	120	220	17.3
Vitamin E (mg)	-	-	113
Arginine (g/16g N)	3.6	6.0	1.33
Histidine (g/16g N)	1.1	2.1	0.61
Lysine (g/16g N)	1.5	4.3	1.32
Tryptophan (g/16g N)	0.8	1.9	0.43
Phenylanaline (g/16g N)	4.3	6.4	1.39
Methionine (g/16g N)	1.4	2.0	0.35
Threonine (g/16g N)	3.9	4.9	1.19
Leucine (g/16g N)	6.5	9.3	1.95
Isoleucine (g/16g N)	4.4	6.3	0.83

Source: Fahey (2005)

2.3.5 ANTI-NUTRITIONAL FACTORS IN MORINGA LEAVES

These are components that limit efficient feed utilisation and affect health of farm animals when present in a diet. Ingestion of anti-nutritional substance by animals could result in interference with digestion and utilization of nutrients and at times even interfered with availability of minerals and other nutrient to the animal; it could affect feed efficiency, hinder growth and may even lead to death of animal. Nityanand (1997) stated that anti-nutitional factors common in non-conventional feed materials could be classified;

1. Based on its chemical nature
2. Based on interference with Nutrients (Protein and carbohydrate) digestibility and utilisation.
3. Based on interference with minerals availability

According to Nityanand (1997); Tannins could be grouped into two : Hydrolysed and condensed tannin. The hydrolysed tannins are very toxic and could be poisonous when large amount is consumed. Tannins could hamper the functions of microbes in the rumen by forming complexes with proteins and other nutrients thus interfering with their availability for optimal growth performance. It is however advised that plant protein sources of high tannin composition should be carefully utilised for health reasons. The inhibition of digestibility and nutrient utilization due to tannins have been reported in ruminants and monogastrics, though washing and soaking of the seeds, leaves and other parts of the plants have been reported to substantially reduce the content of tannin in the plant material.

Another anti- nutritional factor is the saponins. Saponins are bitter tasting and its ingestion can lead to reduced feed intake. Unlike monogastric animals, ruminants can metabolise saponins. The saponin content of plant materials could be reduced by soaking and washing in water (Nityanand, 1997). Phytate is another important anti-nutrient of great concern in animal production. They are found in virtually all feeds of phyto-source and are often associated with proteins, making them of major concern in high protein ingredients like SBM and GNC. They have high chelating capability which makes them chelators of many minerals and thus making them unavailable for use by poultry since they are devoid of the enzyme phytase that breaks the complex between phytic acid and the concerned nutrients.

The utilisation of phytase only in poultry however breaks these complex and thereby avails the monogastric animals of nutrients such as phosphorus for use. Anti-vitamin activities have been reported due to the presence of certain anti-nutrients especially against the fat soluble vitamins (Nityanand, 1997). Processing methods often times employed to improve value of the feedstuff do not totally eradicate these anti-nutrients, rather they reduce their concentrations to tolerable levels in feeds. Changes in some internal organs of an animal are often common indicators used in toxicity studies.

2.3.6 USES OF MORINGA PLANT

Moringa oleifera tree is a multi-purpose plant; all its parts (leaves, fruits, flower, stem and root) are useful and edible. Moringa plant could be used for so many things including but not limited to; green manure fertilizer, animal forage and water purification. Its leaves and stem has lower amounts of harmful compounds, it is not toxic. Moringa leaf meal as been reported to enhanced breast milk production among the African and Asian women (Solvia *et al*., 2005).

2.3.6.1 ANIMAL FEED SUPPLEMENT

Moringa leaf extract is a balanced nutrition for population. It is a very good daily supplement. Moringa saponin extract concentration is very low (4.7-5g/kg on dry matter basis) and as such safe for human and animal consumption without adverse effects and at this level, it may have positive effect on health status. Very good daily supplement and believe its extract is a balanced nutrition for both human and animals. The high protein values of *M. oleifera* qualifies it as supplements for high milk producing cow (Makkar and Becker, 1996) and more protein contents than most conventional protein supplements.

Solvia *et al*. (2005) ranked moringa as a potential replacement for SBM and rape seed meal due to its high fatty acid composition. Its inclusion in low quality diet enhanced intake of dry matter, digestibility and higher milk yield, with no reduction in quality.

2.3.6.2 MORINGA AS BIO-ENHANCER

Some genus of the plant Moringa has been linked with bio-enhancing attributes. Its molecules possess no drug activity of their own but they can enhance the bioavailability or uptake of other drugs thus reducing the effect of toxicity, cost and time in chemotherapeutic treatments. These molecules are referred to as bio-enhancers. In a study to assess the bio

enhancing activity of moringa, it was observed that niaziridin in Moringa pods enhances the activities of conventional antibiotics to combat gram +ve and –ve microbes. The presence of Moringa also increased bioavailability of nutrients and drugs by increasing the rate with which they were absorbed through the membranes of the gastrointestinal tract (Pal *et al*., 2010).

2.3.6.3 MORINGA IN WATER TREATMENT

Good quality water is essential for life as about 80% of human diseases are water related due to indiscriminate human activities in less developed countries. Chemicals used in water treatment at times have a residual deposit on the environment, which in turn can cause serious health hazards to human and animal. For example, the use of aluminum in high amount as coagulants in the treatment of water has raised serious concern due to its associated link with Alzheimer's disease (Mallevialle *et al*., 1984). Moringa is however adopted in clarification of drinking water especially in situations of excess turbidity to lower associated health issues. There has been reports on the use of Moringa in the treatment of waste water especially for the removal of lead, cadmium, nickel and copper (Chen, 2009).

In some rural areas where Moringa have been used in the clarification and treatment of river water, microbial population of water samples were drastically lowered. It was believed that polyelectrolytes from Moringa serves as flocculator and binds particles in suspension thus forming sediments that attach microbes. This observation does not totally guarantee microbe free water since some heat induced microbes can grow within storage tanks but can remove up to 90-100% faecal coliform load. Among the low income rural dweller, a simple, low cost water treatment technique has been developed using Moringa seeds to purify high turbid microbiologically contaminated water. *Moringa* seeds have an antibiotic like attribute, pterygospermin which does the work of destroying microbes in water (Olayemi and Alabi, 1994).

2.3.6.4 MORINGA IN FOOD PRESERVATION

The preservation and protection of food against deterioration is a major public concern especially the food industry. The use of synthetic preservatives in the protection of food though has gained tremendous ground, however, concerns are getting increased on the safety of foods preserved with these substances and the associated risks to the health of the consuming populace. On this note, consumers of foods have their demand and outcry on the need to totally or partially replace the use of synthetic preservatives in the food industry. This outcry has led to increasing interests in natural preservatives especially from plant origins to improve safety and shelf stability of food products (Buker *et al*., 2010). Although there is dearth of information in this regards, there has been recent report on the potential of moringa seeds to exhibit a sanitising and preservating attributes against a range of pathogenic and toxin producing microbes in food and food products.

2.3.6.5 MORINGA IN MEDICINE

Basically, evey parts of the plant are good in treating different diseases. Dolly *et al*. (2009) noted that *Moringa oleifra* leaf extract regulated hyperthyroidism and could be used as ethnomedicine to treat diabetes mellitus. Ghada (2013) also reported the advantageous effect of using the aqueous Moringa leaf extract as potent anti-diabetic treatment.

2.3.6.6 ANTIOXIDANT ACTIVITY OF MORINGA LEAF EXTRACT

Moringa plant is rich in antioxidant, many parts of the plan possess this attribute (Chumark *et al*., 2008). Siddhuraju and Becker (2003) established in a study conducted to examine the antioxidant properties of moringa subjected to different extraction methods, higher levels of antioxidant activities in ethanol and methanol treated moringa. The authors noted that the presence of some phenolic compounds as kaempferol and quercetin may be responsible for this act. A more recent study assessing the antioxidant activity of oil palm and moringa showed that antioxidant activity or scavenging property of moringa was higher than that of oil palm.

Many research reports affirmed moringa leaf and seed as a good antioxidant. Its leaf, seed and fruit aqueous extracts act as good antioxidant (Chumark *et al*. 2008).

2.3.6.7 BIOACTIVE COMPOUNDS IN *MORINGA OLEIFERA*

Investigation on medicinal plants revealed presence of numerous compounds with chemical properties effective at treating illness. These chemical compounds are referred to as phytochemical/bioactive compounds e.g Tannins. phenol, Terpenoids, Saponins, alkaloids. Active bioactive compounds found in medicinal plants had been attributed to phytochemicals (Anwar *et al.,* 2007).

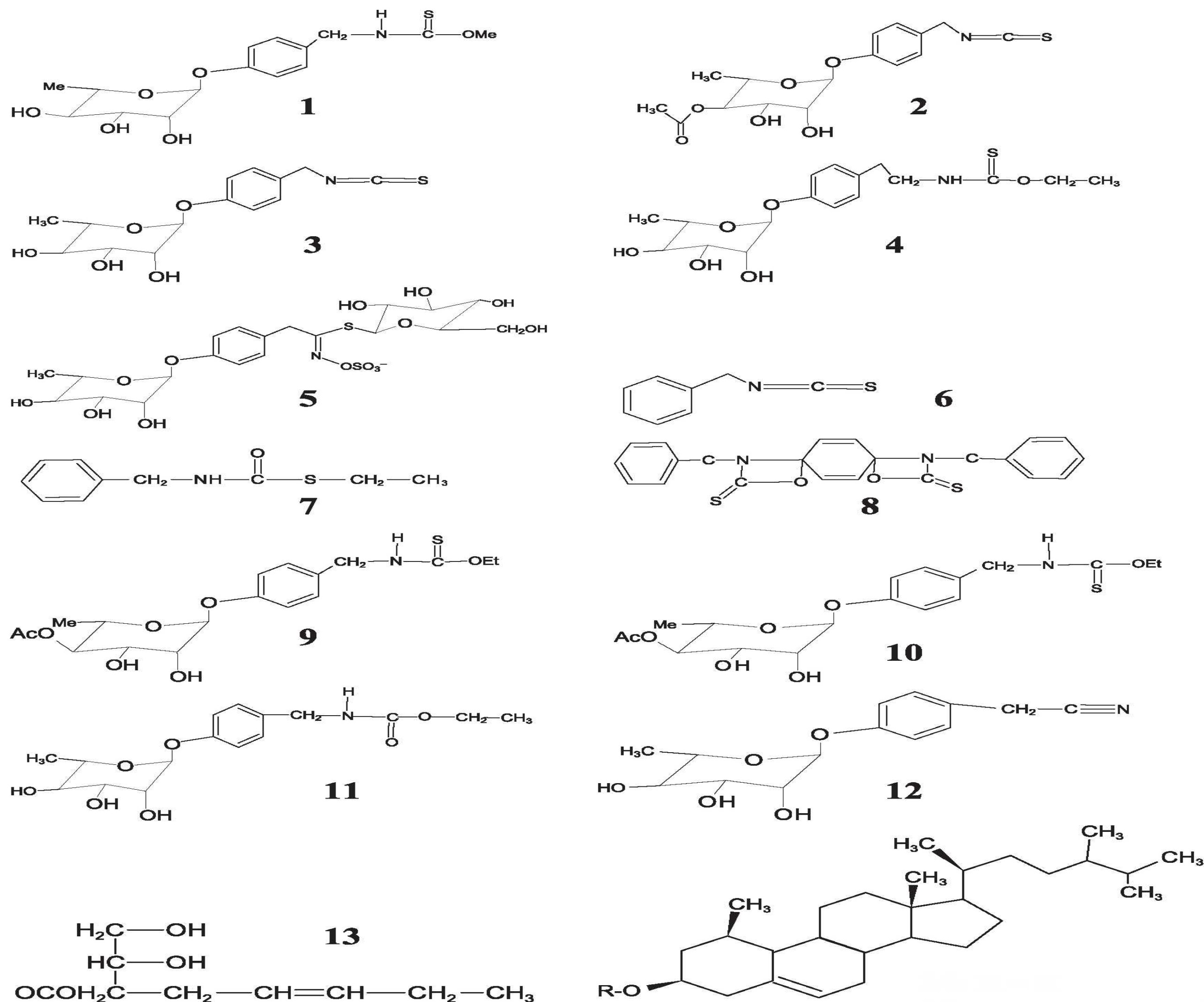

Figure 2.2 **Structures of selected bioactive compound of *Moringa oleifera***

(1), 4-(4'-0-acetyl-a-L-rhamnopyranosyloxy) benzyl isoth-iocyanate, (2) 4-(-L-rhamnopyranosyloxy)benzyl isothiocyanate, (3) niazimicin (4), 4-(a-L-rhamnopyranosyloxy)benzyl glucosinolate, (5) benzyl isothiocyanate, (6) aglycon of deoxy-niazimicine (N-benzyl, S-ethylthioformate) , (7) pterygospermin, (8) niaziminin, (9 + 10), 0-ethyl-4-(a-L-rhamnosyloxy)benzyl carbamate, (11) niazirin, (12)glycerol-1-(9-octadecanoate), (13) sitosterol, (14) 3-0-(6'-0-oleoyl- f-D-glucopyranosyl)-f-sitosterol

Source: Farooq *et al*., (2007

2.3.6.8 ANTIMICROBIAL AND ANTIHELMINTIC ACTIVITIES OF MORINGA LEAF

The discovery of the inhibitory property of moringa against several microbial has confirmed the antimicrobial components in moringa. Saadabi and Abu Zaid (2011) reported the inhibitory effect of moringa against many bacteria in a manner that is dose dependent, against *Mycobacterium phlei* and *B. subtilis* against growth of fungi *Basidiobolus ranarums and Basidiobolus haptosporu*. Moringa has also been implicated in antifungal activity, in a study that compares the antimicrobial extracts from moringa seeds against bacteria and fungi, the authors noted that *B. subtilis* and P. *multocida* were more susceptible to the activities of moringa compared to other microbes due to the influence of cations (Jabeeen *et al*., 2008).

In another study, *P. aeruginosa* and *Candida albicans* were reported to be unaffected by the activities of moringa, the growth of other microbes was however inhibited by moringa. Though when ethanol extract of moringa leaf, seed and roots were used, all microorganisms including previously reported strains of *P. aeruginosa* and *Candida albicans* that were resistant were all inhibited.

2.3.6.9 ANTI-ASTHMATIC ACTIVITY OF MORINGA LEAF

Alkaloids of Moringa have shown a resemblance to ephedrine in its mode of action by relaxing the bronchioles. It hs been reported that, the seed kernels of moringa conferred improvement in the treatment of asthma of the bronchial while decreased symptoms and improved respiratory activities were noted in asthma patients (Agrawal and Mehta, 2008).

2.3.6.10 ANTI-INFLAMMATORY ACTIVITY OF MORINGA LEAF EXTRACT

Root extract of moringa plant has been implicated in anti-inflammatory activity especially on oedema in rat. This characteristics effect has been observed to occur in a dose dependant manner following oral administration using crude ethanol extract of *M. oleifera* (Khare *et al*., 1997). Mahajan *et al*. (2009) noted that butanolic extracts of *M. oleifera* when administered to guinea pigs showed antinflammatory activity against inflammation of air passage.

2.3.6.11 ANALGESIC ACTIVITY OF EXTRACTS OF MORINGA LEAF

The influence of Moringa in pain modulation has been reported due to its analgesic effect.. Also when the seeds and leaves were subjected to alcoholic extraction, a significant analgesic influence was observed when the animals were subjected to hot plate and tail immersion procedures (Sutar *et al*., 2008).

2.3.6.12 CHOLESTEROL LOWERING ACTIVITIES AND ANTI-HYPERTENSIVE EFFECT OF MORINGA LEAF EXTRACTS

Moringa leaves contain bioactive compounds that stabilises blood pressure due to their direct effect in the blood. Anwar *et al.* (2007) reported that the presence of nitrile and thiocarbanate glycosides are crucial in lowering blood pressure. The presence of b-sitosterol in moringa also makes it have a powerful anticholesteric effect as this compound has been reported to suppress serum cholesterol in rats fed high fat diets (Ghasi *et al.,* 2000).

2.3.6.13 ANTI-DIABETIC ACTIVITY OF EXTRACTS OF MORINGA LEAF

There are a number of plants that have been assessed for their medicinal importance especially as therapeutic agents in the treatment of diabetes. Moringa is a plant that has been identified in this regards. Studies in rats have shown that moringa significantly lowered blood glucose in type 2 modelled diabetic cases (Ndong *et al*., 2007). Another study reported a decline in blood glucose within three hours of moringa treatment (Buker *et al*., 2010). Polyphenols present in moringa plant have been implicated in the observed hypoglycaemic activities of this plant. If properly channelled, commercialization of the antidiabetic activities of moringa may be developed to produce conventional drugs that will address ant diabetics using appropriate technology.

2.3.6.14 ANTI-TUMOR ACTIVITY OF EXTRACTS OF MORINGA LEAF

The anticancerous property of moringa plant has also been documented. Among several bioactive compounds identified for their anticancer properties is niazimicin. This thiocarbarnate inhibited teleocidin B-4- induced virus, a tumour promoter in a trial. A study involving many plants in Bangladesh where plant extracts were monitored for

cytotoxicity following shrimp, sea urchin and haemolytic assays using tumour cells showed that moringa is a very potent source of ant cancerous components (Murakami *et al.,* 1998).

