TITLE PAGE

NUTRIENTS AND PHYTOCHEMICAL COMPOSITION OF LESSER - KNOWN VEGETABLES - *PHYLLANTUS NURIRI* AND *MUCUNA PRURIENS* AND THEIR EFFECTS ON IRON AND BETA CAROTENE STATUS OF RATS

BY

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APPROVAL PAGE

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Nomeh Blessing Chinwe, a post graduate student in the Department of Home Science, Nutrition and Dietetics, with registration number PG/MSC/2004/35902, has satisfactorily completed the research work for the degree of MSc in Human Nutrition. The work embodied in this dissertation is original and has not been submitted in part or full for any other diploma or degree of this or any other university.

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DEDICATION

This dissertation is dedicated to God Almighty, the all knowing and all sufficient God.

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ABSTRACT

The study examined the nutrients and phytochemicals composition of some lesser - known vegetables in Nigeria (*Mucuna pruriens* and *Phyllantus nuriri)* and their effects on iron and beta carotene status of rats. Leaf extract was collected from each vegetable. The leaves were separately plucked, sorted and extraneous materials were removed. Then they were washed with deionized water and were pulverized to get the concentrates. Standard methods were used to determine the proximate, some minerals, and vitamins composition as well as phytochemical constituents of the extracts. Animal study was carried out to ascertain the bioavailabihty of these nutrients. Twenty male adult rats were used for the study. They were divided into four groups according to their body weights. The animals were housed individually in metabolic cages. The rats were fed standard rat chow. The extracts were made to provide 0.11 mg/day iron to the rats. The study lasted for twenty eight days. The rat study showed that feeding rats with rat chow supplemented with *Mucuna pruriens* and *Phyllantus nuriri* extract improved the hemoglobin levels. *Mucuna pruriens* contained 11.47% carbohydrates, 3.20% protein, 1.25% ash and 3.82% crude fibre, while *Phyllantus nuriri* contained 15.47% carbohydrate, 3.87% protein, 3.02% ash and 3.20% crude fibre. The vegetables had significant quantities of calcium (23.45mg and 34.71mg), iron (7.45mg and 11.27mg), Vitamin C (26.16mg and 20.13mg/100mg), vitamin E (3.06mg and 3.62mg) and 3 - carotene (3.62 and 3.65mg) per l00g sample. Anti - nutrients and toxicant levels of the vegetables were low. *Mucuna pruriens* had 0.004mg phytate and 0.62mg oxalate while *Phyllantus nuriri* had 1.28mg phytate and 1.31 mg oxalate. Phytochemicals were present in these vegetables. *Mucuna*

pruriens contained 0.003% alkaloids, 0.54% tannins, 7.01% saponins, 3.16% flavonoids, 12.47% cyanogenic glycosides and 1.06% phenols. *Phyllantus nuriri* had 1.68% alkaloids, 0.68% tannins, 3.95% saponins, 6.22% flavonoids, 1.17% cyanogenic glycosides and 0.21% phenols. The hemoglobin level for *Phyllantus nuriri* group was 23.73%, while the *Mucuna pruriens* group was 16.57%. The serum iron, red blood cell count and beta - carotene levels of rats were significantly improved. The recommendations are that the consumption of these vegetables could be useful in addressing some micronutrients and diet - related non – communicable diseases because of their rich and micronutrient and phytochemical constituents. Nutrition educators should use the information from this study to counsel families on the consumption of these lesser - known vegetables. Students should also be assisted to carryout the same study on humans to see the nutritional effect. Organoleptic evaluation of these vegetables should be carried out to evaluate acceptability.

CHAPTER ONE
INTRODUCTION

1.1 Background to the Study

Green leafy vegetables constitute an indispensable constituent of human diet, especially in local delicacies. It is estimated that over 60 species of green leafy vegetables are used as food. Apart from the well-known and easily cultivated green leafy vegetables serving as sources of micronutrients, several wild and ornamental plants are traditionally 'important supplement sources of food nutrients. They are used for various purposes ranging from food for man and animals, to therapeutic and manure for plants. Some of the vegetables are underutilized.

Seasonality partly precipitates micronutrient deficiency, especially during off-season when green leafy vegetables are unavailable. The anti-nutrient content of vegetables, limit the availability of micronutrient present in the vegetables. The issue of micronutrient deficiencies and their consequences on health, learning capability and productivity of affected people constitute a major public health problem globally and is of increasing prominence in Nigeria. The current rate of micronutrient deficiency is 24% among mothers and 48% among pregnant women in Nigeria (FMOH, 2006). There have been several attempts by Nutritionists the world over to proffer adequate solutions to this problem. Part of the efforts is to document the nutrient composition of some green leafy vegetables, which still grow as wild crop or semi-wild crop or unpopular vegetables in

many areas in Nigeria ecosystem, which could contribute to the micronutrient intake of the populace.

Mucuna pruriens leaves and *Phyllanthus nuriri* leaves are unpopular vegetables in Nigeria. *Mucuna* is a leguminous plant (NAS, 1979). A number of species are available in Nigeria for example *M. utilis ("Agbara-orji "), M. Flagellipes-("ukpo'), M Sloanie ("ukpa ').* The leaf of *Mucuna pruriens* is commonly known as "agbara nkita" among the Ibos of Eastern Nigeria. The leaves of *mucuna* are used as medicine or for therapeutic purposes. In the local communities, these leaves are used for the treatment of anaemia (Ezekwesili, 2005).

Phyllanthus nuriri leaf is another wild vegetable, which comes under the botanical family called *Euphorbiaceae*. The most common species of this family in most West African nations are *Phyllanthus amaru* and *Phyllanthus nuriri* popularly called "chancea piedra" in many parts of the world. Among the Ibos of eastern Nigeria it is called "okwonwa". In the local communities, it is used in the treatment of anaemia and malaria (Uzoma, 2004).

1.2 Statement of the Problem

Micronutrient deficiencies and their consequences on health, learning capabilities and productivity of affected people constitute a major problem of public health globally. The problems are of increasing prominence in Nigeria. The deficiencies affect children, pregnant and lactating mothers, and the general populace including adolescents. Green leafy vegetables are rich sources of micronutrients. Little or no information exists in literature for some unpopular, wild or semi-wild vegetables that moderately survive during off seasons. Changes in food habits in Nigeria have greatly affected consumption of local vegetables. Urbanization and globalization have not helped matters either. Many people in different communities do not consume some of the indigenous vegetables due to ignorance of their nutrients composition. Consumption of some vegetables has been known to be associated with poverty.