2.3.6.15 HEPATO-PROTECTIVE ACTIVITY OF EXTRACTS OF MORINGA LEAF

Several studies have reported the hepato-protective attributes of moringa. Ethanol moringa leaf extract reduced damage to the liver that was induced by antitubercular drugs in rats. This hepatoprotective properties is mediated by the activities of serum bilirubin, aspartate amino transferase, alkaline phosphatase, alanine amino transferase and lipids as well as lipid peroxidation activities in the liver (Pari and Kumar, 2002). Methanol and chloroform extracted moringa have also been reported to possess hepatoprotective qualities in rats against induced liver damage as a result of treatment with CCl_4. Aside from the leaf, roots and flowers of this plant have been reported to possess this attributes. Moringa flowers possess quercetin, a flavonoid which is suspected to be responsible for the hepatoprotective tendency. A study recently reported the subsiding effect of moringa seed extract on liver fibrosis, observed that CCl_4 treatment induced fibrosis in the trial by elevating levels of aspartate amino transferase nd globulin in the serum while treatment with moringa invariably lowered the levels of these components suggesting the protective capability of moringa on the hepatocyte.

2.3.6.16 ANTI-FERTILITY ACTIVITY OF EXTRACTS OF MORINGA LEAF

Although there are several advantages to the use of moringa, the root and bark of this plant have been reported to possess anti-fertility effect in rats and in some cases reabsorption of the foetus at the late stage of pregnancy. The evaluation of the estrogenic and progestenic activities of moringa suggest that the aqueous extract induces a lot of antifertility attributes and showed antireproductive attributes with 100% of abortive cases (Nath *et al*., 1992).

2.3.6.17 MORINGA LEAF EXTRACTS AS CARDIAC AND CIRCULATORY STIMULANT

There have been reports that all parts of the moringa plant possess attributes to stimulate the cardiac and circulatory system. This action is conferred through its activities on the

sympathetic nervous system or the obstruction of hyperlipidaemia. Ndong *et al.* (2007) observed prevention of hyperlipidaemia in Wistar rats fed moringa leaf extract due to iron deficiency. Moringa possesses anti-atherosclerotic and hypolipiademic effect by making favourable modulations on biochemical parameters and also prevents damage to histopathogical tissues induced by myocardial infraction (Nandave *et al*., 2009).

2.3.7 PHYTOCHEMICALS IN MORINGA

Evaluation of toxicological attributes of medicinal plants have long been neglected by many local herb practitioners due to the belief that plants do not confer any form of harm and thus their safety for use cannot be totally guaranteed since it is always hard to monitor consequences of their long term consumption. Some plants for example, *Glycyrrhiza glabra* used in the treatment of bronchitis results in conditions like high blood pressure and also have consequential effects on aldosterone and antidiuretic hormone due to long time use. Many plants have been implicated to cause series of disease conditions or ailments in traditional medicine due to prolong consumption. These medicinal have profound effects on organs such as the kidney, liver and heart causing lesions and tumors. Other serious issues have resulted from the use of medicinal when some plants are mistaken for herbs thus resulting to interference with pharmacological therapy. Very few studies have been conducted on the toxicity of moringa. Aqueous extract of *M. oleifera* was found to be unharmful to rats as observed in histological and blood parameters (Adedapo *et al*., 2009).

Ashong and Brown (2011) on the other hand did not notice any immediate or permanent toxicity when poultry were fed varying concentrations of aqueous *Moringa oleifera* leaf extract while ethanol and aqueous extracts of *Moringa oleifera* bark had no influence on growth responses and biochemical profile in rats thus indicating that the plant was safe for consumption. Histology of the renal organ and liver showed a distortion in the integrity of the organs in Guinea pigs fed metabolic extract of moringa (Ferreira *et al*., 2009). Other in vivo study showed that the use of aqueous or methanol extracts of *Moringa Oleifera* was safe (Kasolo *et al*., 2011).

In contrast, the findings of Oluduro and Aderiye (2009) showed that long term consumption of water treated with *M. Oleifera* resulted in liver infraction. Another study by

Maria and Mohammed (2011) suggest that administration of metabolic extract of moringa seeds at low doses was harmless, prolonged consumption however altered some observable parameters suggesting a dose sensitive toxicity after a long time. An assessment of the toxicity effect on cells using various parts of the plants extracted using ethanol showed that there was a pronounced increase in the leakage of lactate dehydrogenase in a dose and time dependent manner in treatments from leaves and seeds extract. Aqueous and ethanol extracts of the leaves or the root did not affect the concentration of LDH. This observation suggests that the compounds in the plant extracted with methanol were toxic while those extracted with water were unharmful

A examination of a specie of Moringa on blood profile and histomorphology of rats showed no observable effect on the kidney when moringa was administered at different dosage (Fahey *et al*., 2001). The authors reported that it will be dangerous to conclude that the plant does not confer any harm until all means of extracting the compounds inherent in the plants have been exhausted and tested to verify the authenticity since every compound has a systematic extraction procedure that is peculiar to it (Fahey *et al*., 2001).

Some compounds from moringa leaves are hypotensive, anticancerous and have antibacterial as well as antioxidant properties. High flavonoid pigment which confers antioxidant activity in moringa have been documented and have been identified as the most potent antioxidant in moringa even more potent than the conventional terpenoids that is equally abundant in moringa while other properties such as bactericidal and fungicidal activities have also been documented (Fahey *et al*., 2001).

2.3.8 BIOACTIVE COMPOUNDS IN *MORINGA OLEIFERA*

The survey conducted by Josephine *et al.* (2010) to identify the various compounds present in moringa using different extraction methods showed that the identified phytonutrients have previously been noted in different parts of the plants by other scientists with flavonoids having a higher portion in the plant and are effective on a different population of microbes by inhibiting the activities of enzymes bound to their membrane thus making them loose their integrity. Other activities of moringa as antioxidants, anti-inflammatory, anticarcinogenic, antimutagenic and reduces blood pressure have been documented.

Anthraquinones present in leaves of *M. Oleifera* have laxative property, while steroids and terpenoids present prevents cancer. The saponins present in *M. Oleifera* due to its foaming ability makes its leaves serve the purpose of soap in some localities and its antioxidant, antiapotosic and immunostimulant prowess suggest that they could be used to address neural aging and other neuro-disorders. Alkaloids present in Moringa have been found to possess antimicrobial effect due to the enabling characteristics of interposing with microbial DNA. While the presence of glucosinolates and thiocarbamite glycosides promotes the treatment of hypertension, modifies tumorihensis and induce apoptotic response in cancer cells in human mammary gland (Bennett *et al*., 2003).

2.4 TANNINS

They are heterogeneous group of plant secondary metabolite that derived its name from a French word 'TAN' which could also be referred to as 'BACK OF TREE'; They are group of phenolic compounds and usually form complexes with proteins, hemicellulose, polysaccharides and cellulose. Tannins have bitter taste and can have a major influence on nutritive values of food and feed for human and animals, respectively. They are common in unripe fruits, in tea, legume forage and grasses. Tannins are used in animal nutrition and health most especially with ruminants like cattle (Frutos *et al*., 2004). Studies revealed tannin's multiple biological activities on the health of humans in the areas of: anti-carcinogenic, cardioprotective and anti-inflammatory. In veterinary medicine, studies reported tannins having the ability to lowering risk of animals' disease and transmission of zoonotic pathogen. Its uses in poultry production are also promising.

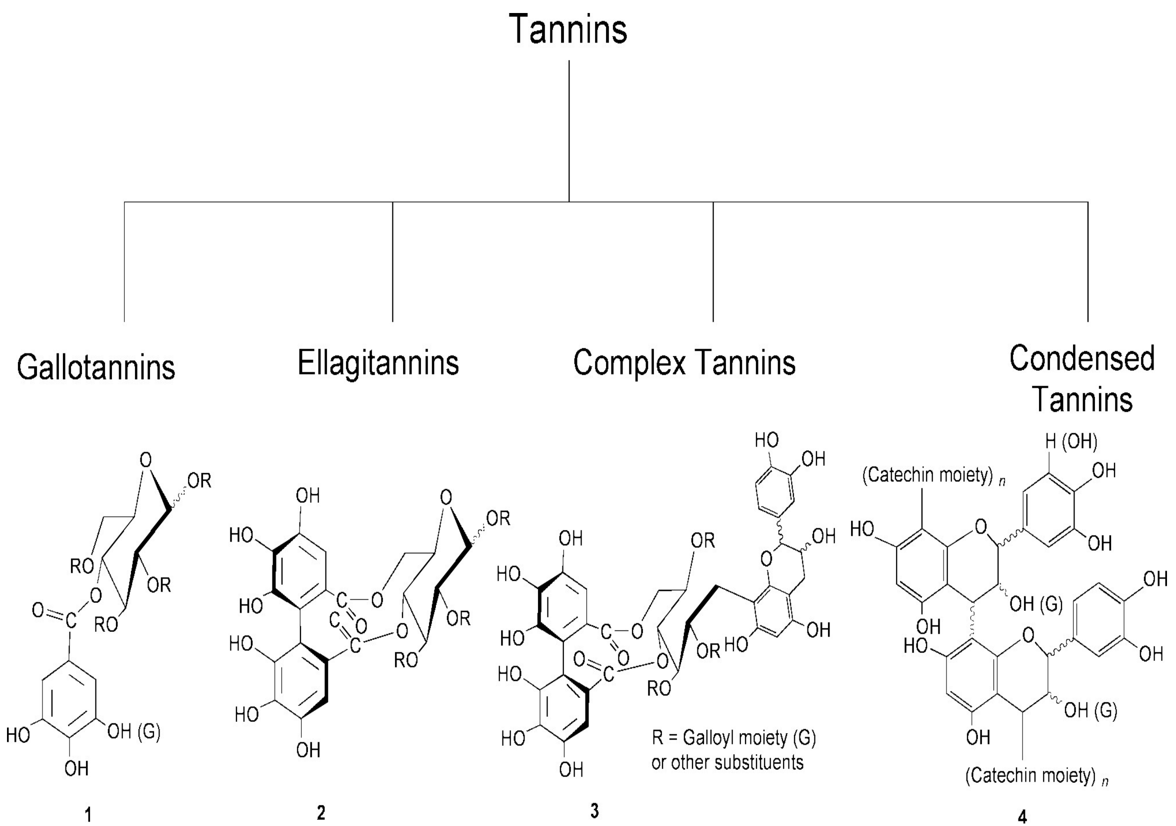

Figure 2.3 Classification based on structural formula

Source: Karamali and Teunts, (2001)

2.4.1 THE DISTRIBUTION OF TANNINS IN NATURE

Tannins are secondary metabolite produced by plant for their defensive mechanism, they are most abundant in plant parts that are valuable and can easily be consumed by livestock or human, for example; the new leaves, the flower and fruits. Environmental, seasonal and physiological factors had been reported to have effect on tannin distribution in plants. Lason *et al*. (1993) noted that plants generally produces a lot of biomass at younger period of growth,.

2.4.2 EFFECTS OF TANNINS ON ANIMAL PERFORMANCE

There had been the general belief that tannins are detrimental to ruminants, but recent research findings have revealed that tannin effects could be harmful or beneficial depending on the type consumed, quantity ingested and the animal species involved. Reduced feed intake and nutrient digestibility have been reported as a result high consumption of tannins while improvements in feed utilisation was reported in animals that consumed low to moderate quantities, which could be as a result of reduced uptake of amino acid due to reduction in protein breakdown in the rumen. Reduced feed intake as a result of reduced palatability could be attributed to bitter taste of tannin, resulting in reduced DM intake, body weight gain and low nutrient digestibility in poultry (Ravindran *et al.,* 2006).

2.4.3 IMPORTANCE OF TANNINS TO ANIMALS

Medical history of tannins shows that tannins could be used to cure diarrhea, haemostatic, and as antihemorrhoidal compound (Cheng *et al*. 2002). It was also established that tannins heal burns and stop bleeding by forming layers on the exposed tissue to prevent further infection of the wound. Reports also revealed tannin ability as anti-viral (Lin *et al*. 2004).

2.5 SAPONINS

They are bioactive compound of plant origin. They are steroids or triterpenoid glycosides manufactured by plants that are nutritionally important for plants and animals. These novel bio-organic complex compounds form soap like foams when mixed with water. Plants contain micro-molecular components; saponins which are glycosides bioactive compounds. Saponins isolated from different sources possess a bitter taste. Steroidal saponins are very common in herbs and used for herbal medicines (Haralampidis *et al*. 2002). Plants like

Capsicum, peppers, tomato, fenugreek *(Trigonellafoenum graecum), Dioscorea zingiberensis* (yellow ginger), *D. floribunda, Panicum dichotomiflorum, Scilla indica,* ginseng and *Lawsonia alba* are the common source of steroidal saponins.

The only enzyme characterized is saponin glucosyl transferases involved in biochemical synthetic pathway for synthesis of triterpenoidal saponins in plants (Haralampidis *et al*. 2002). Young plants have high concentration of saponins than mature or old plants of same species, however, it has been reported that factors like physiological state of plant and environmental conditions influence saponin concentration and its production in plants. High saponin contents have been observed during sprouting while less saponin contents were detected during flowering (Haralampidis *et al*. 2002).

2.5.1 CHEMICAL STRUCTURE AND CLASSIFICATION OF SAPONINS

Saponins are found in both inedible and edible plant as glycosidic compounds, they are bitter in taste. The sugar component is called glycone. Saponin=Sugar [glycone] + Sapogenin [aglycone].

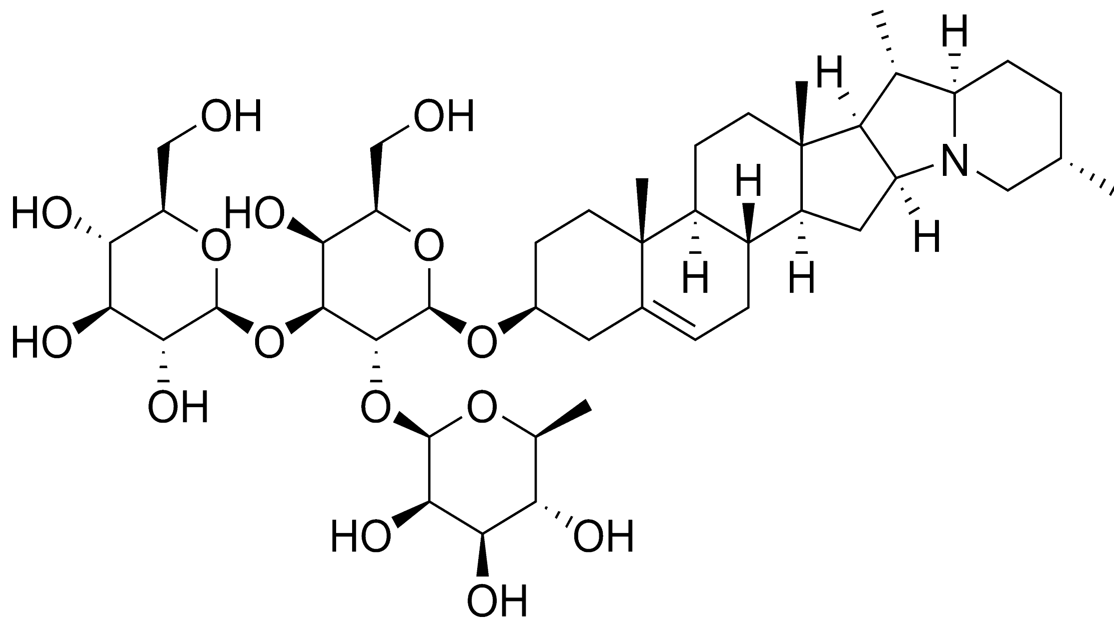

Figure 2.4 Chemical structure of the saponin

Source: Hostettmann and Marston (1995)

2.5.2 USES/IMPORTANCE OF SAPONINS

Saponins are steroidal glycosides which occur widely in plants that are consumed by animals and human. Regarding biological value, saponin has been categorized as anti-nutritional agent in most cases. Sometimes, it has been claimed to lower feed intake, reduced growth rate of monogastric animals. It shows toxicological effects with higher inclusion in diets. Nevertheless, it has the potential as dietary additive with optimum inclusion in diet favoring higher growth rate, better feed efficiency, lower serum cholesterol level (Udea and Shigemizu, 1998) and reducing the emission of ammonia from animals' excreta (Al-Bar *et al*. 1993). In recent time, saponins are being sold as feed additive in poultry production. Scientists are putting their efforts to make saponin as an important additive rather than challenge for better and economic poultry diet formulation.

2.6 ALKALOIDS

Alkaloids are a group of bioactive compound with nitrogen atoms. It derived it name from the word ALKALINE meaning; nitrogen - containing base. Acid-base extraction method is mostly used for extraction; isolation and purification of alkaloids. Some are toxic to other organisms while some are used in drug production for the treatment of diseases, as stimulant; caffeine, cocaine, nicotine, local anesthetic, analgesic morphine, or the anti-malarial drug- quinine.