Based on these observed facts, there is the need to evaluate the nutrients composition of wild, semi-wild and unpopular vegetables used by some communities for therapeutic purposes. The result of the evaluation may shed light to the micronutrients and the possible phytochemicals that are responsible for the therapeutic actions. The vegetables

could be integrated into family meals to address micronutrient problems and their therapeutic uses could be scientifically explored. Since animal food sources of micronutrients are more expensive, there is need to identify those foods that will enhance household food security and improve micronutrient status of the populace. Iron found in vegetables are in a form that is not easily absorbed unless taken with enhancers or processed in a way it will be made available. Biological study on the vegetables with rats will provide evidence – based information on their use for treatment of anaemia.

1.3 General objective of the study

The general objective of this study was to determine the nutrients, anti – nutrients and phytochemicals composition of *Mucuna pruriens* and *Phyllantus nuriri* leaves and their effects on iron and beta carotene status of rats.

1.4 Specific objectives

The specific objectives of the study were to:

1. Determine the levels of some nutrients and anti – nutrients contained in *Mucuna pruriens* and *Phyllanthus nuriri* leaves.
2. Determine the levels of some phytochemicals in *Mucuna pruriens* and *Phyllanthus nuriri* leaves.
3. Determine the effects of the leaf extracts on iron and beta carotene status of rats.

1.5 Significance of the Study

The nutrient composition of the vegetables has not been adequately documented. It is hoped that the result of this study would provide information on the nutrient composition of these vegetables commonly used for therapeutic purposes. The result would serve as baseline information for Nutritionists, Dieticians and Community Health workers to promote the consumption of these lesser-known vegetables if found adequate. The result could also spur interest on other less utilized vegetables that are locally available with a view to using them fill the micronutrient gap that is experience globally.

The study hopefully would generate interest on *Mucuna pruriens* and *Phyllantus nuriri* leaves as wild vegetables that could be consumed during off-season to help address micronutrient deficiency in Nigeria.

The result would provide evidence based information on the use of vegetables for treatment of anaemia.

CHAPTER TWO

LITERATURE REVIEW

2.0 Vegetables

All parts of herbaceous plant eaten as food by human, whole or in part, are normally considered as vegetables (Swedenborg, 2003). Mushrooms though belonging to the biological kingdom fungi are also commonly considered as vegetables. In general, vegetables are savory and not sweet. However there are many exceptions, Nuts, grains, herbs. Spices and culinary fruits are normally not considered vegetables (Swedenborg, 2003). Vegetables can include leaves *(Lettuce),* stems *{asparagus),* roots (carrots), flowers *{broccoli),* bulbs *{garlic),* seeds (peas and beans) and botanical fruits such as *cucumbers, squash, pumpkins,* and *Capsicums* (bell pepper) (Swedenborg, 2003). Botanically, fruits are reproductive organs (ripened ovaries containing one or more seeds) and vegetables are vegetative organs which sustain the plants (Swedenborg, 2003).

In etymology, vegetables are used as a literary term for any plant or vegetable matter from the vegetable kingdom (Swedenborg, 2003).

In the diets, vegetables are eaten in a variety of ways as part of main meals and snacks. The nutrient content of different types of vegetables varies considerably, with the exception of pulses, vegetables contain water soluble vitamins like vitamin B complex and vitamin G, fat-soluble vitamin including vitamin D, vegetables contain carbohydrates, minerals and fibre (Swedenborg, 2003). Vegetables may also contain *antioxidants, antibacterial, anti-fungal, antiviral and anti-carcinogenic* materials. Rotting vegetables (plant matter in general) may have reduced nutrients, but are often full of probiotic bacteria (Anthony and Eko, 1998) as found in fermented vegetables. The green colour of leafy vegetables is due to the presence of the green pigments called chlorophyll and is affected by PH. Some of the acids are released in steam during cooking particularly if cooked in pots without cover (Swedenborg, 2003).

The red/blue colourings of some vegetables (for example red cabbage) are due to *anthocyanins,* which are sensitive to changes in PH. When PH is neutral, the pigments are purple. These pigments are highly soluble in water.

Green leafy vegetables constitute an indispensable constitute of human diet, especially in local delicacies. It is estimated that over 60 species of green leafy plants are used as food (Anthony, 1998). Besides, well-known and easily cultivated green leafy vegetables used as sources of micronutrients, several wild woody plants are traditionally consumed as supplementary sources of micro nutrients for many people (Price, 1997). Most indigenous vegetables are under utilized. A few are popularly used by small groups or people in ever limited geographical area. The disuse endangers the survival of some indigenous species (Anthony *et al.*$_y$ 1998). However, these species of seemingly lower potentials are actually untapped natural resources that when properly cultivated and harnessed will decrease rate of micronutrient deficiency. These species may lead to sustainable production system, provide diversity in diet, supply micronutrients, reduce seasonality in vegetable supply, provide extra income for farmers and prevent the loss of genetic diversity (Price, 1997). Consumption of vegetables will be of immense advantage especially to the elderly, since consumption of vegetables will reduce high incidence of obesity, diabetes, cardiovascular diseases, high blood pressure which are associated with high intake of fatty foods.

2.1 NUTRIENT COMPOSITION OF VEGETABLES

Vegetables contain non-volatile acids, organic acids, mineral salts, volatile sulphuric compounds and tannins which impact flavor in diets (Uwaegbute, 1989). Different types of vegetables contain different nutrients. The carbohydrates in vegetable consist mainly of indigestible fibrous materials such as cellulose, hemicelluloses and lignin. Vegetables contain small quantities of sugars such as glucose, fructose and sucrose. However, the proportion of fibre in vegetables depends on stages of maturity (Uwaegbute 1989). Many vegetables contain a substance known as carotene which is converted to vitamin A. Several green leafy vegetables contain riboflavin, a member of vitamin B complex, (Oguntona, 1998). Several vegetables and green leafy vegetables are good sources of vitamin C, such as tomatoes, spinach and cabbage.

The proximate analysis of leaves of important wild vegetables showed that they are valuable sources of mineral, proteins, fats and oils (Okafor, Okolo and Ejiofor, 1996). Gnetam, Africana (“okazi”), *Gongronema latifolium (“utazi”), peper guineense (“uziza")*

and veronia *amygdalina ("olugbu")* are good sources of minerals sodium, potassium, calcium, manganese and iron. The fat content of G. *Latifolium, G.AJricanum and V. amygdalina* are significant (18.77,14.20, and 4.50%, respectively) (Okafor *et al,* 1996) Generally, vegetables contain non-volatile acids, organic acids, mineral salt and vitamins (Uwaegbute, 1989). The carbohydrate in vegetables consists mainly of indigestible fibrous materials such as cellulose, hemicellulose and lignium.

These are in addition to small quantities of sugars such as glucose, fructose and sucrose.

The proportion of fibre in vegetable depends on the stage of maturity (Uwaegbute, 1989).

Vegetables contain non volatile acids, organic acids, mineral salts, volatile sulphuric compounds and tannins which impart flavour in diets (Uwaegbute, 1989). Different types of vegetables contain different nutrients.

Carbohydrate: Carbohydrates are one of the three calonic-containing nutrients found in foods. The other two nutrients are protein and fats; carbohydrate contains four calories per grain, as does protein. Fat is more calorie-dense. Carbohydrates are metabolized by the body into blood glucose. Carbohydrate in glucose form provides energy to cells in the body, particularly the brain, the only carbohydrate dependent organ in the body (Effiong *et al,* 2009).

Green leafy vegetables are poor sources of energy because of the low dry matter contents of many leaves. This may be one of the reasons much work has not been conducted on components of leafy vegetables.

Protein: Green leafy vegetables have crude protein content that ranges from 1.5 to 1.7%. However, some workers (Aleter and Adeogun 1995) have obtained a mean of 4.2% for seventeen (17) of such vegetables. When dried samples were used, the crude protein content ranged from 15.0 to 30% and the mean is usually around 20% (Aleter and Adeogun, 1995).

The quality of the protein in green leafy vegetables is almost 75% total nitrogen in most vegetables in protein nitrogen. Many reports showed that leafy vegetables 8protein is low in sulphur amino acids (Eka, 1998).

The Recommended Dietary Intake (RDI) for children is 27g of protein (FAO, 1987).

2.2 Mineral Composition

Among the factors that influence minerals composition of green leafy vegetables, soil fertility (or type of fertilizer) is probably the most important. This is perhaps the reason for the wide variation observed in some published work (Eka, 1998). Green leafy vegetables provide some health benefits as dietary components because they contain significant quantities of minerals like (Zn, Fe, Ca, Mg, P) (Nnam *et al,* 2009).

Zinc is important for the formation of bone tissues, healing of wounds and sores and production of proteins. It protects the skin and improves resistance to infectious diseases, inflammations and allergies (Nnam *et al,* 2009). The range observed for some indigenous vegetables were from 0.80 to 2.05mg and 3.00 and 23.00mg/100g respectively (Okafor *et al*, 1998) (Nnam and Ngwa, 2010). Lippard and Berg (1994) stated that zinc is a trace mineral element because they play a catalytic role in enzymes and the RDA < 200mg/day.

Mineral are known to play important metabolic and physiologic roles in the living system (Enechi and Odonwodo, 2003). Iron, zinc, selenium and manganese strengthen the immune system as antioxidants. Similarly, magnesium, zinc and selenium are known to prevent cardionyopathy, muscle degeneration, growth retardation, alopecia, dermatitis, immunologic dysfunction, gonadal malformations and bleeding disorder (Chaturvedi *et al,* 2004).

The highly soluble minerals calcium, magnesium, phosphorus, iron and potassium help in the maintenance of acid base balance of the hydrogen ion concentration of the body tissues. They help complete the absorption of vitamins, proteins, fats and carbohydrates in food (Islam *et al,* 2004). Iron and calcium furnish all cells and tissues of the body with the elements and nutritional enzymes which they need. Calcium is required for bone and teeth formation and in the proper functioning of the nervous system (Olaiya *et al,* 2010). Calcium is a common electrolyte and also structural component for muscle and digestive systems. It builds bones, neutralizes acidity, clears toxins and helps blood stream (Nelson and Cox, 2000).

Maundu (2006) and Nnamani, Osebele and Agbatutu (2009), reported 47.00 to 442.00mg/100g and 54.06 to 90.10mg/100g values respectively for some underutilized

indigenous vegetables. The RDI for calcium is llOmg for pregnant and lactating women and 450mg for adults (FAO, 2002).

Magnesium and potassium are needed for the acid-base and electrolyte balance in the body. They are therefore good for hypertension patients since magnesium is useful in the reduction of blood pressure. Magnesium is an obligate co-factor for DNA synthesis and the proportion supplied by vegetables can be used to supplement low magnesium based staple foods such as cassava in Nigeria (Olaiya *et al,* 2010). Okafor *et al* (1996) and Nnamani *et al* (2009) reported 0.04 to 8.20mg/100g and 3.00 to 57.00mg/100g respectively on underutilized indigenous vegetables. Green leafy vegetables are also good sources of potassium which is required in muscle and nerve function. Taiye and Asiebey - Berko (2001) and Nnamani *et al* (2009) reported these values 214.42 - 591.00mg/100g and 37.00 - 39.'00mg/100g, respectively for some green leafy vegetables.

Sodium is a very common electrolyte not generally found in dietary supplements despite being needed in greater quantities, with < 200mg/day (Nelson and Cox, 2000). Nnamani (2000) and Taiye *et al* (2001) reported values for sodium, for some underutilized vegetables 7.00 - 21.00mg/100g and 6.44 - 21.82 mg/l00g, respectively. The ion is very common in food, typically as sodium chloride or common salt.

2.2.1. Vitamins

The name 'vitamin' has given to a group of food substances other than fat, proteins, carbohydrates and salts that occur in small quantities in natural food materials. They are essential for growth, for reproduction and for the maintenance of health. Green leafy vegetables and other yellow leafy vegetable are important sources of vitamin A. These leafy green and yellow vegetables contribute about 33 percent of the vitamin A supplied by the major food crops. They supply also about 25% of ascorbic acid and appreciable quantity of other vitamins. The concentration of vitamin A and C will contribute significantly to the daily requirement (Ujowundu *et al,* 2010). The RDA for vitamin A is 400 — 600 p.g (FAO, 2002). Vitamin C maintains blood vessel flexibility and improves circulation in the arteries of smokers (Okudu, 2007). The RDA for vitamin C is 20mg for children 1 - 1 2 years and 30mg for others (FAO, 2002). The most important benefit claimed for vitamins A and C is their role as antioxidants in which they scavenge oxygen

free. These chemically active particles are by-products of many of the body's normal chemical processes. Their numbers are increased by environmental assaults such as smoking chemicals, toxins and stress (Okudu, 2007).