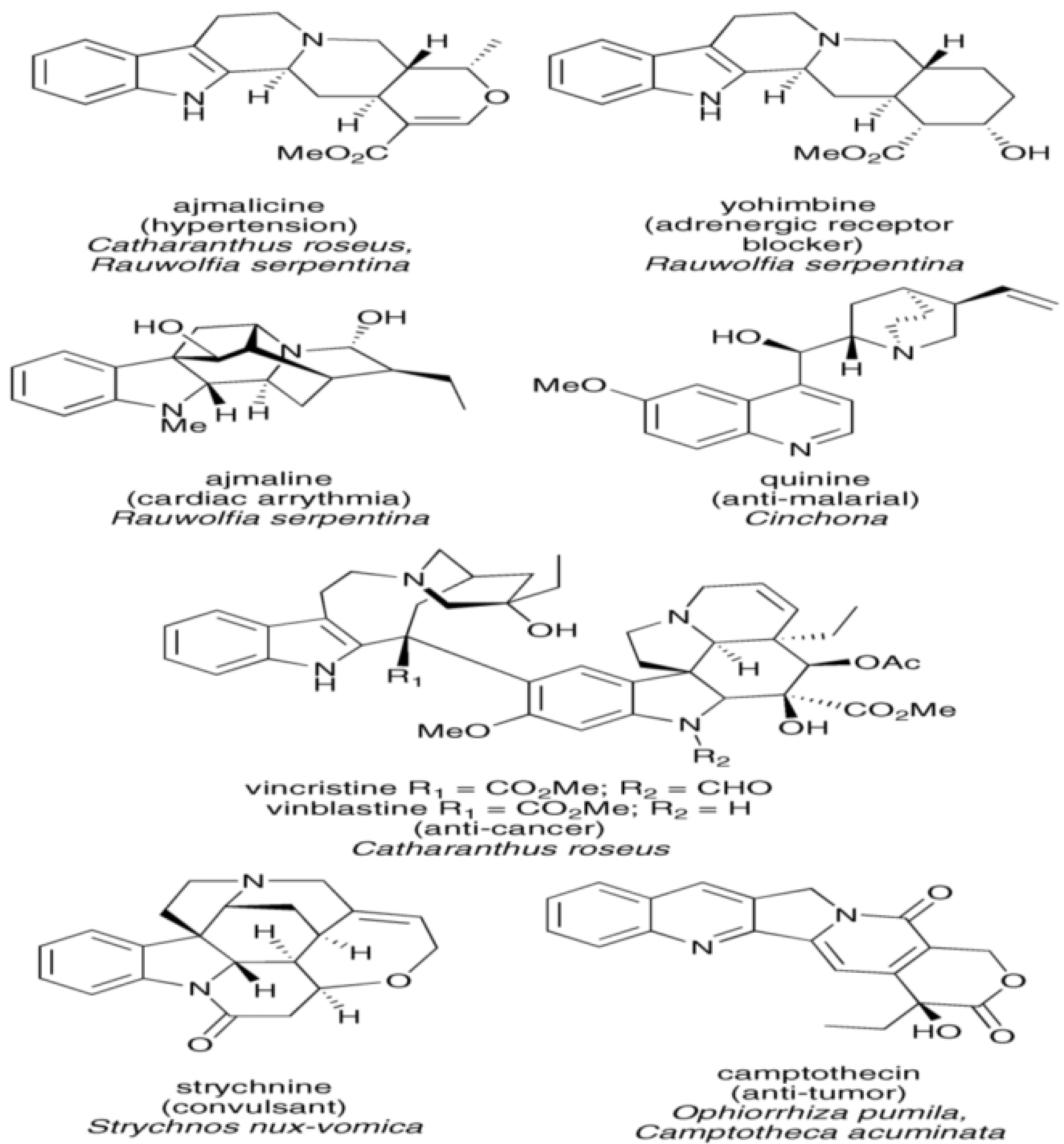

Figure 2.5: Physiologically active Alkaloids and plants that produce them

Source: Serah *et al*., (2006)

2.6.1 USES / IMPORTANCE OF ALKALOIDS TO ANIMALS

Alkaloids are very important to plant that produce them and at the same time very useful to man though some are toxic. Some alkaloids can cause paralysis while some stimulate the nervous systems. Some of the alkaloids are used as tranquilizers, to reduce pain, with anti-microbial properties. Thus, Lipinski *et al*. (2001) suggested alkaloids as suitable candidates for orally bio-available drugs. A mixture of mataranine-A and B was found active *in vitro* against *Plasmodium falciparum.* While 25 to 50% of the current pharmaceuticals, including anti-malarial drugs, were derived from plants, no ideal anti-bacterial drugs (antibiotics) have yet been developed from plants (Cowan, 1999).

2.7 LEAF MEAL NUTRITIONAL EVALUATION ON THE PERFORMANCE OF ANIMALS

The use of leaf meal as an unconventional feedstuff in partial substitution for grains in animal feed had been very successful and found fit in supplying adequate nutrients to the animals. For example**,** the use of *Leucaena leucocephala* at low inclusion rate for rabbit diet made differences in DM intake, daily weight gain, litter size at weaning and milk yield of does. In another study by Iheukwumere *et al*. (2007) on Anak broiler fed cassava leaf meal (0, 5, 10, or 15%.) to check for performance, haematology and carcass yield,and observed better performance in the control (0% leaf meal) compared to the groups on 10 and 15% cassava leaf meal.

The authors noted that biochemical and haematological parameters measured for broilers on diets without cassava leaf meal supplementation and 5% cassava leaf meal supplementation were better than the values observed in birds on 10% and 15% leaf meal, but the cholesterol, creatinine and urea values were not different among the treatment groups. Carcass quality of the control experiment was better and significantly higher than in broilers fed 5%, 10% and 15% leaf meal in terms of carcass yield. Iheukwumere *et al*. (2007) concluded that 5% inclusion rate was safe and will not have a negative effect on performance, haematology and carcass yield of broiler chicken. Odunsi *et al*. (2002) fed gliricidia leaf meal (GLM 0, 5, 10 or 15%) to laying bird and reported significant reduction in feed intake, body weight changes, hen-day egg production and feed conversion efficiency. Egg quality attributes observed in the study were either not significantly

affected or were lowered by the inclusion of GLM in the diets of the birds. Of the entire internal egg attributes observed yolk colour was the most favourably influenced by GLM as birds on the different levels of the inclusion had enhanced colour of the yolk. When subjected to cooking however, reduction in yolk colour was observed though shell and egg membrane were heavier. The authors concluded that GLM supplementation beyond 5% in the diet of laying hen might not be appropriate for improved performance and productivity and thus suggested lower inclusion rate.

Smith (1988) conducted a study on the degradability of some selected foliages (cassava, *Leucaena leucocephala*, oil palm, bamboo and *Gliricidia sepium leaves*) in cattle and goats. The author observed higher degradability in cassava leaf within 48hours compared to other leaves.

The author suggested that cassava leaf meal is high in some yellow pigments that enhances the pigmentation of egg yolk and as such may be an appropriate alternative to alfalfa in laying hen production. Famounya and Meffega (1986) in their study observed reduction in body weight of rabbits on sun-dried cassava leaves diets. The authors concluded that the reduction in weight gain was due to low feed intake by the rabbits which was not totally attributed to the cassava leaves but to the nature or the form in which the feed was presented. They however, suggested that pelletizing the feed will improve the compactness of the feed and thus may increase its acceptability by the rabbits.

Bamikole *et al*. (2005) studied the effect of Mulberry leaf meal (100:0, 75:25, 50:50, 25:75, 0:100) potential, concluded that mulberry leaves at very low inclusion level could enhance feed intake, nutrient digestibility and weight gain in rabbits, and could reduce dependence on expensive concentrate diets. Ghasi *et al*. (2000) extracted moringa juice from leaves and orally administered a dose of 1 mg/g, with a high fat diet daily over a period of 30 days on Wistar rats, and described leaf extract as an active hypocholesterolemic agent while reduction in blood lipids were observed in rats and broiler finishers fed diets supplemented with neem leaf meal.

2.8 MORINGA LEAF MEAL COMPOSITION AS INFLUENCED BY THE TYPE OF EXTRACTION SOLVENT AND STAGES OF MATURITY

Nutritional information is of great importance in determining the right harvesting stage of plant for animal consumption. Better nutritional benefits can be derived from plant materials when harvested at appropriate maturity stage. Based on nutritional requirement of different animals, the use of leaf meal extract in animal diet should be hinged on nutritional composition of the leaves at different stages of growth, thus the need to determine the appropriate stage of harvesting that will supply enough nutrients for animal consumption.

Recent work on *Acacia angustissima* leaf materials revealed variation in nutrient composition at different stages of growth. Gusha *et al*. (2013) harvested *Acacia angustissima* leaf material at mid maturity stage (15week), at matured age and harvested leaves irrespective of age, respectively, in feeding goat and reported variation in chemical composition of the leaves. Yu *et al*. (2004) reported an increased dry matter value with increased ages of maturity of plant, meaning the more matured the leaves, the lower the moisture content of the leaves. The younger the leaf materials, the more the moisture content and the lower the total solid. Yu *et al*. (2004) reported that stages of maturity of plant affected the nutrient composition of the leaves. Therefore, it is necessary to choose a suitable stage of harvesting.

Varying levels of nutrients and phytochemicals are present in plants based on the stage of growth. At younger age, the leaves contain more crude protein and condensed tannins but less crude fiber, indicating that the leaves are better harvested at early or mid-age, if the target is as protein supplement in animal feed. Also, extraction solvent exerted significant effect on nutrient, phytochemical composition and antioxidant activities as reported by Siddhuraju and Becker (2003). The authors reported the best extracting solvent varies from plant to plant, targeted type and classes of phytochemical and the corresponding antioxidant capacity. The antioxidant activities and phytochemical content of fresh moringa leaves are influenced by age of the plant and the extracting solvent used, as well as the interaction between them. Generally, organic solvent (Ethanol) was more efficient for extraction of flavonoid while methanol was better for production of Polyphenol- rich extract. Both solvent exhibited greater antioxidant activities than aqueous extract.

2.9 THE IMPACT OF NUTRITION ON BIOCHEMICAL AND HAEMATOLOGICAL COMPONENTS OF FARM ANIMALS

Blood cell are very important component of human and animal body, it contains a large volume of organic and inorganic substance dissolved in it and also functions in circulation of nutrients, oxygen, enzymes, drugs and waste product within the arteries, veins and capillaries, maintaining organ functions and homeostasis of the internal environment. The feed quality and level of anti-nutritional factors goes a long way in affecting serum biochemistry and haemological components of the blood. Protein quality and feed toxicity are monitored by haematological indices. Low level haemoglobin (Hb) is also an indication of low protein quality in the diet (Oyawoye and Ogunkunle, 1998). When protein content of a diet is poor, it negatively affects the oxygen transportation to the peripheral tissues. Presence of toxin in the blood always shows decline in pack cell volume concentration (Oyawoye and Ogunkunle, 1998).

Increase in counts of foreign bodies in the blood increases the white blood cell count and production of antibodies to defend the system while low quality protein in the blood is usually revealed by high urea value of the. Esonu *et al*. (2001) noted that biochemical and haematological components measures the physiological responsiveness of the animal to both its internal and external environments.

Biochemical and haematological analysis are important to check health status of an animal and physiological alertness to factors internal and external to the animal. Total protein of the serum shows an indication of retention of protein fed to an animal; total blood protein on the other hand makes reference to the quantity and quality of dietary protein fed while high level of serum creatinine as a result of muscle wasting, a process observed during creatinine phosphate catabolism. Increase in activities of urea enzymes orminthine, carbonyl transferase and orginase is an indication of increased serum urea concentration, revealing possible kidney damage (Esonu *et al*., 2001). Ologhobo *et al*. (2014) reported that normal blood sugar values are an indication that the animal is not surviving at the expenses of the body tissues. Increase in blood neutrophils is usually observed during acute

infections while eosinophils are normally increased in some instances of chronic infections due to parasites or allergy.

CHAPTER THREE

MATERIALS AND METHODS

STUDY ONE:

3.1 DETERMINATION OF CHEMICAL CONSTITUENT OF *Moringaoleifera* LEAF MEAL AT DIFFERENT STAGES OF GROWTH

3.1.1 CULTIVATION OF MORINGA PLANT

A total of twenty (20), six week old seedlings were purchased from the National Cereal Research Institute, Ibadan (NCRI) and transplanted on a plot of land located at Obada-oko area of Abeokuta, Nigeria located on latitude 7^{0}07'N, 3^{0}29' E. It was planted at a spacing of 2m by 2m and harvested at 12, 14, 16, 18 and 20 weeks of age. Water was supplied through natural precipitation.

3.1.2 PREPARATION OF MORINGA LEAF MEAL

Fresh leaves of *Moringa oleifera* plant were harvested at random by cutting the plant from the stem at two-week interval and the leaves removed from the stem by hand and spread on a clean flat table under shed to air dry for a period of 14 days when a constant weight was attained. The leaves were turned twice a day to prevent growth of fungi and enhance proper drying. The dried leaves were ground in a motorized electric blender and stored in a sealed container to prevent absorption of moisture.

3.1.3 PREPARATION OF MORINGA LEAF EXTRACTS OBTAINED FROM MORINGA LEAVES AT DIFFERENT AGES

The dried Moringa leaf meal samples obtained at 12, 14, 16, 18 and 20 week were extracted. 100g of dried powdered samples were soaked in 500 mL of distilled water, methanol and ethanol, respectively for 12 hr. to obtain the leaf extracts. The three extracted solvents were compared in terms of rate of recovery to determine which solvent gives the highest quantity of extract. This was done by weighing the quantity of extract and the

residue. The extract from Methanol solvent (been the solvent with the highest recovery rate) was used for the determination of proximate, elemental and phytochemical quantity.

3.1.4 PROXIMATE COMPOSITION ANALYSIS

This was carried out using AOAC (2000), AOAC 900.02A and AOAC 978.04 methods for the determination of moisture content, total ash content, crude lipid content, and crude protein content.

3.1.5 MINERAL COMPOSITION

The Potassium, Magnesium, Calcium, Sodium, Iodine, Phosphorus, iron and Chloride in leaf meals were measured with the aid of Atomic absorption spectrophotometer (Aas-buck 205) as described by Association of Official Analytical Chemist (AOAC, 1990). All determinations were done in triplicates.

3.1.6 PHYTOCHEMICAL ANALYSIS

3.1.6.1 QUALITATIVE ANALYSIS

This was done using standardized procedures of Tiwari et *al.* (2011). The extract was subjected to phytochemical screening for alkaloids (Mayer's test and Wagner's test), saponins (Froth test and Foam test) (Obadoni and Ochuko, 2001) tannins (Gelatin test) Van-Burden and Robinson (1981), flavonoids (Alkaline reagent test and lead acetate test) Sofowara (1993), phenols and cyanide Vedula *et al*. (2011).

3.1.6.1.1 ALKALOIDS

Exactly 0.5 g of the extracts (ethanol, aqueous and methanol) were mixed in 8ml of 1% HCL, this was warmed and filtered. 2ml of each the filtrates was treated with Mayer's reagent. The present of alkaloid shows turbidity or precipitate formation.

3.1.6.1.2 TANNINS

Exactly 0.5 g of the extracts (ethanol, aqueous and methanol) was dissolved in 2 ml of distilled water, 2 drops of diluted ferric chloride solution was added. A dark green or blue green coloration indicates the presence of tannins

3.1.6.1.3 PHENOLS

Exactly 10mg extracts were treated with 2 drops of ferric chloride solution. Formation of bluish black colour indicated the presence of phenol.

3.1.6.1.4 CYANIDE

Exactly 0.5g of each extract was desolve in methanol solvent, add iron sulphate, the resulting mixture was acidified with mineral acid. The Prussian blue colour is an indication of cyanide.

3.1.6.2 PHYTOCHEMICAL QUANTITATIVE ANALYSIS

3.1.6.2.1 DETERMINATION OF ALKALOIDS

This was carried out using methods of Harborne (1999). Exactly 0.5 g of methanol extracts was dissolved in 50mls of the solvents. Concentrated ammonium hydroxide was added to the extract solutions drop wise until precipitation was completed. The whole solutions were allowed to settle and the precipitates were collected via filtration (using Wattman filter paper no 24). The residues were weighed and the percentages of total alkaloid contents were calculated as;

$$\text{Percentage of total alkaloids (\%)} = \frac{W2-W1}{W3} * 100$$

3.1.6.2.2 TANNIN DETERMINATION

This was carried out using the procedure of Van-Burden and Robinson (1981**).** Exactly 500mg of the sample was measured into a glass bottle, 50 ml of distilled water added and shaken in a mechanical shaker for a period of 1 h, it was filtered in a 50 ml volumetric flask and made up to the mark. Exactly 5 ml of the filtrate was pipetted into the test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M $K_4Fe(CN)_6$ (potassium ferrocyanide). The absorbance was measured at 120 nm within 10 mins.

3.1.7 Statistical analysis

Data obtained were analysed using descriptive statistics (SAS 2003).

STUDY TWO

3.2 BIOCHEMICAL RESPONSES OF WEANLING WISTAR RATS ADMINISTERED CRUDE ALKALOID EXTRACT OF *Moringa oleifera* (Lam) LEAF

3.2.1 CULTIVATION OF MORINGA PLANT:

A total of twenty (20), six week old seedlings were purchased from the National Cereal Research Institute, Ibadan (NCRI) and transplanted on a plot of land located at Obada-oko area of Abeokuta, Nigeria located on latitude $7^0$07'N, $3^0$29' E. It was planted at a spacing of 2m by 2m and harvested at 12, 14, 16, 18 and 20 weeks of age. Water was supplied through natural precipitation.

3.2.2 PREPARATION OF DRY MORINGA LEAVES MEAL:

Fresh leaves of *Moringa oleifera* plant were harvested at random by cutting the plant from the stem at two-week interval and the leaves removed from the stem by hand and spread on a clean flat table under shed to air dry for a period of 14 days when a constant weight was attained. The leaves were turned twice a day to prevent growth of fungi and enhance proper drying. The dried leaves were ground in a motorized electric blender and stored in a sealed container to prevent absorption of moisture.

3.2.3 EXTRACTION AND ISOLATION OF MORINGA ALKALOIDS

METHANOLIC EXTRACTION

700g of powdered Moringa leaves was taken and extracted with 4 litre of 100% methanol (x3) at room temperature for 72 hours each time. Extracts were pooled together and concentrated to dryness on a rotary evaporator yielding 90g.

ACID-BASED EXTRACTION OF THE N-BUTANOLIC FRACTION (MOBF)

The n-butanol fraction was dissolved in water (200ml), acidified with 5% HCl and partitioned with dichloromethane and concentrated. This was then basified with ammonia, and the librated bases extracted with di-chloromethane. The organic solvent was removed to give an extract which tested positive with Draggendorffs 'reagent for Alkaloids. The crude alkaloids isolated were kept in the refrigerator for proper preservation

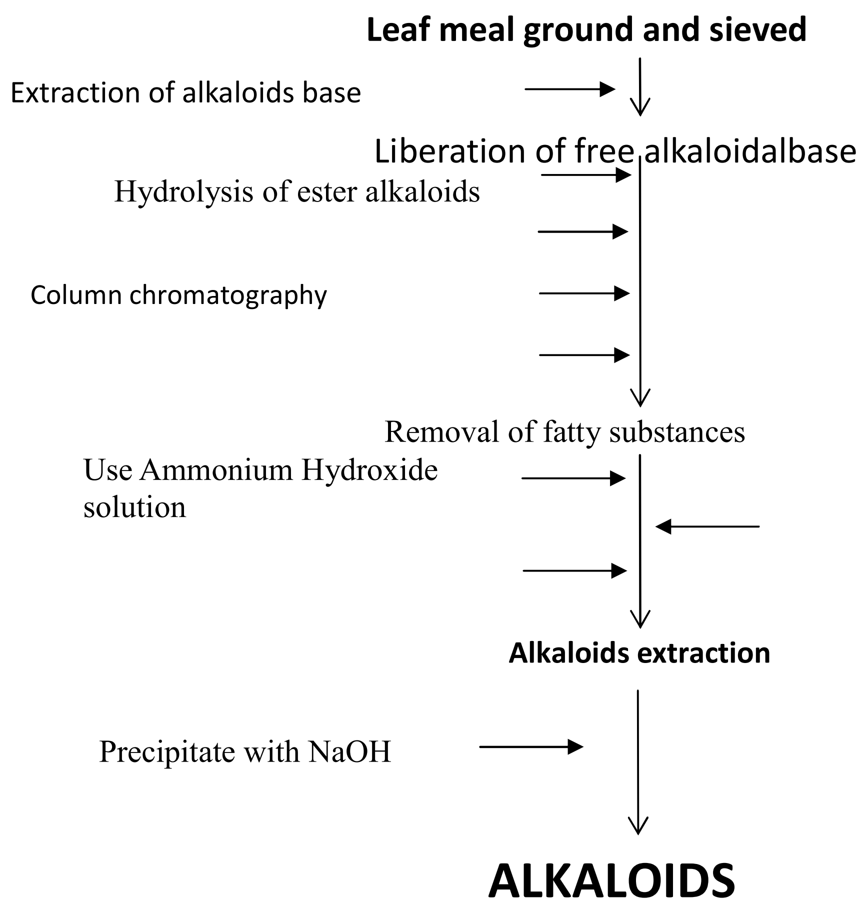

Figure 3.1: FLOW CHART FOR ISOLATION OF ALKALOIDS

3.2.4 PREPARATION OF CRUDE EXTRACTS SOLUTION

1gm of crude alkaloid extract was dissolved in 10 mL of distilled water to produce homogenous solution from which three different concentration; 1.0 mg/mL, 1.5 mg/mL, 2.0 mg/mL, were administered orally. Similarly, three volumes of the Moringa extracts were also administered orally. All rats were fed the basal diet; distilled water served orally as control.

3.2.5 EXPERIMENTAL SITE

The experiment was conducted at the Rat Room in the Department of Animal Science, University of Ibadan, located on latitude 7^{0}20'N, 3^{0}50'E, 200m above sea level.

3.2.6 EXPERIMENTAL ANIMALS AND DESIGN

A total of 70 weanling 4-week old Wistar rats were assigned to seven dietary treatments of ten rats each in a completely randomised design. The rats were fed *ad libitum* with the basal diet (Table 3.1) for 21 days. Rats were given the extracts orally each morning after which feed and water were served two hours later.

Table 3.1: Gross composition of basal diet fed to weanling Wistar rats

INGREDIENTS	INCLUSION (%)
Corn starch	58.87
Casein	10.53
Glucose	5.00
Sucrose	7.00
Cellulose	5.00
Soya oil	10.00
Vitamin premix*	0.30
Salt	0.30
Di-calcium phosphate	2.00
Limestone	1.00
Total	100.00

3.2.7 DATA COLLECTION

3.2.7.1 FEED INTAKE (g)

Feed intake was determined by subtracting the amount of feeds left over feed from the quantity supplied to the rats and were weighed daily for each treatment. The daily feed intake of each animal was calculated by finding the differences between the feed supplied and left over feed.

3.2.7.2 BODY WEIGHT GAIN (g)

Weekly body weight was measured by subtracting the previous week's body weight from the current week's body weight, values obtained was used to calculate the average body weight gain per replicate.

3.2.7.3 FEED CONVERSION RATIO

This was calculated from the value of average weight gained and average feed consumed by the rats in each treatment, using the fomular below;

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Average feed intake}}{\text{Average daily gain}}$$

3.2.7.4 BLOOD COLLECTION

For biochemical and haematological analyses, three rats were randomly selected from each replicate per treatment. The rats were sacrificed under anaesthesia and 2 ml of blood was collected from the jugular. Blood collection for biochemical analysis was achieved with the use of Gel clot activator and sodium fluoride tubes while K_3 EDTA tubes were used to collect blood for the haematology samples. The blood samples were centrifuged at 2146 xg for 3 minutes to collect the plasma and serum. Samples were then subjected to analysis; Packed Cell Volume (PCV) was determined by microhaematocrit method, Haemoglobin (HB) content was determined by cyanomethaemoglobin methods. The Red Blood Cell (RBC), White Blood Cell (WBC), Platelets (PLAT), Lymphocyte (LYM), Neutrophil

(NEUT), Monocyte (MON) and Eosinophil (EO). were determined using an automatic haematological analyser (Model 7020, Hitachi, Tokyo, Japan).

The plasma total proteins (g/dL) were determined according to the method described by Henry (1974). The determination of plasma albumin (g d/dL) based on a colorimetric method described by Doumas *et al.* (1971). Globulin was calculated by subtraction of plasma albumin from plasma total protein. The enzyme activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined calorimetrically by using available commercial kits. The immunoglobulin D, E, G and M were determined using the immunoturbidimetric method and spectrophotometer. The immunoglobulin reagent and the Chemwell auto-analyzer were pre-warmed to 37°C. Ten microlitre (10µl) of reagent was used for IgM, while 7µl was used for IgG and IgD respectively.

3.2.7.5 RELATIVE ORGAN WEIGHTS MEASUREMENT

At the end of the experiment, three rats per replicate were sacrified and the brain, heart, liver, spleen and kidney removed, weighed with a digital scale and the organs kept in formaling well labelled for each treatment. The organ weigh was then calculated in relation with the final weigt of each rat.

3.2.8 STATISTICAL ANALYSIS

Data were analysed using Analysis of Variance (SAS, 2012), means were separated using Duncan's multiple range test.

STUDY THREE:

3.3 BIOCHEMICAL RESPONSE OF WEANLING WISTAR RATS ADMINISTERED CRUDE SAPONIN EXTRACT OF *Moringa oleifera* (Lam) LEAF

3.3.1 CULTIVATION OF MORINGA PLANT:

A total of twenty (20), six week old seedlings were purchased from the National Cereal Research Institute, Ibadan (NCRI) and transplanted on a plot of land located at Obada-oko area of Abeokuta, Nigeria located on latitude $7^0$07'N, $3^0$29' E. It was planted at a spacing of 2m by 2m and harvested at 12, 14, 16, 18 and 20 weeks of age. Water was supplied through natural precipitation.

3.3.2 PREPARATION OF DRY MORINGA LEAVES MEAL:

Fresh leaves of *Moringa oleifera* plant were harvested at random by cutting the plant from the stem at two-week interval and the leaves removed from the stem by hand and spread on a clean flat table under shed to air dry for a period of 14 days when a constant weight was attained. The leaves were turned twice a day to prevent growth of fungi and enhance proper drying. The dried leaves were ground in a motorized electric blender and stored in a sealed container to prevent absorption of moisture.

3.3.3 EXTRACTION AND ISOLATION OF MORINGA SAPONINS:

METHANOLIC EXTRACTION

700g of powdered Moringa leaves was taken and extracted with 4 litre of 100% methanol (x3) at room temperature for 72 hours each time. Extracts were pooled together and concentrated to dryness on a rotary evaporator yielding 90g.

ACID-BASED EXTRACTION OF THE N-BUTANOLIC FRACTION (MOBF)

The n-butanol fraction was dissolved in water (200ml), acidified with 5% HCl and partitioned with dichloromethane and concentrated. This was then basified with ammonia, and the librated bases extracted with di-chloromethane. Saponin was extracted and isolated as shown in the chart below;

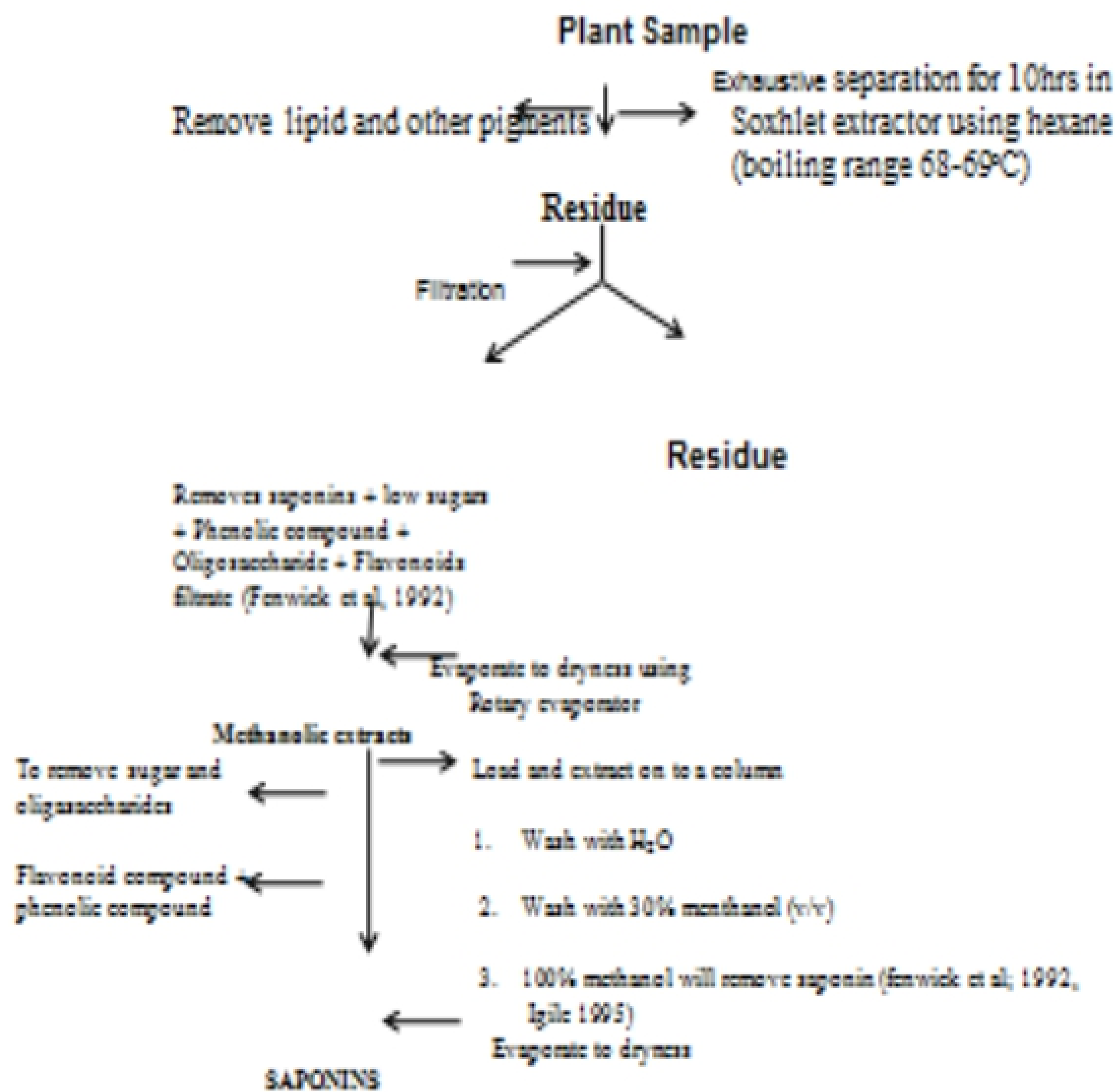

Figure 3.2: Flow Chart for isolation of saponin

3.3.4 PREPARATION OF CRUDE EXTRACTS SOLUTION

One gram of crude alkaloid extract was dissolved in 10 mL of distilled water to produce homogenous solution from which three different concentration; 1.0 mg/mL, 1.5 mg/mL, 2.0 mg/mL, were administered orally. Similarly, three volumes of the Moringa extracts were also administered orally. All rats were fed the basal diet; distilled water served orally as control.

3.3.5 EXPERIMENTAL SITE

The experiment was conducted at the Rat Room in the Department of Animal science, University of Ibadan, located on latitude $7^0$20'N, $3^0$50'E, 200m above sea level.

3.3.6 EXPERIMENTAL ANIMALS AND DESIGN

A total of 70 weanling 4-week old Wistar rats were assigned to seven dietary treatments of ten rats each in a completely randomised design. The rats were fed *ad libitum* for 21 days. Rats were given the extracts orally each morning after which feed and water were served two hours later

Table 3.1: Gross composition of basal diet fed to weanling Wistar rats

INGREDIENTS	INCLUSION (%)
Corn starch	58.87
Casein	10.53
Glucose	5.00
Sucrose	7.00
Cellulose	5.00
Soya oil	10.00
Vitamin premix*	0.30
Salt	0.30
Di-calcium phosphate	2.00
Limestone	1.00
Total	100.00

3.3.7 DATA COLLECTION

3.3.7.1 FEED INTAKE (g)

Feed intake was determined by subtracting the amount of feeds left over feed from the quantity supplied to the rats and were weighed daily for each treatment. The daily feed intake of each animal was calculated by finding the differences between the feed supplied and left over feed.

3.3.7.2 BODY WEIGHT GAIN (g)

Weekly body weight was measured by subtracting the previous week's body weight from the current week's body weight, values obtained was used to calculate the average body weight gain per replicate.

3.3.7.3 FEED CONVERSION RATIO

This was calculated from the value of average weight gained and average feed consumed by the rats in each treatment, using the fomular below;

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Average feed intake}}{\text{Average daily gain}}$$

3.3.7.4 BLOOD COLLECTION

For biochemical and haematological analyses, three rats were randomly selected from each replicate per treatment. The rats were sacrificed under anaesthesia and 2 ml of blood was collected from the jugular. Blood collection for biochemical analysis was achieved with the use of Gel clot activator and sodium fluoride tubes while K_3 EDTA tubes were used to collect blood for the haematology samples. The blood samples were centrifuged at 2146 xg for 3 minutes to collect the plasma and serum. Samples were then subjected to analysis; Packed Cell Volume (PCV) was determined by microhaematocrit method, Haemoglobin

(HB) content was determined by cyanomethaemoglobin methods. The Red Blood Cell (RBC), White Blood Cell (WBC), Platelets (PLAT), Lymphocyte (LYM), Neutrophil (NEUT), Monocyte (MON) and Eosinophil (EO). were determined using an automatic haematological analyser (Model 7020, Hitachi, Tokyo, Japan).

The plasma total proteins (g/dL) were determined according to the method described by Henry (1974). The determination of plasma albumin (g d/dL) based on a colorimetric method described by Doumas *et al.* (1971). Globulin was calculated by subtraction of plasma albumin from plasma total protein. The enzyme activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined calorimetrically by using available commercial kits. The immunoglobulin D, E, G and M were determined using the immunoturbidimetric method and spectrophotometer. The immunoglobulin reagent and the Chemwell auto-analyzer were pre-warmed to 37°C. Ten microlitres (10µl) of reagent was used for IgM, while 7µl was used for IgG and IgD respectively.