Vitamin E is a powerful antioxidant. It contributes to a healthy circulatory system and improves wound healing carotenoid is essential for maintaining normal vision, regulation of gene expression and immunity and growth and development. It also helps in red blood cell production (Aaron, 2009). The RDI for vitamin E for adult men, women and pregnant women should be about 22 IU per day. Breastfeeding mothers need 28 IU per day (Aaron 2009).

2.3. Nutrient Composition of Green Leafy Vegetables

Green leafy vegetables are good sources of minerals in addition to vitamins and dietary fibre (Uwaegbute, 1989). It is pertinent to note however, that these values vary according to geographical location, age and species of vegetable (Uwaegbute , 1989). Barmunas *et al.* (1998) studied six non-conventional leafy vegetables consumed largely by the rural populace of Nigeria. They reported that *aranthus spp* and *Adansaum digitata* leaves contained the highest level of iron (38.4mg/100g and 30.6mg/100mg, respectively). Nnamani *et al* (2009) reported 4.17 and 11.04mg/100g and Nnam (2010) also reported 0.07 to 8.90mg/100g for some underutilized vegetables. Meanwhile, the RDA for Iron is 12.00mg per day.

Chinma and Lgyyor (2006) in their study on micronutrients and antinutrient contents of selected tropical vegetables grown in South East, Nigeria. These include "*oha " (Pterocarpus soyaumii), "ukazi" (Gnentum ofericanum). "Nchuarmu*" *(ocimum viride) "utabanzi"(gongronema ratifolia) "uziza" (peper guineses).* The result showed that vitamin C composition varied from 14.61 to 30-84mg and calcium and magnesium content of these vegetables ranges from 204.26 to 305.5 lmg, and 195.57 to 304.39mg, respectively.

Talinum tringullara, cor chorus olitorius and *piper guineense* have crude protein on 20.59 to 38.18%, fat from 5.90 to 12.73%, fibre from 6.20 to 7.20% and ash from 8.00 to 25.49%, respectively. Sodium ranges from 0.64 to 8.97g/100g and potassium from 1.34 to 3.34g/100g with the most abundant minerals (Akindahusi *et al.,* 2006) *Solarium*

macrocarpon (eggplant) leaves is also a good source of zinc (93.4 to 5.7mg/kg) and calcium (12.0 to 18.2mg/kg) depending on the different conventional food processing techniques they are subjected to (soaking, blanching, abrasion with or without salt). (Akindahusi *et al,* 2006).

According to Olaiya, and Adebisi (2010) the carbohydrate contents of the vegetables are relatively higher in comparison with their lipid contents. The least carbohydrate of 4.0g/100g was observed in *C.argentea* while the highest value of 6.2g/100g was observed in *B.alba.* The protein contents of the vegetables were between 2.1g/100g (in *B.alba)* and 6.4g/100g (in *T.occidentalis).* All the vegetables had low fat contents with the least value of 0.0lg/l00g in *C.argentea* and highest value of 1.80g/100g in *A.spinosus.*

The moisture content of the leafy vegetables studied are relatively high ranging from 75.0% in *S.aethiopicum* to 91.5% in *T.traingulare.* The high moisture content of vegetables makes them to aid the digestion of food (Olaiya *et ah,* 2010).

In another work on proximate composition of underutilized green leafy vegetables in southern Karnataka namely: *Brassica aleracea, Amaranthus sp, kadakesa,* the proximate compositions of the vegetables were stated as shown in Table 2.1.

Table 2.1: Proximate Composition of *Brassica aleracea* and *Amaranthus sp*

Botanical name	Moisture (%)	Protein (gm)	Fat (gm)	CI+O (gm)	Iron (mg)	Ca (mg)	Ascorbic acid (mg)	Oxalic Acid (mg)
Brassica aleracea	85	3.5	0.4	6.4	13.30	740	157	16.87
Amaranthus sp	93	3.2	0.3	1.3	21.03	305	30	30.28
L.N Kadakesa	75	1.8	0.9	15.2	16.55	280	3	51.55
L.N Yelsun soppu	16	2.2	2.1	11.6	18.34	187	16	36.83
Psidium guagara	80	0.8	1.2	3.9	13.12	75	14	23.46
Hibiscus sp	97	1.7	1.3	6.3	3.68	274	57	88.21
Basella sp	93	3.3	1.9	0.4	5.45	187	15	60.84
Amaranthus viridis	91	2.0	0.9	2.2	18.16	188	17	56.37

Source: K.. Sheela, Kamal Ci. Nath, L). Vijayalakshimi, Ueeta M. Yamkanchi and Roopa B. Patil (2004)

The green leafy vegetables are good sources of minerals. The highly soluble minerals calcium (ca), magnesium (mg), phosphorus (p), iron (fe), and potassium (K) help in the maintenance of acid base balance of the hydrogen ion concentration of the body tissues. They help complete the absorption of vitamins, proteins, fats and carbohydrate (Islam *et al.,* 2004) calcium and iron furnish all the cells and tissues of the body with the elements and the nutritional enzymes which they need. The higher calcium contents of *C.pepo* 94.20mg/100g) and *SModijlorum* (5.60mg/100g) suggests that they would be more advantageous to the body in the functions associated with the minerals (Olaiya *et al*., 2010) calcium is required for bone and teeth formation and in proper functioning of the nervous system.

According to Ujowundu *et al.* (2010) the nutrient composition of *combretum Zenkeri* leaves show.

Table 2.2: Proximate composition of *C. Zenkeri* leaf (%)

Nutrients	% Composition
Moisture	11.3250 ±1.32
Ash	0.1386 ±0.03
Crude protein	20.5398 ±1.15
Crude fat	2.2700 ± 0.73
Crude fibre	47.6300 ±1.27
Total Carbohydrate	65.7266±1.27

Source: Ujonwundu, C. O; Okafor, O. E; Agha, N. C; Nwaogu, L. A; Igwe, K. O and Igwe, C. U (2010)

Values are mean + standard deviation of triplicate determinations.