3.3.7.5 RELATIVE ORGAN WEIGHTS MEASUREMENT

At the end of the experiment, three rats per replicate were sacrified and the brain, heart, liver, spleen and kidney removed, weighed with a digital scale and the organs kept in formaling well labelled for each treatment. The organ weigh was then calculated in relation with the final weigt of each rat.

3.3.8 STATISTICAL ANALYSIS

Data were analysed using Analysis of Variance (SAS, 2012), means were separated using Duncan's multiple range test.

STUDY FOUR

3.4 BIOCHEMICAL RESPONSE OF WEANLING WISTAR RATS ADMINISTERED CRUDE TANNIN EXTRACT OF *Moringa oleifera* (Lam) LEAF

3.4.1 CULTIVATION OF MORINGA PLANT:

A total of twenty (20), six week old seedlings were purchased from the National Cereal Research Institute, Ibadan (NCRI) and transplanted on a plot of land located at Obada-oko area of Abeokuta, Nigeria located on latitude $7^0$07'N, $3^0$29' E. It was planted at a spacing of 2m by 2m and harvested at 12, 14, 16, 18 and 20 weeks of age. Water was supplied through natural precipitation.

3.4.2 PREPARATION OF DRY MORINGA LEAVES MEAL:

Fresh leaves of *Moringa oleifera* plant were harvested at random by cutting the plant from the stem at two-week interval and the leaves removed from the stem by hand and spread on a clean flat table under shed to air dry for a period of 14 days when a constant weight was attained. The leaves were turned twice a day to prevent growth of fungi and enhance proper drying. The dried leaves were ground in a motorized electric blender and stored in a sealed container to prevent absorption of moisture.

3.4.3 EXTRACTION AND ISOLATION OF MORINGA ALKALOIDS

METHANOLIC EXTRACTION

700g of powdered Moringa leaves was taken and extracted with 4 litre of 100% methanol (x3) at room temperature for 72 hours each time. Extracts were pooled together and concentrated to dryness on a rotary evaporator yielding 90g.

ACID-BASED EXTRACTION OF THE N-BUTANOLIC FRACTION (MOBF)

The n-butanol fraction was dissolved in water (200ml), acidified with 5% HCl and partitioned with dichloromethane and concentrated. This was then basified with ammonia, and the librated bases extracted with di-chloromethane. Tannins were extracted and isolated as shown in the chart below;

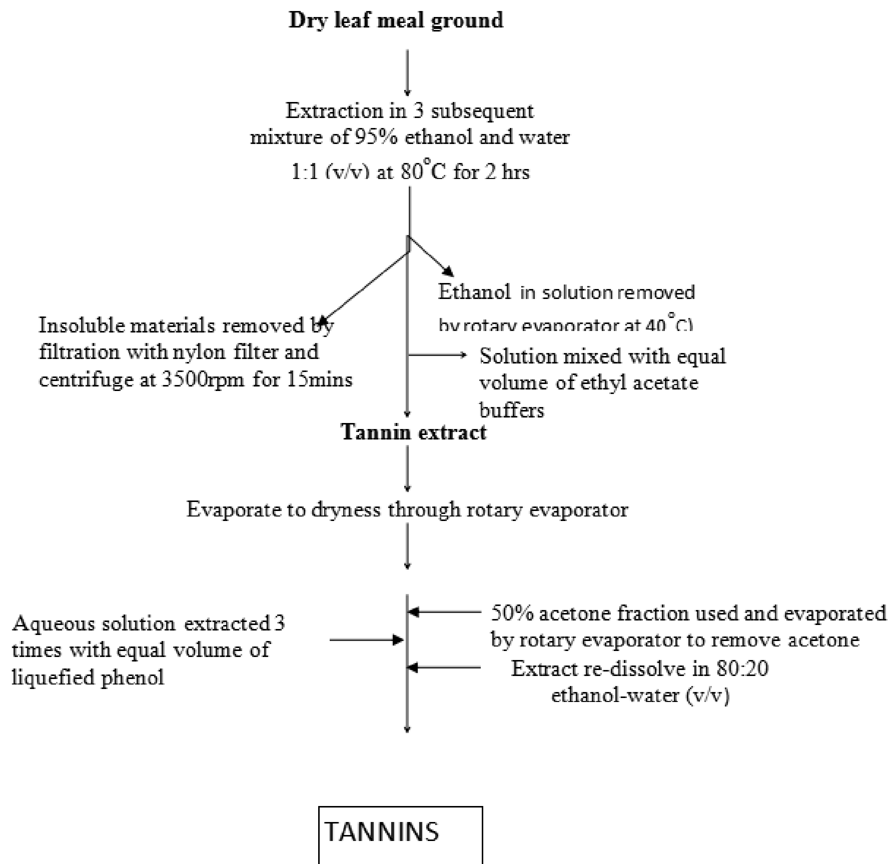

Figure 3.3: **FLOW CHART FOR ISOLATION OF TANNINS**

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3.4.4 PREPARATION OF CRUDE EXTRACTS SOLUTION

Exacly 1.0g of crude alkaloid extract was dissolved in 10 mL of distilled water to produce homogenous solution from which three different concentration; 1.0 mg/mL, 1.5 mg/mL, 2.0 mg/mL, were administered orally. Similarly, three volumes of the Moringa extracts were also administered orally. All rats were fed the basal diet; distilled water served orally as control.

3.4.6 EXPERIMENTAL ANIMALS AND DESIGN

A total of 70 weanling 4-week old Wistar rats were assigned to seven dietary treatments of ten rats each in a completely randomised design. The rats were fed *ad libitum* for 21 days.Rats were given the extracts orally each morning after which feed and water were served two hours later

Table 3.1: Gross composition of basal diet fed to weanling Wistar rats

INGREDIENTS	INCLUSION (%)
Corn starch	58.87
Casein	10.53
Glucose	5.00
Sucrose	7.00
Cellulose	5.00
Soya oil	10.00
Vitamin premix*	0.30
Salt	0.30
Di-calcium phosphate	2.00
Limestone	1.00
Total	100.00

3.4.7 Data collection

3.4.7.1 FEED INTAKE (g)

Feed intake was determined by subtracting the amount of feeds left over feed from the quantity supplied to the rats and were weighed daily for each treatment. The daily feed intake of each animal was calculated by finding the differences between the feed supplied and left over feed.

3.4.7.2 BODY WEIGHT GAIN (g)

Weekly body weight was measured by subtracting the previous week's body weight from the current week's body weight, values obtained was used to calculate the average body weight gain per replicate.

3.4.7.3 FEED CONVERSION RATIO

This was calculated from the value of average weight gained and average feed consumed by the rats in each treatment, using the fomular below;

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Average feed intake}}{\text{Average daily gain}}$$

3.4.7.4 BLOOD COLLECTION

For biochemical and haematological analyses, three rats were randomly selected from each replicate per treatment. The rats were sacrificed under anaesthesia and 2 ml of blood was collected from the jugular. Blood collection for biochemical analysis was achieved with the use of Gel clot activator and sodium fluoride tubes while K_3 EDTA tubes were used to collect blood for the haematology samples. The blood samples were centrifuged at 2146 xg for 3 minutes to collect the plasma and serum. Samples were then subjected to analysis; Packed Cell Volume (PCV) was determined by microhaematocrit method, Haemoglobin (HB) content was determined by cyanomethaemoglobin methods. The Red Blood Cell

(RBC), White Blood Cell (WBC), Platelets (PLAT), Lymphocyte (LYM), Neutrophil (NEUT), Monocyte (MON) and Eosinophil (EO). were determined using an automatic haematological analyser (Model 7020, Hitachi, Tokyo, Japan).

The plasma total proteins (g/dL) were determined according to the method described by Henry (1974). The determination of plasma albumin (g d/dL) based on a colorimetric method described by Doumas *et al.* (1971). Globulin was calculated by subtraction of plasma albumin from plasma total protein. The enzyme activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined calorimetrically by using available commercial kits. The immunoglobulin D, E, G and M were determined using the immunoturbidimetric method and spectrophotometer. The immunoglobulin reagent and the Chemwell auto-analyzer were pre-warmed to 37°C. Ten microlitres (10µl) of reagent was used for IgM, while 7µl was used for IgG and IgD respectively.

3.4.7.5 RELATIVE ORGAN WEIGHTS MEASUREMENT

At the end of the experiment, three rats per replicate were sacrified and the brain, heart, liver, spleen and kidney removed, weighed with a digital scale and the organs kept in formaling well labelled for each treatment. The organ weigh was then calculated in relation with the final weigt of each rat.

3.4.8 STATISTICAL ANALYSIS

Data were analysed using Analysis of Variance (SAS, 2012), means were separated using Duncan's multiple range test.

CHAPTER FOUR

RESULTS

4.1 PROXIMATE COMPOSITION OF *Moringa oleifera* LEAF AT DIFFERENT AGES OF GROWTH

Proximate analyses of *moringa* leaf meal at different stages of growth are shown in Table 4.1. Leaves harvested at 12- week old had the highest mean value for moisture content of 8.96% and 20 week old leaves with the least mean moisture content of 7.82%, which were similar to values of the mean obtained for all the treatments. The crude protein content in leaves harvested at 12 week old had the highest mean value of 22.82% while the least mean value of 22.11% was obtained in leaves harvested at 20 week old. There were no differences in crude protein, ether extract and ash content of the leaves from 12 week to 20 week of age. The fibre content follows the same pattern as the ash content in the leaves.

4.2 MINERAL COMPOSITION OF *MORINGA OLEIFERA* LEAF AT DIFFERENT AGES OF GROWTH

The result of mineral composition of *M. oleifera* leaves harvested at different ages is presented in Table 4.2. All the minerals follow the same pattern as there were no differences in mineral composition of the leaves at different ages.

4.3 COMPOSITION OF SECONDARY METABOLITES OF MORINGA OLEIFERA LEAF HARVESTED AT DIFFERENT AGES OF GROWTH

Tables 4.3 and 4.4 show the results of qualitative and quantitative phytochemical screening of *Moringa oleifera* leaves at different ages of growth. The leaf meal was extracted with three different solvents, for both the quantitative and qualitative phytochemical screening. Five different phytochemicals were detected out of six tested for, it was observed that the leaves contain Saponins, tannins, phenols, alkaloids, flavonoids and cyanide was not detected. Saponins, Tannins and Alkaloids were more abundantly found than others. It was also observed that methanol solvent extracted more than other two solvents (aqueous and

ethanol). The result as shown on Table 4.4, displayed an increase in the content of Phytochemicals with increase in ages.

4.4 RATE OF RECOVERY OF EXTRACT FROM MORINGA LEAF MEAL USING DIFFERENT SOLVENTS

Three different solvents were used (aqueous, methanol and ethanol). After the extraction process, it was observed that methanol solvent extracted more than any other solvents, been an organic solvent it extracted more than aqueous.

Table 4.1: Effect of ages of growth on proximate composition of *Moringa oleifera* leaves

Parameters(%)	WEEK 12	WEEK 14	WEEK 16	WEEK18	WK 20
Moisture	8.96±0.52	8.81±0.39	8.56±0.14	7.93±0.49	7.82±0.60
Crude Protein	22.82±0.40	22.66±0.24	22.36±0.07	22.13±0.29	22.11±0.31
Ether extract	2.38±0.08	2.42±0.04	2.48±0.07	2.48±0.02	2.53±0.07
Ash	5.59±0.66	6.16±0.09	6.26±0.01	6.36±0.11	6.51±0.26
Crude Fibre	7.16±0.70	7.90±0.16	8.39±0.33	8.41±0.35	8.45±0.39
NFE	53.09±0.70	52.05±0.34	51.95±0.44	52.69±0.30	52.58±0.19

NFE- Nitrogen free extract

Table 4.2: Effect of ages of growth on mineral compositions of *Moringa oleifera* leaves

Parameters(mg /g)	Week 12	Week 14	Week 16	Week 18	Week 20
Calcium	741.67±26.33	761.67±6.33	766.67±1.33	770.00±2.00	800.00±32.00
Iron	17.32±0.51	17.28±0.47	16.63±0.18	16.44±0.37	16.39±0.42
Magnesium	128.33±7.45	130.55±5.23	136.67±0.89	141.66±5.88	141.67±5.89
Sodium	611.67±15.22	625.56±1.33	627.78±0.89	632.22±5.33	637.22±10.33
Chlorine	305.56±27.77	328.335. ±00	340.00±6.67	345.67±12.34	346.111±2.78
Potassium	238.33±16.89	250.00±5.22	253.33±1.89	264.44±9.22	270.00±14.78
Iodine	0.28±0.05	0.30±0.03	0.30±0.03	0.36±0.03	0.40±0.07
Phosphate	658.11±36.38	694.89±0.00	697.17±2.28	705.56±10.67	705.11±10.22

Table 4.3: Qualitative phytochemical screening of moringa leaf extract

SOLVENTS	SAPONINS	TANNINS	FLAVONOIDS	PHENOL	ALKALOIDS	CYANIDES
METHANOL	++	++	+	+	+++	-
ETHANOL	+	+++	-	-	++	-
AQUEOUS	+	-	++	++	+	-

NOTE;

+ Present in a small quantity

++ Moderately present

+ + + Present in appreciable quantity

- Not present

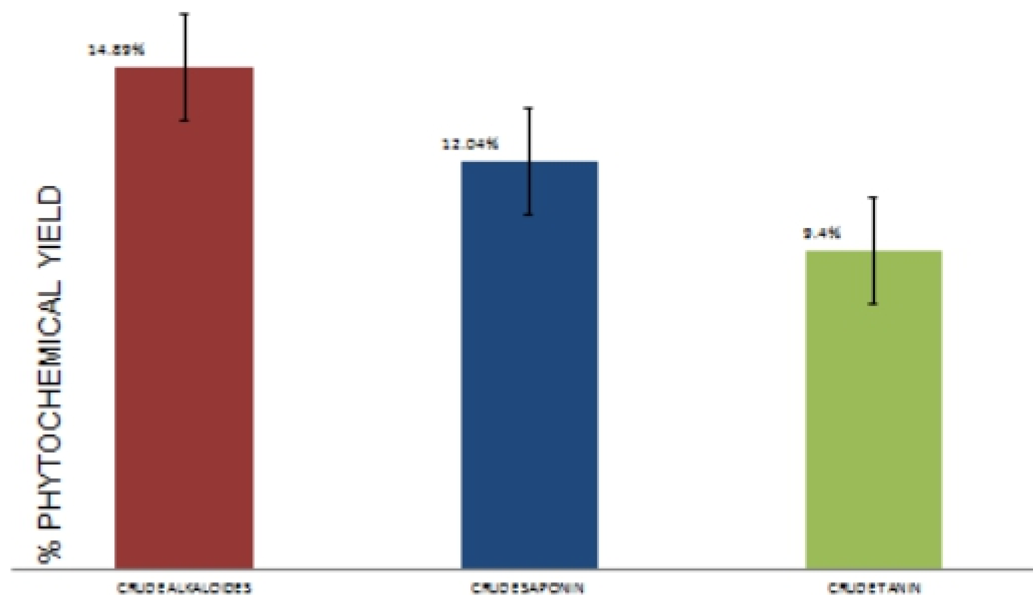

Figure 4.1: PHYTOCHEMICAL YIELD OF ALKALOID,SAPONIN AND TANNIN FROM *Moringa oleifera* LEAF MEAL

Table 4.4: Quantitative phytochemical constituent of *Moringa oleifera* leaves at different ages of growth

PARAMETER (mg/g)	**Week 12**	**Week 14**	**Week 16**	**Week 18**	**Week 20**
Alkaloids	235.56±8.66	242.78±1.44	244.22±0.00	247.78±3.56	250.00±5.78
Saponins	153.27±4.28	154.22±3.33	159.16±1.61	159.55±2.00	161.33±3.78
Tannins	132.22±17.56	146.11±3.67	150.56±0.78	150.56±0.78	169.44±19.99

4.5 BIOCHEMICAL RESPONSE OF WEANLING WISTAR RATS ADMINISTERED CRUDE EXTRACTS OF *Moringa oleifera* (LAM) LEAF

4.5.1 GROWTH PERFORMANCE OF RATS FED PHYTOCHEMICALS EXTRACT FROM MORINGA LEAVES

The effect of moringa alkaloids on growth performance of rats is as shown in Table 4.5. There was decline in the quantity of feed intake with elevated dosage of moringa extract ingested. Feed intake ranged from 30.40g for rats on 1 mg/ mL moringa leaf extract down to 24.25g for rats on 2 mg/mL moringa extract and was significantly ($P<0.05$) lower than those animals on the control. Crude moringa extract and crude moringa alkaloids inclusion at higher dosage (1.5 and 2.0 mg/mL) depressed feed intake compared to the control. These were also reflected in the value of weight change and consequently on the feed conversion ratio.