Ujowundu *et al* (2010) reported that C. *Zenkeri* leaf is also a rich source of minerals and vitamins especially vitamin C and A. the table show vitamins and mineral reported in (Mg/l00g).

Table 2.3: Mineral and Vitamin Composition of *C. zenkeri*

Nutrients	% Composition
Calcium	11.4774 ±3.1329
Phosphorous	0.6208 ± 0.274
Magnesium	0.3875 ±0.953
Manganese	0.0473 ±0.016
Iron	0.00834 ± 0.06
Zinc	0.0076 ± 0.007
Selenium	0.2752 ± 0.086
Potassium	17.5000 ± 1.414
Sodium	2.000 ±1.414 '
Vitamin A	9.51 ±0.11
Vitamin C	28.482 ±0.85

Source: Ujonwundu, C. O; Okafor, O. E; Agha, N. C; Nwaogu, L. A; Igwe, K. O and Igwe, C. U (2010)

2.4 Nutrient Composition of *Mucuna Pruriens*

The proximate composition of *Mucuna pruriens* leaf on a dry weight basis, are as follows: protein 15.1%, fat 2.1%, fibre 19.3% and ash 14.9%. Digestible protein is 10.7%, digestible carbohydrate is 49.6%.

2.5 Nutrient Composition of *Phyllantus Nuriri*

The extract of *Phyllantus nuriri* contains carbohydrate 45.52%, protein. 6.10%, moisture 11.05%, ash 6.80%, fat 6.03%, Saponin 24.05%, Tannin 17.50%, Oxalate 5.47% and fibre 24.50% (Ujuwondu *et al,* 2010).

2.6 Phytochemical Content of Vegetables

Vegetables are referred to as functional foods because of the phytochemicals they contain. This term indicates that the food provides health benefits beyond what is supplied by the traditional nutrients (Wardlaw and Kessel, 2002). Many nutritionists

believe that the integration of micronutrient rich foods into the diets is the only sustainable way to improve micronutrients status in human body (Ali and Teoy,
1997). Phytochemicals are non-nutritive chemical substances in plants that provide protection against the development of diseases and health related problems. They protect the organs, act as antioxidant and reduce the cell damage effect of free radicals, protect against cancer, diabetes and heart diseases (Nnam *et al,* 2009).

Among edible plants with health promoting phytochemicals, *Brassica* vegetable (broccoli, cauliflower, cabbage, kale, bmsselle sproutes) are currently used for the treatment of respiratory *papillomatosis tumours* caused by the human *papilIonia* virus due to the presence of the phytochemical-Di indolymethane. Sometimes, some of the compounds in plants with potent medical properties may not necessarily be chemicals but elements such as selenium found abundantly in *brassica* vegetables with potent anti-viral and anti-cancer properties (Uzoma, 2004). Selenium supplementation has been shown to reduce the HIV viral load and is currently recommended world wide by physicians as adjuvant nutritional supplement to AIDS treatment. It has also been shown to reduce mortality among prostrate cancer patients (Uzoma, 2004).

Some phytochemicals present in vegetables are anthocyanins which reduce the coagulation of blood platelets, inhibit formation of blood clots involved in stroke, pulmonary embolism, peripheral vascular disease and heart attack. Anthocyanins promote higher levels of "good" cholesterol, HDL and inhibit oxidation of "bad" cholesterol, LDL. Some phytochemicals neutralize oxygen radicals and down-regulate enzymes, lead to inflammatory reactions that cause pain and stimulate other diseases (Erdman *et al,* 2007). Anthocyanins mostly found in fruits such as blue and black berries and red raspberries contain several hundred mg of active chemicals per l00g serving. Most of the intake is degraded in the digestive tract after within hours of meal (Prior *et al.,* 2007). Current research shows that strawberry leaf is an excellent source of anthocyanins (Gross, 2007). Some phytochemicals inhibit the pathways of the release of antagonistic or destructive by-products. The phytochemicals include indoles, isothiocyanates and dithiolthiones which block various hormone actions and metabolic pathways that are associated with the development of cancer, Indole-3 - carbinol induce enzymes known to prevent estrogen action and thereby reduce the risk of breast and

uterine cancers, Sulforaphane a phytochemical found in broccoli and watermelon is potential for treatment of cancer.

Other phytochemicals are flavonoids (quercetin) found in some vegetables. They extend vitamin C activity when they act as antioxidants. Carotenoids are plant pigments found in bright yellow, orange and red fruits and vegetables. They are responsible for the pink colour of flamingoes and shellfish and the egg yolk. There are more than 600 naturally occurring carotenoids divided into two distinct types: Carotenes and xanthophylls. Carotenoids are generally well-known as vitamin A precursors, meaning that once ingested in the body, it is converted to vitamin A. However, less than 10% carotenoids function in this way. In the carotene group only alpha, beta and epsilon carotene function as vitamin A precursors. Beta carotene is the most active of the three. Xanthophylls protect vitamin A. The vitamin A precursors as well as other carotenoids such as gamma carotene, lycopene and lutein have been shown to be protective against lung, breast, uterine and prostrate cancers. Zeaxanthin and lutein may prevent loss of sight in persons over fifty years (Nyam News, 2005). Another xanthophylls (cryptoxanthin) commonly found in mangoes are thought to have a protective effect on the female reproductive tissues namely virginal, uterine and cervical tissues. There are ranges of other phytochemicaLs that have been found to boost cancer fighting enzymes (e.g. organic sulphur compounds); promote growth of beneficial bacteria (e.g. organic acids and polysaccharides) and assist in blood clotting (e.g., phytosterols and fatty acids) (Nyam News, 2005).

Phytosterols are plant sterols that occur in most plant species but appear to be most abundant in seeds of green and yellow vegetables. They are important in human diet because they reduce the amount of dietary cholesterol absorbed by the body by blocking uptake in the intestine. They also facilitate cholesterol excretion from the body. This is, especially important as cholesterol is a well established risk factor for heart disease (Nyam News, 2005).