There were significant ($P<0.05$) reduction in feed consumption of the animals with increase in dose of both moringa extract and crude moringa saponin with a consequential reduction in weight change and increase in feed conversion ratio as shown in Table 4.6. In all, the feed consumption was lower than that of the control which significantly reflected in the weight change of the animals. Just as was observed with crude moringa alkaloid and saponin, effect of crude moringa tannin shown in Table 4.7 also revealed a significant ($P < 0.05$) reduction in feed intake and weight change as the dose of tannins ingested increased. All the phytochemical and their extract impacted a major reduction in feed consumed, weight change and increase in value of feed conversion ratio in all the rats as the dosage of the extract increased. That is, the higher the dosage, the less the feed intake and weight change.

Table 4.5: Effect of *moringa leaves* alkaloid extract on the growth performance of rats

Parameters	Control (mg/mL)	Moringa extract (mg/mL)			Crude Moringa Alkaloids (mg/mL)			SEM
		1.0	1.5	2.0	1.0	1.5	2.0	
Feed intake (g)	32.20^{b}	30.40^{b}	27.48^{bc}	24.25^{c}	37.25^{a}	36.23^{a}	23.60^{c}	1.44
Weight change (g)	39.08^{a}	24.00^{b}	16.75^{c}	16.00^{c}	25.25^{b}	13.00^{d}	8.20^{e}	1.06
FCR	0.82^{d}	1.27^{c}	1.64^{b}	1.52^{b}	1.48^{cb}	2.79^{a}	2.88^{a}	0.08

Table 4.6: Effect of moringa leaves saponin extract on the growth performance of rats

Parameters	Control (mg/mL)	Moringa extract (mg/mL)			Crude Moringa Saponins (mg/mL)			SEM
		1.0	1.5	2.0	1.0	1.5	2.0	
Feed intake (g)	32.20[a]	30.40[a]	27.48[b]	24.25[c]	31.00[a]	27.48[b]	24.73[c]	1.44
Weight change (g)	39.08[a]	24.00[b]	16.75[c]	16.00[c]	16.25[c]	10.00[d]	8.45[e]	1.11
FCR	0.82[d]	1.27[c]	1.64[b]	1.52[b]	1.91[ab]	2.75[a]	2.93[a]	0.12

Table 4.7: Effect of moringa leaves tannins extract on the growth performance of rats

Parameters	Control (mg/mL)	Moringa extract (mg/mL)			Crude Moringa Saponins (mg/mL)			SEM
		1.0	1.5	2.0	1.0	1.5	2.0	
Feed intake (g)	32.20[a]	30.40[b]	27.48[bc]	24.25[bc]	32.25[a]	31.60[a]	21.38[c]	1.49
Weight change (g)	39.08[a]	24.00[b]	16.75[c]	16.00[c]	17.25[bc]	16.75[c]	11.50[d]	0.97
FCR	0.82[d]	1.27[c]	1.64[b]	1.52[b]	1.87[a]	1.87[a]	1.86[a]	0.10

4.5.2 RELATIVE ORGAN WEIGHTS OF WISTAR RATS FED PHYTOCHEMICAL EXTRACT FROM MORINGA LEAVES

Effect of *Moringa oleifera* alkaloids on relative organ weights of rats is as sown in Table 4.8. A significant ($P<0.05$) reduction in mean organ weights of the kidney, heart and brain was observed with increase in dosage of extracts. For kidney weight, rats administered with 1.0 mg/mL moringa extract had the highest kidney weight (0.69g) simiar to the control (0.63g) while rats on the 2.0 mg/mL moringa extract showed depressed growth.

For heart weight, control had the highest mean weight of 0.34g followed by 1.0 mg/mL moringa extract and were significantly higher than those on 2.0 mg/mL of moringa extract. For brain weight, group given 1.0 mg/mL crude moringa alkaloids had the highest mean weight gain of 1.41g which was significantly higher than the control and significantly higher than rats given 2.0 mg/mL crude moringa alkaloid. Rats on 2.0 mg/mL crude moringa alkaloid which had the least mean weight..

There was no significant difference ($P > 0.05$) between mean value of organ weights of control groups and other treatments for liver and brain. However, significant differences were observed between the organ weights for kidney, spleen and heart. Group given 1.0 mg/mL moringa extract had the highest mean value for kidney which was not significantly different ($P > 0.05$) from control. Group given 2.0 mg/mL moringa extract had the least weight. Control group had highest organ weights for heart and liver. Group given 1.5 mg/mL of moringa extract had the least mean value.

Table 4.10 shows the effect of moringa tannins leaf extracts on organ weights of rats. There were significant differences between the control and all the treatments for kidney, spleen, heart and brain. kidney, spleen and brain given 1.0 mg/mL moringa extract had the highest weight followed by the control group and were statistically higher than group given 2.0 mg/mL of crude moringa tannins. However, for heart, control group had the highest weight followed by group given 1.0 mg/mL of moringa extract while group given 2.0 mg/mL crude moringa tannins had the least weight.

Table 4.8: Effect of moringa extract and crude alkaloids on relative organ weight of rats

Parameters	Control	Moringa extract (mg/mL)			Crude Moringa Alkaloids (mg/mL)			SEM
(%)	(mg/mL)	1.0	1.5	2.0	1.0	1.5	2.0	
Kidney	0.63[a]	0.69[a]	0.52[b]	0.51[b]	0.59[ab]	0.56[ab]	0.34[c]	0.03
Spleen	0.28[ab]	0.36[a]	0.19[c]	0.33[a]	0.24[b]	0.13[c]	0.08[d]	0.03
Heart	0.34[a]	0.30[a]	0.22[b]	0.26[ab]	0.25[ab]	0.24[b]	0.19[c]	0.02
Liver	2.23	2.24	1.94	2.00	2.09	1.78	1.43	0.12
Brain	1.28[b]	1.33[ab]	1.27[b]	1.28[b]	1.41[a]	1.22[b]	0.92[c]	0.05

Table 4.9: Effect of moringa extract and crude saponins on relative organ weight of rats

Parameters (%)	Control (mg/mL)	Moringa extract (mg/mL)			Crude Moringa Saponins (mg/mL)			SEM
		1.0	1.5	2.0	1.0	1.5	2.0	
Kidney	0.63[a]	0.69[a]	0.52[b]	0.51[b]	0.53[b]	0.52[b]	0.48[c]	0.03
Spleen	0.28[ab]	0.36[a]	0.19[c]	0.33[a]	0.21[b]	0.12[c]	0.11[c]	0.03
Heart	0.34[a]	0.30[a]	0.22[b]	0.26[b]	0.25[b]	0.24[b]	0.23[b]	0.02
Liver	2.23	2.24	1.94	2.00	2.09	2.05	1.76	0.12
Brain	1.28[b]	1.33[a]	1.27[b]	1.28[b]	1.35[a]	1.26[b]	1.17[c]	0.05

Table 4.10: Effect of moringa extract and crude tannins on relative organ weight of rats

Parameters	Control	Moringa extract (mg/mL)			Crude Moringa Tannins (mg/mL)			SEM
(%)	(mg/mL)	1.0	1.5	2.0	1.0	1.5	2.0	
Kidney	0.63^{a}	0.69^{a}	0.52^{b}	0.51^{b}	0.61^{ab}	0.53^{b}	0.29^{c}	0.04
pleen	0.28^{b}	0.36^{a}	0.19^{c}	0.33^{a}	0.17^{c}	0.18^{c}	0.08^{d}	0.03
Heart	0.34^{a}	0.30^{a}	0.22^{b}	0.26^{ab}	0.28^{ab}	0.22^{b}	0.14^{c}	0.02
Liver	2.23	2.24	1.94	2.00	2.04	1.83	1.42	0.16
Brain	1.28^{b}	1.33^{a}	1.27^{b}	1.28^{b}	1.32^{a}	1.31^{a}	0.65^{c}	0.08

4.5.3 HEAMATOLOGICAL RESPONSE OF RATS FED PHYTOCHEMICALS EXTRACT OF MORINGA LEAVES

From Table 4.11, the effect of crude moringa extract and crude moringa alkaloids was not significantly different from control for PCV, Hb, RBC, lymphocytes, neutrophils and monocytes. For WBC, platelets and eosinophil, there were differences between the mean values. White Blood Cell (WBC) value increased with increase in dosage for both moringa extract and crude moringa alkaloid and in both cases significantly higher than that of control but with significant drop in the value of platelets with increase in dosage of the phytochemicals. For eosinophil, treatments in which control group had the highest mean value of 3.5% was significantly different from group given 1.5 mg/mL and 2.0 mg/mL crude moringa alkaloids that had the lowest values.

Effect of *Moringa oleifera* saponins on heamatological parameter of rats is shown in Table 4.12. The effect of moringa extract and crude moringa saponins did not influences PCV, Hb, RBC, Lym, and Mono. Control had the highest mean for EO which was significantly higher ($P<0.05$) than 1.5 mg/mL and 2.0 mg/mL crude moringa saponins. Effect of moringa crude saponins increased with increased dosage for Plat.

Table 4.13 shows the effect of *Moringa oleifera* tannins on the heamotological parameters of rats. No significant difference was observed in the mean values for all the treatments for PCV, Hb, RBC, lym, Neut and Mono, with control having the highest mean value for PCV,Hb,RBC,plateletandEO

Table 4.11: Effect of moringa alkaloids on haematological parameters of rats

Parameters	Control	Moringa Extract (mg/mL)			Crude Moringa Alkaloids (mg/mL)			SEM
	(mg/mL)	1.0	1.5	2.0	1.0	1.5	2.0	
PCV(%)	36.75	33.75	31.75	29.50	39.67	33.75	32.00	1.32
Hb.(g/dl)	11.88	11.05	10.73	9.83	13.23	11.20	10.78	0.45
RBC($x10^6$/ul)	6.05	5.44	5.31	5.09	6.68	5.82	5.40	0.22
WBC($x10^3$/ul)	8.13^d	8.79^d	11.19^b	12.29^a	10.47^c	11.84^b	12.64^a	1.30
PLAT($x10^3$/ul)	2.92^a	2.11^a	1.69^{bc}	1.62^c	2.73^a	1.91^b	0.88^e	3.11
LYM(%)	65.00	60.25	70.00	71.50	62.25	64.00	70.00	1.88
NEUT(%)	26.75	23.75	25.00	34.75	21.67	33.50	33.00	2.0
MON(%)	2.25	2.00	2.00	2.25	2.00	2.00	2.25	0.19
EO(%)	3.50^a	3.00^b	2.75^c	2.75^c	3.00^b	1.75^d	2.00^{cd}	0.16

a,b,c,d….Mean with different superscripts along the same row are significantly ($P<0.05$) different.

PCV—packed cell volume , WBC –white blood cells , NEUT—neutrophils ,HB –heamoglobin concentration PLAT – platelets ,MON --- monocytes ,RBC—red blood cells, LYM –lymphocyte, EO –eosinophil SEM=-Standard Error of Mean

Table 4.12: Effect of crude extract saponins from moringa leaves on hematological parameters of rats

Parameters	Control	Moringa Extract (mg/mL)			Crude Moringa Saponins (mg/mL)			SEM
	(mg/mL)	1.0	1.5	2.0	1.0	1.5	2.0	
PCV(%)	36.75	33.75	31.75	29.50	33.50	35.75	35.67	1.29
Hb (g/dl)	11.88	11.05	10.73	9.83	11.18	11.60	11.98	0.44
RBC($x10^6$/ul)	6.05	5.44	5.31	5.09	5.37	5.83	6.02	0.22
WBC($x10^3$/ul)	8.13^d	8.79^c	11.19^b	12.29^a	8.63^c	10.03^b	13.40^a	1.41
PLAT ($x10^3$/ul)	2.92^a	2.11^c	1.69^d	1.62^d	8.00^b	12.12^a	13.40^a	1.05
LYM(%)	65.00	60.25	70.00	71.50	58.75	61.25	66.67	2.01
NEUT(%)	26.75^c	23.75^c	25.00^c	34.75^a	36.25^a	34.25^a	31.00^b	2.06
MON(%)	2.25	2.00	2.00	2.25	2.25	2.00	2.00	0.19
EO(%)	3.50^a	3.00^b	2.75^c	2.75^c	3.00^b	2.25^d	2.00^e	0.14

a,bc…Mean with different superscripts along the same row are significantly ($P<0.05$) different

PCV—packed cell volume , WBC –white blood cells , NEUT—neutrophils ,HB –heamoglobin concentration , PLAT – platelets ,MON --- monocytes ,RBC—red blood cells, LYM –lymphocyte, EO –eosinophil; SEM- Standard Error of Mean

Table 4.13: Effect of *Moringa oleifera* tannins on hematological parameters of rats

Parameters	Control	Moringa Extract (mg/mL)			Crude Moringa Tannins (mg/mL)			SEM
	(mg/mL)	1.0	1.5	2.0	1.0	1.5	2.0	
PCV(%)	36.75	33.75	31.75	29.50	35.50	34.67	31.75	1.08
Hb (g/dl)	11.88	11.05	10.73	9.83	11.58	11.37	10.88	0.37
RBC($x10^6$/ul)	6.05	5.44	5.31	5.09	5.82	5.85	5.09	0.19
WBC($x10^3$/ul)	8.13^c	8.79^c	11.19^b	12.29^a	8.21^c	8.10^c	8.13^c	1.34
PLAT($x10^3$/ul)	2.92^a	2.11^a	1.69^b	1.62^b	1.68^b	1.02^c	0.75^d	0.29
LYM(%)	65.00	60.25	70.00	71.50	77.50	68.50	65.00	2.13
NEUT(%)	26.75	23.75	25.00	34.75	31.67	26.50	16.75	2.05
MON(%)	2.25	2.00	2.00	2.25	3.00	2.75	1.33	0.22
EO(%)	3.50^a	3.00^b	2.75^c	2.75^c	3.00^b	2.75^c	2.25^d	0.53

abc………Mean with different superscripts along the same row are significantly ($P<0.05$) different. PCV—packed cell volume , WBC –white blood cells , NEUT—neutrophils ,HB –heamoglobin concentration, PLAT – platelets ,MON --- monocytes , RBC—red blood cells, LYM –lymphocyte, EO –eosinophil, SEM- Standard Error of Mean

4.5.4 SERUM BIOCHEMISTRY INDICES OF RATS FED PHYTOCHEMICAL EXTRACT FROM MORINGA LEAVES

From the result (Table 4.14), there were no significant differences (P > 0.05) in the mean values of Total protein, Globulin and Urea of rats in all the treatments. For Albumin, rats administered 1.0 mg/mL of crude moringa alkaloids had the highest mean value (3.48g/dL) while the rats given 2.0 mg/mL of crude moringa extract had the least mean value (2.26g/dL). No significant difference in the mean value of control, 1.0 mg/mL and 1.5 mg/mL moringa extract and 1.5 mg/mL moringa alkaloids. Also no difference between the mean of 2.0 mg/mL and 1.5 mg/mL moringa alkaloids, control and all the three graded level of moringa extract. There was decrease in Albumin level of rats from 1.0 mg/mL to 2.0 mg/mL dosage for crude moringa extract and crude moringa alkaloids which indicated that as the dosage of the extract given to the rats increased Albumin level decreased.

There was no statistical difference between the mean values for rats at the three graded levels for crude moringa extract, but there was statistical difference for crude moringa alkaloids. For Alb-Gld ration, rats given 1.0 mg/mL crude moringa alkaloids had the highest mean value (0.86) followed by 1.5 mg/mL crude moringa alkaloids (0.78g/dL) with 2.0 mg/mL moringa extract having the least value (0.53 g/dL). Significant difference was not observed within the three graded levels for both moringa extract and crude moringa alkaloids.

Table 4.15 shows the effect of M*oringa oleifera* saponins on serum metabolism of rats. Mean value of Albumin, Globulin and Urea were not significantly different (P>0.05) but there was significant difference (P<0.05) between the mean value of Total protein and Globulin. For total protein, rats given 2.0 mg/mL crude moringa extract had the least mean (6.5g/dL) while rats given 1.0 mg/mL crude moringa saponins leaf extract had the highest mean (7.98g/dL). Control is not different for moringa extract and crude moringa saponins at 1.0 mg/mL.

For Globulin, 2.0 mg/mL crude moringa saponins had the highest mean value of 4.83g/dL with 1.0 mg/mL of crude moringa saponins having least value(3.70 g/dL). The effect of *Moringa oleifera* tannins on serum metabolism of rats is shown in table 4.16 below. There was no significant difference (P>0.05) in the mean value of Total protein, Albumin, Globulin, .Alb-Glob

ratio and Urea for all the treatments. For all the parameters measured, rats given 2.0 mg/mL moringa extract had the least mean value while rats given 1.0 mg/mL crude moringa tannins for Total protein, Albumin, Alb-Glb ratio, urea and creatinine had the highest mean value, but for Globulin, rats given 2.0 mg/mL crude moringa tannins had the highest mean.

Table 4.14: Effect of *Moringa oleifera* alkaloid on serum biochemical indices of rats.

Parameters	Control (mg/mL)	Moringa Extract (mg/mL)			Crude Moringa Alkaloids (mg/mL)			SEM
		1.0	1.5	2.0	1.0	1.5	2.0	
Total protein (g/dL)	7.18	7.33	7.00	6.50	7.53	7.20	6.55	0.15
Albumin (g/dL)	3.15^{ab}	3.40^{a}	2.85^{b}	2.26^{c}	3.48^{a}	3.15^{ab}	2.40^{c}	0.01
Globulin (g/dL)	4.03	3.93	4.15	4.24	4.05	4.05	4.15	0.09
Alb-Glb Ratio	0.78^{ab}	0.87^{a}	0.69^{b}	0.53^{c}	0.86^{a}	0.78^{ab}	0.58^{c}	0.03
Urea (mg/dL)	15.25	14.75	15.25	14.33	15.67	14.50	16.00	0.22
Creatinine (mg/dL)	0.70^{b}	0.73^{b}	0.68^{c}	0.63^{c}	0.80^{ab}	0.68^{c}	1.00^{a}	0.04

Table 4.15: Effect of *Moringa oleifera* saponin on serum biochemical indices of rats

Parameters	Control (mg/mL)	Moringa Extract (mg/mL)			Crude Moringa Saponins (mg/mL)			SEM
		1.0	1.5	2.0	1.0	1.5	2.0	
Total protein (g/dL)	7.18	7.33	7.00	6.50	7.98	7.65	6.57	0.14
Albumin (g/dL)	3.15[ab]	3.40[a]	2.85[b]	2.26[c]	3.15[ab]	3.15[ab]	2.87[b]	0.09
Globulin (g/dL)	4.03	3.93	4.15	4.24	3.70	4.65	4.83	0.11
Alb-Glb Ratio	0.78[ab]	0.87[a]	0.69[b]	0.53[c]	0.85[a]	0.68[b]	0.59[c]	0.03
Urea (mg/dL)	15.25	14.75	15.25	14.33	15.25	15.25	15.00	0.17
Creatinine(mg/dL)	0.70[ab]	0.73[ab]	0.68[b]	0.63[c]	0.67[b]	0.73[ab]	0.85[a]	0.04

Table 4.16: Effect of *Moringa oleifera* tannins on serum biochemical indices of rats

Parameters	Control (mg/mL)	Moringa Extract (mg/mL)			Crude Moringa Tannins (mg/mL)			SEM
		1.0	1.5	2.0	1.0	1.5	2.0	
Total protein (g/dL)	7.18	7.33	7.00	6.50	7.40	7.25	7.25	0.15
Albumin (g/dL)	3.15^{ab}	3.40^{a}	2.85^{b}	2.26^{c}	3.35^{a}	3.00^{ab}	2.80^{b}	0.1
Globulin (g/dL)	4.03	3.93	4.15	4.24	4.05	4.25	4.45	0.09
Alb-Glb Ratio	0.78^{ab}	0.87^{a}	0.69^{b}	0.53^{c}	0.80^{ab}	0.68^{b}	0.58^{c}	0.06
Urea (mg/dL)	15.25	14.75	15.25	14.33	15.25	15.00	14.50	0.21
Creatinine(mg/dL)	0.70^{b}	0.73^{b}	0.68^{c}	0.63^{c}	0.93^{a}	0.75^{b}	0.68^{c}	0.04

4.5.5 EFFECT OF *MORINGA OLEIFERA* PHYTOCHEMICALS ON SERUM ENZYME OF RATS

There was no significant difference ($P>0.05$) between the mean value for AST and ALT for all the treatments, but significant difference ($P<0.05$) was observed in the mean for ALP. Moringa extract at 1.0 mg/mL had the least mean of 106.75u/L for ALP with 2.0 mg/mL moringa extract having the highest mean of 122.67u/L. Moringa extract at 2 mg/mL is statistically higher than 1.0 mg/mL moringa extract and control, meaning ALP mean value for moringa extract increase from 1.0 mg/mL level of 106.75u/l to 2.0 mg/mL level of 122.67u/l. Also for crude moringa alkaloid, value increased from 1.0 mg/mL to 2.0 mg/mL.

. From the result (table 4.18), there was no significant different ($P>0.05$) between the mean of all treatments for ALT and AST, while significant difference was observed for ALP. For ALP, rats given 1.0 mg/mL moringa extract had the least mean value followed by 1.5 mg/mL crude moringa saponins while 2.0 mg/mL moringa extract had the higthest. Increase in the ALP value for rats given moringa extract increased from 1.0 mg/mL dosage level to 2 mg/mL dosage level while that of crude moringa saponins decreased. The result on effect of moringa oleifera tannins on serum enzyme of rats is shown in the table 4.19. There was no significant difference for AST and ALT, which shows that for ALT and AST, *Moringa oleifera* tannins had no effect on the rat serum enzyme. For ALP, rats given 1.0 mg/mL moringa extract had the lowest mean value while rats given 2.0 mg/ml of moringa extract had the highest mean of 122.67u/l. There was increase in the mean value of rats for AST from 1.0 mg/mL to 2.0 mg/mL level for both moringa extract and crude moringa tannins. For ALP, moringa extract 1.0 mg/mL had the least mean value of 106.75u/L and 2.0 mg/mL moringa extract had the highest mean value of 122.67u/l. Rats given 2.0 mg/mL moringa extract had mean value which was not statistically different from the control, moringa extract at 1.0 mg/mL, crude moringa tannins at 1.5 mg/mL and 2.0 mg/mL crude moringa tannins.

Table 4.17: Effect of *Moringa oleifera* alkaloid on serum enzymes of rats

Parameters (u/l)	Control (mg/mL)	Moringa extract(mg/mL)			Crude Moringa Alkaloids (mg/mL)			SEM
		1.0	1.5	2.0	1.0	1.5	2.0	
AST	41.50	38.33	41.00	45.25	40.33	42.00	40.75	0.80
ALT	29.50	29.50	28.33	30.50	28.33	30.75	28.50	0.58
ALP	111.50[c]	106.75[d]	117.50[c]	122.67[b]	114.00[c]	118.75[c]	173.00[a]	2.35

AST---- Aspartate amino transferase ,ALT--Alanine amino transferase ,ALP ---Alkaline phosphatase

Table 4.18: Effect of *Moringa oleifera* saponins on serum enzymes of rats

Parameters (u/l)	Control	Moringa extract (mg/mL)			Crude Moringa Saponins (mg/mL)			SEM
	(mg/mL)	1.0	1.5	2.0	1.0	1.5	2.0	
AST	41.50	38.33	41.00	45.25	37.00	39.00	39.00	0.79
ALT	29.50	29.50	28.33	30.50	26.67	27.00	28;00	0.54
ALP	111.50^{b}	106.75^{c}	117.50^{b}	122.67^{a}	107.00^{c}	112.00^{b}	116.25^{b}	2.44

AST-- Aspartates amino transferase, ALT --Alanine amino transferase, ALP --Alkaline phosphatase

Table 4.19: Effect of *Moringa oleifera* tannins on serum enzymes of rats

Parameters	Control	Moringa extract (mg/mL)			Crude Moringa Tannins (mg/mL)			SEM
(u/l)	(mg/mL)	1.0	1.5	2.0	1.0	1.5	2.0	
AST	41.50	38.33	41.00	45.25	42.50	44.75	44.00	0.77
ALT	29.50	29.50	28.33	30.50	31.00	30.50	31.50	0.52
ALP	111.50^{c}	106.75^{d}	117.50^{b}	122.67^{a}	108.25^{d}	110.00^{c}	114.25^{b}	1.33

AST-- Aspartates aminotransferace, ALT---Alanine aminotransferase, ALP ---Alkaline phosphatase

4.5.6 IMMUNE RESPONSE OF RATS FED PHYTOCHEMICAL EXTRACT FROM MORINGA LEAVES

Group given 1.0 mg/mL crude moringa alkaloids had lowest mean value for Zn, IgE and IgG while group given 1.0 mg/mL moringa extract had the least mean value, even lower than control. For all the parameter measured, there was increase in mean value from 1.0 mg/mL to 2.0 mg/mL for moringa extract, while for crude moringa alkaloids there was increase in mean value as the dosage level increased from 1.0 mg/mL to 2.0 mg/mL. Control was significantly higher than all the groups given 1.0 mg/mL, 1.5 mg/mL and 2.0 mg/mL of moringa extract and significantly lower than 1.0 mg/mL crude moringa alkaloids.

Table 4.21 shows the effect of *Moringa oleifera* saponins on the immune response of rats. Significant difference ($P<0.05$) was observed between the mean of all the treatment for Zn, IgD ,IgE, IgG, IgM with control having significant highest mean value . With group given 1.0 mg/mL crude moringa Saponins having the least significant mean value. For both moringa extract and crude moringa saponin, mean value for all parameters measured increased from 1.0 mg/mL to 2.0 mg/mL.

The effect of *Moringa oleifera* tannins on immune response of rats is shown in table 4.22. For all the parameters measured (Zn, IgD, IgE, IgG and Ig.M), there was significant difference between the mean value of all the treatments with group given 1.0 mg/ml of moringa extract having the least significant mean value and control having the highest significant mean value. Also, for all the parameters measured, mean value increased from 1 mg/mL to 2 mg/mL of moringa extract, whereas for crude moringa tannins,mean value decreased from 1.0 mg/mL to 1.5 mg/mL and later increased at 2.0 mg/mL.

Table 4.20: Effect of *Moringa oleifera* alkaloid on immune response of rats

Parameters	Control	Moringa extract (mg/mL)			Crude Moringa alkaloids (mg/mL)			SEM
(mg/dl)	(mg/mL)	1.0	1.5	2.0	1.0	1.5	2.0	
Zn	49.76^{b}	38.14^{c}	39.48^{c}	41.41^{bc}	52.40^{a}	52.79^{a}	54.48^{a}	1.45
IgD	323.41^{b}	247.89^{c}	256.64^{c}	269.14^{c}	340.59^{a}	343.16^{a}	354.13^{a}	9.46
IgE	0.67^{b}	0.51^{c}	0.53^{c}	0.57^{bc}	0.70^{a}	0.71^{a}	0.73^{a}	0.02
IgG	938.68^{b}	719.47^{c}	744.88^{c}	781.14^{c}	988.53^{b}	996.00^{b}	1027.84^{a}	27.27
IgM	180.15^{b}	138.08^{c}	140.96^{c}	148.62^{c}	189.72^{ab}	191.75^{a}	191.75^{a}	5.39

Zn—zinc, IgD—immoglobulin, IgE--immoglobulin E, IgM--immoglobulin M,IgG --- immoglobulin G

Table 4.21: Effect of *Moringa oleifera* saponin on immune response of rats

Parameters	Control	Moringa extract (mg/mL)			Crude Moringa Saponins (mg/mL)			SEM
(mg/dl)	(mg/mL)	1.0	1.5	2.0	1.0	1.5	2.0	
Zn	49.76[a]	38.14[b]	39.48[b]	41.41[ab]	30.64[d]	34.35[c]	36.64[c]	1.22
IgD	323.41[a]	247.89[cd]	256.64[c]	269.14[c]	299.13[b]	232.29[d]	238.16[d]	8.19
IgE	0.67[a]	0.51[c]	0.53[c]	0.57[b]	0.41[d]	0.46[cd]	0.49[cd]	0.02
IgG	938.68[a]	719.47[d]	744.88[c]	781.14[b]	777.96[b]	748.06[c]	791.23[b]	23.58
IgM	180.15[a]	138.08[c]	140.96[b]	148.62[b]	110.92[e]	124.38[d]	132.66[c]	4.57

zn—zinc , igD—immoglobulin D , igE--immoglobulin E , igM--immoglobulin M , igG immoglobulin G

Table 4.22: Effect of *Moringa oleifera* tannins on immune response of rats

Parameters (mg/dL)	Control (mg/mL)	Moringa extract (mg/mL)			Crude Moringa Tannins (mg/mL)			SEM
		1.0	1.5	2.0	1.0	1.5	2,0	
Zn	49.76[a]	38.14[c]	39.48[c]	41.41[cb]	45.10[b]	46.17[b]	46.68[b]	0.83
IgD	323.41[a]	247.89[e]	256.64[d]	269.14[c]	293.17[b]	300.11[b]	303.41[b]	5.60
IgE	0.67[a]	0.51[c]	0.53[c]	0.57[bc]	0.61[b]	0.62[b]	0.63[b]	0.01
IgG	938.68[a]	719.47[e]	744.88[d]	781.14[c]	850.88[b]	871.05[b]	880.63[b]	16.53
IgM	180.15[a]	138.08[d]	140.96[c]	148.62[c]	163.30[b]	164.84[b]	169.01[b]	3.39

Zn—zinc , igD—immoglobulin D, igE--immoglobulin E, igM--immoglobulin M, igG--- immoglobulinG

4.5.7 THE EFFECT OF *MORINGA OLEIFERA* PHYTOCHEMICALS ON LIPID PROFILE OF RATS

Table 4.23 shows the effect of *Moringa oleifera* alkaloids on cholesterol level of rats. There was significant difference among the treatments for Cholesterol, Trig., HDL and LDL. For cholesterol, rats given 1.0 mg/mL of crude moringa alkaloids had the highest mean value followed by 1.0 mg/mL moringa extract, with 2.0 mg/mL crude moringa extract having the least value. In the case of Trig., HDL and LDL, the same trend that was observed for cholesterol was repeated for moringa extract. For all the four parameters measured, mean value decrease as the dosage increase for both moringa extract and crude moringa alkaloid.

Table 4.24 shows the effect of *Moringa oleifera* saponins on the cholesterol level of albino rats. There was significant difference in the mean value of cholesterol, Trig, LDL and HDL between the treatments. For cholesterol level, group given 1.0 mg/mL moringa extract had the highest mean value of 51.75 mg/dL with group given 2.0 mg/mL crude moringa saponins having the least mean value of 40.50 mg/dL.

For Trig., group given 2.0 mg/mL crude moringa extract had the least mean value which was not significantly different from the control and rats given 1.0 mg/mL moringa extract had the highest. The result of effect of *Moringa oleifera* tannins on Cholesterol level of rats is shown in table 4.25 below. From the table there was no significant difference ($P > 0.05$) between the mean value for Trig., and HDL while LDL shows significant difference .

For cholesterol, rats given 1.0 mg/mL moringa tannins had the highest value of 53.75mg/dL followed by 1.0 mg/mL crude moringa extract with 51.75mg/dL which were not significantly different from the control. Cholesterol level decreased as dosage level increased for moringa extract and moringa tannins. For Trig., there was no significant difference.

Table 4.23: Effect of *Moringa oleifera leave* alkaloid on lipid profile of rats

Parameters	Control	Moringa extract (mg/mL)			Crude MoringAlkaloids(mg/mL)			SEM
(mg/dl)	(mg/mL)	1.0	1.5	2.0	1.0	1.5	2.0	
Total cholesterol	52.75[a]	51.75[a]	43.67[b]	42.25[b]	55.67[a]	45.50[b]	44.25[b]	2.34
Triglycerides	51.25[a]	52.75[a]	52.50[a]	51.33[a]	52.33[a]	50.25[a]	42.00[b]	3.28
HDL	28.75	28.00	28.50	28.50	28.00	28.50	29.67	0.77
LDL	24.00[b]	23.75[b]	15.17[c]	13.75[d]	27.67[a]	17.00[c]	14.58[d]	0.35

HDL-High density lipoprotein , LDL-Low density lipoprotein ,

SEM- Standard Error of Mean

Table 4.24: Effect of *Moringa oleifera* leave saponin on lipid profile of rats

Parameters	Control	Moringa extract (mg/mL)			Crude Moringa Saponins (mg/mL)			SEM
(mg/dl)	(mg/mL)	1.0	1.5	2.0	1.0	1.5	2.0	
Cholesterol	52.75[a]	51.75[a]	43.67[b]	42.25[b]	46.75[ab]	42.67[b]	40.50[c]	2.96
Triglycerides	51.25[a]	52.75[a]	52.50[a]	51.33[a]	46.50[ab]	42.00[b]	38.67[c]	2.87
HDL	28.75[c]	28.00[c]	28.50[c]	28.50[c]	29.02[b]	36.28[a]	38.00[a]	1.65
LDL	24.00[a]	23.75[a]	15.17[b]	13.75[c]	17.73[b]	6.39[d]	2.50[e]	0.34

HDL ------ High density lipoprotein , LDL-------- Low density lipoprotein

Table 4.25: Effect of *Moringa oleifera leave* tannins on lipid profile of rats

Parameters	Control	Moringa extract (mg/mL)			Crude Moringa tannins (mg/mL)			SEM
(mg/dl)	mg/mL	1.0	1.5	2.0	1.0	1.5	2.0	
Total Cholesterol	52.75[a]	51.75[a]	43.67[c]	42.25[c]	53.75[a]	49.25[b]	43.75[c]	2.86
Triglycerides	51.25	52.75	52.50	51.33	53.73	49.25	48.67	2.76
HDL	28.75	28.00	28.50	28.50	26.00	26.75	31.00	0.57
LDL	24.00[b]	23.75[b]	15.17[c]	13.75[d]	27.75[a]	22.50[b]	22.75[b]	0.50

HDL-High density lipoprotein , LDL-Low density lipoprotein, VLDL-Very low density lipoprotein

CHAPTER FIVE

DISCUSSION

5.1 CHEMICAL CONSTITUENT OF *Moringa oleifera* LEAF MEAL AT DIFFERENT AGES OF GROWTH

5.1.1 RATE OF RECOVERY

The rate of recovery for Ethanol, Methanol and aqueous solvents were examined. Methanol solvent was the highest and the aqueous the least, this might be because organic solvent have been proved to extract better. Ezekwe *et al*. (2013). Extracts of M. oleifera from the three different solvents shown that tannins, alkaloids and saponins are significantly presenct compeared to others, though, more was been recovered with methanol than ethanol solvent with aqueous solvent having the least rate of recovery.This was in line with the report of Bukar and Oyeyi (2010), that tannins are more detected in ethanol leaf extract which was in line with the result of this study.