Alkaloids are the most efficient therapeutically significant plant substance. Pure isolated alkaloids and synthetic derivatives are used as the basic medicinal agent. This is because of their analgesic antispasmodic and bacterial properties (Stray, 1998). Saponins constituents are responsible for the possession of hemolytic property. Plants with high

content of saponin have cytoloxic effect such as permealization of intestine. It gives the plant leaves the bitter taste. Saponin has relationship with sex hormones like oxytocin (Okwu and Ekeke, 2003). Vegetables contain some pharmaceutical components with medicinal value. The problem is that many of the phytochemicals are destroyed or removed by modem food processing techniques, including cooking. For this reason, it is believed that industrially processed foods are less beneficial (contain less phytochemical) than unprocessed foods. Vegetables contain flavonoids such as quercetin, astrsigin, quescitrin, isoquuercitrin and rutin (Uzoma, 2004). According to Uzoma (2004) these flavonoids act as anti-oxidants to clean up free radicals. They protect the cells against the breakdown of arachidonic acid, an unsaturated fatty acid that keeps cell membrane healthy and permeable. Flavonoids lower high blood pressure as well as cholesterol in animal studies and have strong anti - inflammatory properties. Flavonoids are potent anti - oxidants, anti - carcinogenic binding toxic materials and escort them out of the body. They also inhibit low density lipoprotein (LDL) oxidation by free radicals (Verena, Mario, and Karl, 2006).

Quercetin and other flavone glycosides (a type of flavonoids) found in *Phyllanthus nuriri* leaf are suggested to improve blood circulation and prevent formation of clots in blood vessels. They increase the strength of capillary walls, reduce inflammation and prevent blood cells and proteins from seeping into the tissues (Uzoma, 2004). Terpene lactones found in *Chanca piedra* include cymene, lupeol acetate and limonene. They bring oxygen to the tissues and enhance the absorption of glucose by the body. Terpene lactones also protect nerve cells from damage during even brief periods of oxygen deprivation that precipitates strokes. These chemical constituents appear to be responsible for the ability of *Chanca piedra* leaf to improve mental function, alertness and promotion of recovery from stroke (Uzoma, 2004).

Some vegetables have L-Dopa and bioactive alkaloids mucunine, mucunadine, prurienine, glutathione, lecithing nemolic and gellic acids. L-Dopa is a neurotransmitter precursor, an effective drug for relief in Parkinson disease. The main plant chemicals found in *Mucuna* include alkaloids, alkylamines, arachidonic acid, beta carboline, Beta-sito serol, crystine, linolenic acid, dopamine, these are important brain chemical involved in mood, sexuality and movement. *Mucuna pruriens* supplements influence dopamine

content in the cortex of the brain *Mucuna pruriens* has antioxidant properties. There is also presence of lysine mannose, fatty acid, serine, serotonin, phenylalanine, saponins and serine (Sathyanarayanan and Aruthmozhi, 2007). Some of the health benefits of phytochemicals show that these phytochemicals either alone or in combination with others, are powerful tools to combat various ailments especially cancer. Phytochemicals include saponins, alkaloids, tamins, deoxy sugar, cardia glycoside, anthroquqinone, terpene; flavonoid and carotenoid. These phytochemicals reduce the risk of certain types of cancer and coronary heart diseases, improve lung function and reduce complications associated with diabetes (Nnam, Udofia and Atasit, 2009). Tannins have antioxidant effects which help in cancer prevention. The safe level for tannins is 0.20% (as recommended by Schiavone, Guo and Tassone, 2008). Saponins lower cholesterol levels due to the hypocholesterolenic effects of saponins (Nnam *et al,* 2009).

Other studies (Onyeka and Nwanbekwe, 2007) showed that other common green leafy vegetable had varied phytochemical content. The phytochemical content of raw vegetables was higher than the cooked. In their study on eight common green leafy vegetable in the raw and cooked forms as natural source of phytochemical revealed these green leafy vegetable content alkaloid, steroid, tannin, anthocyanin, carotenoids and flavonoid.

The phytochemical content of the vegetables varied significantly ($p = 0.05$) among the green leafy vegetable. "Onugbu" (*Vernoria Amygdalnd)* had the highest steroid content of 0.27g/100g while "nchuanwu" (*Ocimum viridd)* had the lowest (0.07g/100g). "Oha" (*Pheherocapus soyallxii)* followed by "ahihara" *(cochorus oliforus*) was the most enriched with respect to the tested phyto chemical (Onyeka *et al,* 2007).

Differences in phytochemical content between raw and cooked green leafy vegetables were not significant ($p = 0.05$) with the exception of flavonoid and alkaloids. Generally the green leafy vegetable showed a low content of anthocyanin and carotenoids while alkaloid was most abundant in them. Alkaloid content of the green leafy vegetable in raw and cooked form, some were 1.28,2.96g/100g and 0.30- 0.84g/100 respectively (Onyeka *et al,* 2007).

2.7. Antinutrients

Anti - nutrients are natural or synthetic compounds that interfere with the absorption of nutrients. One common example is phytate which forms insoluble complexes with calcium, zinc, iron and copper. Proteins can also be anti - nutrients such as the trypsin inhibitors and lectins found in legumes (Sarkiyayi and Agar, 2010).

These are chemical components in foods that affect the consumption, digestion, absorption and utilization of specific nutrients in the body. They can be classified as non-fibrous natural substance with negative effect on growth and health of man and livestock. It is important to note that these substances give the plants a natural protection against attacks from animals, moulds, bacteria, insects and birds (Huisman and Toiman, 1999). The anti-nutrients found in vegetables include phytic acid or phytates, polyphenols and tannins. Several studies indicate that the easiest treatment is heat application because most of them are heat labile. Wilting (spreading vegetables under the sun for 30 - 60 minutes) proved to imprpve the palatability of most tropical foods by diluting the effect of certain astringent components of vegetables (Smith and Van Hautert, 1987). Tannins are high molecular weight polyphenolic compound. They interfere with protein digestibility by either inactivating the digestive enzymes or reducing the susceptibility of the substrate protein (Sarkiyayi *et al* ,2010).

Some common anti-nutrients in plants are cyanogenic glycosides found in sweet potatoes and lima beans. These cause gastrointestinal inflammation and inhibition of cellular respiration in humans. Gossypol in cotton seed reduces iron uptake. Phenols are mostly found in fruits, vegetables and cereals. Thiamin raises cholesterol and estrogen-mimic while oxalate in spinach and tomato reduces solubility of calcium, iron and zinc (Geo - Pie Project, 2002).