5.1.2 PROXIMATE ANALYSIS

The proximate analysisshows that they are rich in nutrients and revealed that they can be grouped as good source of carbohydrate. Generally, vegetables are made up of essential components that supply protein, calcium, iron, vitamins and other nutrients. Adenipekun and Oyetunji (2010). Moisture content of the leaves ranged from 8.58-8.96%. The lower the moisture contents of the leaves, the longer the shef life of the leaf meal and the lower the tendency of microrgamism development on the meal. Low ash content recorded for the leaf meal (5.96-6.51%) especially at the younger age, was an indication that more minerals are deposited as the plant increase in age. Crude protein of moringa leaves ranged from 22.13-22.82% for the different ages of growth. However, Ray-Yu Yang (2006), recommended that moringa leave meal could be used to improve nutrition and immune functions.

5.1.3 PHYTOCHEMICALS

Phytochemicals which can also be refer to as bioactive counpounds are non nutritive plant chemicals with ability to instill physiological effects on farm animals. The quantitative result revealed that the leaves contain tannins. Saponins and alkaloids in appreciable amount. The beneficial effects had been reported in the area of improving immune system, blood cholesterol level lowering, bone health and treatments of some certain diseases. Both tannins and alkaloids have also been reported to having the ability to prevent some basic animal and human diseases (Kasolo *et al*., 2010; Elvin-lewis *et al*., 1977 and Reed, 1995).

5.2 BIOCHEMICAL RESPONSE OF WEANLING WISTAR RATS ADMINISTERED CRUDE EXTRACTS OF *Moringa oleifera* (Lam) LEAF

5.2.1 GROWTH PERFORMANCE OF RATS FED ALKALOIDS, SAPONINS, TANNINS EXTRACT OF MORINGA LEAVES

Administrative effect of methanol leaf extracts of moringa on feed consumption is determined by its taste to the animal, the bitter taste of the extracts usually affect the feed intake. From 1.5 mg/mL and above for all extracts, feed intake of rats was depressed. This could suggest a duration dependent effect of alkaloids, Saponins and tannins on feed intake. Oyewo *et al*., (2012[b]) attributed the decreased appetite to aqueous moringa extract administered to high level of saponin it contains. Evers, (2008) reported that high rate of saponins caused significant low dietary nutrient absorption in the gastrointestinal tract. This might have played a part in reduction of intake of the animals. However, it was evident across all phytochemical groups that 1.5 claims that the administrations of the moringa leaf extract lower feed consumption of rats, depending on the level of inclusion (Oyewo *et al*. 2013b).

Therefore, with increase in exposure duration, weight decrease was observed; the rats administered moringa treatments loss weight in line with the level of ingestion,according to Stanek *et al*., (2015) report that rats were sensitive to the bitter taste of alkaloids, resulting in lower body weight gains of rats. On the contrary, Sobotka *et al.,* (2013), reported that rats

response to dietary alkaloid was low, but with non-significant decreased in the growth rate. Similarly, Butler *et al*., (1996); Robbins *et al*., (1996) reported reduction in feed intake in the first two weeks of feeding, which was traced to alkaloid intake. Similar trend was observed in moringa tannin with its effect on weight loss of rats administered 1 mg/mL to 2 mg/mL, with increase in crude moringa tannin administration, the more the weight loss.

Increase weight depression was observed in rats administered crude moringa saponin with increase in length of administration. The result obtained was collaborated by report of Igwilo *et al.,* (2013) that soaked moringa oleifera seed does not support growth of albino rats. Similarly, Oyewo *et al.,* (2013) reported weight increase in rats administered 250mg/kg BW of aqueous moringa extract, and at higher dose (500-1000mg/kg BW) the extract decrease weight gain.The trend obtained indicated crude moringa extract and its phytochemicals possess growth depressive effect on rats with increase in length of administration.

5.2.2 RELATIVE ORGAN WEIGHT OF RATS FED PHYTOCHEMICAL EXTRACT FROM MORINGA LEAVES

Moringa extract and crude moringa alkaloids reduced weight of all organs assessed with increase in dosage. Crude moringa tannin reduced kidney, heart, brain and live weights of rat according to the level of ingestion compare to rats on the control. However, liver weights were not affected by moringa tannin administration. This was contrary to Ezejindu *et al*., (2014) report that relative weights of organs of rats are not influenced by moringa oleifera aqueous extract. Moringa saponin did not influence the kidney, spleen, heart, liver and brain of rat compared to rats on control. However, live weight of rats was depressed according to the level of ingestion. This study was in agreement with Singh *et al*., (2009) that extract of *Moringa oleifera* has significant hepatoprotective activity. This is contrary to Pushpa Latha *et al.* (2011) that *Achyranthes aspera* saponin extract reduced liver and kidney weights in rats fed high fat diets. An indication that saponin has a more potent effect on obese rats. The trend of result shows that moringa saponin is more beneficial on organ development in rats compare to alkaloid and tannin. However, the three had a dose dependent depression on live weights of rats over 15 days of administration. This was

not in line with Ezejindu *et al*. (2014) report that relative weights of organ of rats are not influenced by moringa oleifera aqueous extract.

5.2.3 HAEMATOLOGICAL RESPONSE OF RATS FED PHYTOCHEMICAL EXTRACT OF MORINGA LEAVES

Moringa extract and its alkaloid did not influence hematological indices of rats except eosinophils. However, 1 mg/mL of crude moringa alkaloid apparently enhanced erythrocytic indices of rats compared to other treatments. Crude moringa tannin and saponin did not influence all haematological indices and all values obtain are in line with values as reported by Mitruka and Rawsley (1977). This was contrary to Elekofehinti *et al*., (2012) who reported that oral administration of *Solanum anguivi* fruit saponin reduced red blood cells (RBC), Haemoglobin concentration and WBC counts of *rattus novergicus* (20-100mg/kg) and was dose dependent.

Elekofehinti *et al*., (2012) suggested that the observed red blood cell count reduction could be traced to suppressive effect of saponin from *S. anguivi* on bone marrow. Another probable reason for the observed decrease in RBC count may be due to haemolysis caused failure of J. erythropoietin production mediated via saponin from *Solanum anguivi* fr uits (Elekofehinti *et al.*, (2012). This affirms that the dosage of administered moringa tannin and saponin did not compromise haemopoesis.

The effect of the three moringa phytochemicals were not evident in rats heamatographic parameters over the levels of administration, this suggested that the phytochemicals does not compromised the heamatology of the rats,which was in line with the report of Oyeyemi *et al*., (2015) who reported that saponin extract from *vernonia amygdalina* does not influence the haemogram of male wistar rats. Values obtained in this study suggested that the animals are in normal health status in line with reports of Mitruka and Rawsley (1977). Similar to result obtained in this work is the report of Elekofehinti *et al*., (2012) that the effect of saponin from Solanum anguivi did not affect WBC differential count in Rats. This means that saponin may not be able to protect much against some form of infections at the dosages under investigation. Contrariwise, this work's result contradicted Elekofehinti *et al*. (2012) who reported reduction in

WBC and the neutrophils of rats administered oral saponin from Solanum anguivi, suggested selective and localized toxicity (Ashafa *et al*. 2011).

5.2.4 SERUM BIOCHEMICAL INDICES OF RATS FED PHYTOCHEMICAL EXTRACT FROM MORINGA LEAVES

The liver and kidney contain numerous enzymes, some of which are also present in the serum in very low concentrations. These enzymes which play specific roles in the normal functioning of the organs have no known functions in the serum other than corroborating or indicating damage to the hepatocytes and nephrons. Furthermore, the alterations in the secretory, synthetic, and excretory biomolecules of the liver, such as albumin , bilirubin, globulin as well as the creatinine, urea, uric acid, and serum electrolytes of the kidney can also be used as indicators of impaired organ function or organ dysfunction (Yakubu and Musa, 2012).

Serum total protein of rats administered moringa extracts decreases apparently with increase in dosage. Albumin, albumin- globulin ratio and Serum total protein of rats show similar trend in moringa alkaloids. However, rats administered moringa extract and its alkaloids possess similar serum metabolites as rats on control, this suggests normal protein metabolism and utilization, absence of protein and muscle wastage. Yakubu and Musa (2012) suggests elevated serum albumin coupled with reduction in the level of globulin, a reliable indicator of the synthetic function of the liver.

All serum metabolites assessed were not influenced by moringa extract and its tannin administration to rats. Values obtained are similar to those reported by Duncan and Prasse (1987). Effect on serum protein profile and creatinine by moringa saponins was not significant as compared to the control. However, 1.5 mg/mL of moringa saponin significantly increased serum protein and globulin in rats compared to other dosage. Similarly, Ajibade and Famurewa, (2012) reported significant increase in urea, uric acid, creatinine, plasma protein and blood glucose in rabbits treated with saponin of P. nirurido.

Yahaya *et al*. (2012) reported better serum protein in rats exposed to cement dust and administered moringa extract compared to rats exposed to only cement dust, suggesting prophylactic effect of moringa in rats. Creatinine is formed by non-enzymatic breakdown of

creatinine, and changes in the serum concentration could be the result of renal blood flow, renal function and or urine flow (OECD, 2002; Kataya and Hamza, 2000). The elevated serum concentrations of creatinine, a reliable indicator of impaired glomerular filtration is reported by Yakubu and Musa (2012) in the administration of Senna alata crude alkaloid leaves to pregnant rats. This is contrary to indications from this work. Reports by Ola-Davies *et al*., (2014) in female rats administered aquoues root extract of *Moringa olieferaa*nd Ewuola *et al*., (2012) in rabbits administered *Moringa oliefera* leaf meal reported that serum protein, albumin, globulin, urea and creatinine were not influenced by *Moringa olifera*, supported the report of this work. The liver and kidney contain numerous enzymes, some of which are also present in the serum in very low concentrations. These enzymes which play specific roles in the normal functioning of the organs have no known functions in the serum other than corroborating or indicating damage to the hepatocytes and nephrons. Furthermore, the alterations in the secretory, synthetic, and excretory biomolecules of the liver, such as albumin , bilirubin, globulin as well as the creatinine, urea, uric acid, and serum electrolytes of the kidney can also be used as indicators of impaired organ function or organ dysfunction (Yakubu and Musa, 2012).

5.2.5 EFFECT OF PHYTOCHEMICALS FROM MORINGA ON SERUM ENZYME OF RATS

Alkaline phosphatase is a ubiquitous enzyme localized within the plasma membrane and can be used to measure the integrity of the plasma membrane, whereas GGT, a microsomal enzyme present in the hepatocytes, renal tubules, pancreas, and intestine is also located within the cell membrane where they transport peptides into the cell and across the cell membrane (Gowda *et al.* 2009). Crude moringa extracts increase alkaline phosphatase activity in rats compared to rats on control. Crude moringa alkaloids do not affect serum AST and ALT activity of rats administered 1 mg/mL to 2 mg/mL dosage. Values obtained for rats administered crude moringa alkaloids are similar to those from rats on control. The findings on AST and ALT in this study are similar to report of Yakubu and Musa (2012).Values of serum enzyme obtained for rats administered crude moringa tannin are similar to those from rats on control. Values obtained for AST and ALT of rats administered crude moringa extract were apparently higher than those of rats administered moringa saponin and tannin at similar doses. This suggests that a complex of

the phytochemicals increases liver and kidney enzyme activities. However, rats on control possess similar enzyme activities as rats administered moringa saponin.Similar trend of elevated levels of AST were observed in rats administered soaked moringa (Igwilo *et al*. 2013), which is an indication of myocardial infarction and increased haemolysis, since it is found at higher concentrations in the heart, muscles and erythrocyte (Nelson and Cox, 2008). Oliveira *et al*., (1999) reported that feeding rats with moringa seed meal caused enlargement of the liver and some other vital organs of rats. This is contrary to Igwilo *et al*., (2013) report, hat normal level of ALP, ALT and Bilirubin in rats administered soaked moringa indicated that it poses no threat to liver.

5.2.6 IMMUNE RESPONSE OF RATS FED PHYTOCHEMICAL EXTRACT FROM MORINGA LEAVES

Saponins, which concentrations are high in moringa (Oyewo *et al*., 2013), have modulating effects on the immune system by serving as adjuvant at low concentrations by inducing the production of interleukins and stimulate cell mediated immune system. (Oda *et al*. 2000; Zahid *et al*. 2007).Crude moringa alkaloid significantly enhanced serum zinc compared to crude moringa extract. However, lower dosage of crude moringa alkaloid elicited higher effect on all immune response assessed. Contrary wise moringa extract elicit lower serum immunological values in rats compared to rats on control. This is an indication that alkaloid of moringa possess higher enhancive ability on immunology of rats compared to moringa extracts.

Contrary to result of crude moringa alkaloids, crude moringa tannin did not increase immunology of rat, also evident was increase in values obtained with increase in dosage. Which could suggest higher dosage may be required to improve immunology of rats compared to control. However, least response values were obtained in crude moringa extract administered rats. Similar to result of crude moringa tannins, crude moringa saponins did not increase immunology of rat, also evident was increase in values obtained with increase in dosage which could suggest higher dosage, may be required to improve immunology of rats compared to control. However, values obtained in crude moringa extract administered rats were higher significantly compared to moringa saponin administered rats. The level of alkaloids in aqueous

extract from the leaves of Moringa, *may* suggest that the extract has immunomodulatory activity (Oyewo *et al*. 2013).

In comparison, moringa alkaloid is suggested to be a significant contributor to immunological response observed in moringa oleifera administration. This is inferred from results obtained across the three phytochemicals of moringa in which alkaloid had a significantly improvement on immune response of the rats compared with the other two phytochemical, and crude moringa extract.

5.2.7 EFFECT OF PHYTOCHEMICALS FROM MORINGA LEAF EXTRACTS ON CHOLESTEROL LEVEL OF RATS

Results obtained shown that moringa extract and its crude alkaloids possess hypocholesterolemic effect in rats, with an increase potency with increase in dosage. However, HDL activity was increased with 1 mg/mL dosage of moringa extract and its crude alkaloids. The result of increase in triglyceride in alkaloid treated groups was similar to report of Ibekwe *et al*., (2011) in rats administered Garcina kola alkaloids. In this study, crude moringa tannin did not significantly influence the cholesterol profile of rats compared to rats administered control. Rats administered 1.5 mg/mL of crude moringa tannin had higher cholesterol and HDL profile compared to other tannin treated groups. Crude moringa tannins possess lower efficacy on cholesterol profile of rat compared to alkaloids

Crude moringa saponin reduced significantly cholesterol profile of rats according to the concentration; this demonstrated its hypocholesterolemic potential. Results obtained shown that alkaloids and saponins possess higher hypocholesterolemic than moringa tannins. This could explain the contributing phytochemicals to the cholesterolemic effect of moringa. This is exemplified by their sole potency in reducing cholesterol profile of rats at the dose of administration. Cholesterol would be transported from peripheral tissues to the liver for excretion and this could be the reason for the reported trends in the serum cholesterol concentration in rats administered the saponin, alkaloid and tannin extracts of moringa.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

CONCLUSION

This study revealed variation in chemical profile of *Moringa oleifera* leaf extracts harvested at different ages of growth under Nigeria (Abeokuta), southwest climatic condition. The results from the studies showed that all the stages of growth under examination were good and rich in nutrients and Phytochemicals.Although, the leaves of all the ages have varying percentages of the nutritional composition, it was observed that those leaves at older ages of 20 week contain the highest quantity of phytochemicals .

All the phytochemicals did not impacted any negative effect on the haematological indices of the rats, indicating that these phytochemicals are not toxic at the dosage levels of these studies, revealing that the rats were in good health status.

It was observed that administration of moringa leaf extracts and its constituent phytochemicals did not influence serum enzyme and metabolite negatively. The trend of result obtained indicated that crude moringa extract and its phytochemicals possess growth depressive effect on rats with increase in length and dosage. Moringa extract and its phytochemicals are more beneficial on organ development over a short period of administration. Moringa alkaloids and saponins significantly contributed to improved immune response of the rats, with moringa alkaloids influencing more than moringa saponins. This showed that moringa alkaloids and saponins possess higher hypocholesteroilemic potential than moringa tannins.

RECOMMENDATION

Moringa oleifera leaf extracts can be used as an environmentally friendly feed additive in animal ration, regardless of the age at harvest. The study further revealed *Moringa oleifera* leaf extracts as dense source of nutrients and phytochemicals that could be used as an effective strategy for organic meat, milk and egg production. Methanol leaf extracts of Moringa was

therefore recommended to be included in animal diet as additive up to 2.0 mg/mL in the drinking water to improve performance, reduce cholesterol and improve immune response of farm animals.

However, more research work needs to be conducted on the possibility of using it as an alternative for antibiotics and also to study any possible negative effect of the extracts on the animals, when fed at doses higher than 2.0 mg/mL over a longer period of time.

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