The anti-nutrient composition of some vegetables grown in South Eastern Nigeria ranges are phytic acid 34.70 to 68.50mg/100g, tannins 0.32 to 0.83g/100g and oxalic acid 24.65 to 42.15mg/g, respectively. These include "oha" *(pherocarpus soyauxii),* "ukazi" (*gnetum ofericannum),* "nchuanwu'[7] (*ocimum vinda*), "utabanzi" *(gongronema rat if olid),* "uziza" *(piper guinenses), and* "inene" (*amaranthus spinosus).* (Chinma and Igyor, 2006). Ukachukwu *et al.* (1997) reported that *Mucuna pruriens* contains tannins 5.54mg/g. There is also some food toxicants found in green leafy vegetables. Food toxicants are

chemical components in food substances that affect the body systems such that normal homeostasis may become distorted leading to disease and even death. The examples include cyanides, especially hydrocyanic acids (HCN) in cassava., heamagglutinin, in soyabeans, trypsin inhibitor, goitrogens, oxalates and urease. On the other hand, anti-nutrients affect the metabolism of nutrients. In all these cases, the bio-availability of the nutrient and general well being of the body is affected. Most foods undergo one processing or the other to improve on organoleptic and nutrient qualities. The impact of the effect of these anti-nutrients is seen more in browse vegetables because they affect livestock negatively (Eagle *et al.,* 2000). Phytic acid and oxalic acid usually forms insoluble salts with mineral element such as zinc, calcium and iron to prevent their utilization. The low level of these anti - nutrients would therefore permit the absorption of these elements, which form complexes with them (Sarkiyayi *et al*, 2010). The safe level for phytates and oxalates are 35mg/100g and 2.20mg/100g, respectively (Munro and Bassir, 1969).

Anti-nutrient levels in some green vegetables are the example of cyanide found in eggplant (2.0mg/kg) and *Mucuna pruriens* (40.0mg/kg), respectively. In Piper *guineese "uziza"* the hydrocyanic acid is (85.9mg/100mg). The *glucosinolates* in piper *guineese* is (0.20mg/100g). The *oxalic* acid in six green leafy vegetables *pterocarpus soyauzii, gnetum afericanum, ocinum virde, Gongronema ratifolia, piper guinenses* and *Amaranthus spinosits* ranges from 34.70 to 68.50mg/100g.

2.8. Agronomy and Characteristic Features of *Phyllanthus Nuriri*

Phyllcintus nuriri is found in many countries of the world. This herb tends to grow widely and can be found in the following habitats, bushes, river banks, abandon fields and forest edges (Uzoma, 2004).

2.8.1 Plant Description

Phyllanthus nuriri is a common annual plant that does not grow more that 30-60cm tall. The name *phyllanthus* reflect the unique arrangement of flowers on the leaves. In Greek "*phyllon* refers to a leaf and "*anthos* " to flowers, thus describing the flowers hanging from the leaves.

Stem: The plant is erect, slender and profusely branched. It is brownish at the base but greenish from the middle to upper parts.

Taproot: The taproot is slender, hairy with lateral branches that are evenly distributed. Leaves: The leaves are small, simple, numerous, green, oblong and feathered. Sometimes, there are about 15-30 leaves arranged alternatively in two rows along 5-10mm long, 3 -4mm wide. They are oblong to asymmetrically ovate or elliptic pale green above with clearly visible veins below. When the plant is picked, the leaves fold in, completely closing themselves as though taking position (Uzoma, 2004).

2.8.2 Phenols

Phenolic acids are plant metabolites widely spread throughout the plant kingdom. Recent interest in phenolic acids stems from their potential protective role, through ingestion of fruits and vegetables, against oxidative damage diseases (coronary heart disease, stroke, and cancers). Phenolic compounds are essential for the growth and reproduction of plants, and are produced as a response for defending injured plants against pathogens. The importance of antioxidant activities of phenolic compounds and their possible usage in processed foods as a natural antioxidant have reached a new height in recent years (Ray Sahelian, 2006).

Phenolic acid compounds seem to be universally distributed in plants. They have been subjects of a great number of chemical, biological agricultural and medical studies. Phenolic acids form a diverse group that includes the widely distributed hydroxybenzoic and hydroxycinnamic acids. Hydroxycinnamic acid compounds occur most frequently as simple esters with hydroxyl carboxylic acids or glucose. Hydroxybenzoic acid compounds are present mainly in the form of glucosides. (Ray Sahelian, 2006).

Phenolic acids in coffee induce an increase in the resistance of LDL cholesterol to oxidative changes, probably as a result of the incorporation of phenolic acids found in coffee into LDL cholesterol. Phenolic acids are found in berries, spices and herbs. Other natural substances with phenolic acids compounds are cannabinoids, the active constituents of cannabis, cresols from coal tar and creosote. Guaiacol (2- methoxyphenol) from roasted coffee, whisky; and smoke that has a smoky flavour salicylic acid, a plant

hormone and analgesic, and inflammatory drug, precursor compound to Aspirin. Aspirin (acetylicylic acid) is still the most commonly used salicylate (Ray Sahelian, 2006).

2.9 Agronomy/Characteristic Features of *Mucuna Pruriens*

Mucuna is a herbaceous annual or perennial vine, usually a vigorous climber, deep rooted and has high agronomic potential in terms of yield (Chilaka and Ukachukwu, 1995). *Mucuna* species resist most pests and diseases and some strains of nematodes common to legumes (Skerman *et al,* 1988). The green forage yield is also high and ranges between 11 and 19 tones per hectare (Chilaka and Ukachukwu, 1995).

Mucuna is exceptionally promising in sustaining agricultural yield in extreme heat, humidity and heavy rainfall of the tropics. The bean is tolerant to drought conditions and show considerable promise on dry land famine (Kay, 1979). It tolerates a wide range of soils from sandy to clays and grows on soils of appreciable acidity. It thrives in altitudes of between sea level and 2100m (NAS, 1979; Skerman *et al,* 1988).

2.9.1. Current Status and Use *of Mucuna Pruriens*

Most *Mucuna pruriens* species still grow as wild crop in many areas of Nigeria. In other places, they thrive as semi-wild or protected wild crop. In a few localities, however, the rural populace cultivates them (Ukachukwu and Obioha, 1997). *Mucuna* serves as green manure and as cover crop for erosion control and weed suppression (Buckles *et al,* 1998). Some research institutions like International Institute for Tropical Agriculture (IITA), Ibadan and National Root Crops Research Institute (NRCRI) Umudike are actively involved in the seed multiplication. This is mainly in relation to its utilization in weed management and as cover crop (Maunyong *et al,* 1999).

Mucuna pruriens leaves are claimed to be good remedies for anaemia. (Ezekwesili, 2006). Anemia is a haematological disorder characterized by a reduction in the number of circulating red blood cells (Allan, 2002). Anaemia is the most common disease affecting mankind, particularly in African. It may be caused by a decreased production of red cell or increase destruction of red cell. Anaemia is a leading cause of increased maternal and infant mortality in developing countries (Ezekwesili, 2006). Anaemia is usually associated with decrease in the packed cell volume (PCV), hemoglobin concentration and

total count. Estimation of the level of these hematological parameters gives an insight into the severity of the malady (INACG, 1995).

Mucuna pruriens leaves are mostly in abandoned farmlands in the tropics. In the southern part of Nigeria, water extract of the leaves is administered as in herbal tea form as blood tonic. Other biological actions of *Mucuna pruriens* leaves documented include central system effect (Mahagans, 1996; 2006) and *hypoglycaemic* effect (Akinkumi, 2006).

2.10. Induction of Anaemia

It is an established fact that a rabbit has between 57.7mls and 70mls of blood per kilogram body weight. Also a loss of about one - third of the total blood volume of an animal is known to precipitate anaemia (Santhrani, Saraswathy and Maheswari, 2011**).**

2.10.1 Micronutrient Malnutrition Approach

Micronutrient malnutrition constitutes the most wide-spread form of malnutrition in the world (FAO, 1996). Such deficiency affects both the rich and the poor, with women and children being mostly affected. More than one billion people in the world suffer from various nutrient deficiencies. Vitamin A, iron and iodine deficiency causes serous health problem including anaemia, blindness, mental retardation and reduced resistance to infectious diseases that cause death. These are currently receiving public health attention. These dietary deficiencies were weakening individual households, community and nation, causing loss of human potential and productivity. Unlike the impediment of social and economic development, these deficiencies could be reduced with relatively small investments. The technology is available to address many of them but they still persist for a variety of reasons including insufficient awareness.

2.10.1.1. Vitamin A Deficiency (VAD)

Vitamin A deficiency (VAD) is the most serious childhood nutritional diseases and is usually accompanied with protein - energy malnutrition (PEM). Vitamin A deficiency causes night blindness and may cause total blindness and increase the risk of infection and death. Each year, an estimation of 300,000 children in developing countries lose their sights because of vitamin A deficiency and two - thirds of these children are at risk of

dying. VAD does not only affect children but also adults, pregnant and lactating mothers are at particular risk (McGuire, 1993). McGuire further explained that VAD manifests when a child or adult does not consume enough vitamin A, so when the diet is low in fat only small amount of vitamin A are absorbed. Vitamin A deficiency is aggravated by health problems such as measles and diarrhea, which also augment vitamin A needs. Vitamin A deficiency is particularly common in areas of rainfall with distinct wet and dry season, and where the availability and regular consumption of green vegetables and yellow and orange - coloured fruits are seasonal. Its presence in a community may, sometimes be confirmed by combination of low consumption of food rich in vitamin A. The best way to prevent VAD is to encourage families to cultivate and consume foods that are rich in vitamin A all year round. The foods include use of dark green leafy vegetable and yellow or orange - coloured fruits as well as the wide range of individual leafy vegetables. Green vegetables are good sources of micronutrients.

McGuire (1993) reported that there is accumulating evidence that VAD increases risk of developing respiration disease and the children who are vitamin A deficient are more likely of developing respiration diseases and the children who are vitamin A deficient are more likely to suffer from chronic ear infections. Prevention of VAD is particularly important to reduce the incidence and severity of respiratory tract infection of which pneumonia is most serious. Emphasis on prevention of VAD by dietary improvement; fortification and/or supplementation is aimed at ameliorating infectious diseases through effects on immunity and/or epithelial tissue.

2.10.2. Iron

2.10.2.1. The Role of Iron in Human Metabolic Processes

Iron has several vital functions in the body. It serves as a carrier of oxygen to the tissues from the lungs by red blood cell haemoglobin, as a transport medium for electrons within cells, and as an integrated part of importance (Lynch, 2007). Iron also plays a vital role in oxidative metabolism, cellular proliferation and many other physiological processes. It has a key property that allows it to coordinate electron donors and to participate in redox processes. This property also accounts for its potential to cause toxic effects through the generation of free radicals. Furthermore, iron is an essential nutrient for all known

pathogens. Freely available, iron may greatly increase their virulence. It is therefore not surprising that the human body has tightly regulated processes for absorbing, transporting and storing iron (Lynch, 2007). Most of the iron in the body is present in the erythrocytes as haemoglobin, a molecule composed of four units, each containing one heme group and one protein chain. The structure of hemoglobin allows it to be frilly loaded with oxygen in the lungs and partially unloaded in the tissues (e.g. in the muscles). The iron containing oxygen storage protein in the muscles, myoglobin, is similar in structure to hemoglobin but has only one heme unit and one globin chain. Several iron - containing enzymes, the cytochromes, also have one heme group and one protein globin chain. These enzymes act as election carrier within the cell and their structures do not permit reversible loading and unloading of oxygen. Their role in the oxidative metabolism is to transfer energy within the cell and specifically in the mitochondria (Lynch, 2007). Other key functions for the iron - containing enzymes (e.g. cytochrome p450) include the synthesis of steroid hormones and bile acids; detoxification of foreign substances in the liver and signal controlling in some neurotransmitters, like dopamine and serotonine systems in the brain. Iron is reversibly stored within the liver as ferritin and hemosiderin, whereas it is transported within different compartments in the body by the protein transferring.

Approximately 75% of the iron in the body is present in metabolically active compounds. The remaining 25% constitutes a dynamic store that is turned over constantly. This store ensures an adequate supply for normal organ function despite short - term variations in absorption or loss from the body. It also supplies its immediate needs when requirements are increased. The iron reserves that have then been utilized are then gradually replaced by increased absorption. The circulating transferrin pool supplies almost all functional requirements. It contains only about 3mg iron in adults, but ten times as much (± 35mg) moves through the pool each day, roughly 80% destined for red blood cell production. Most of the iron that is transferred from the dynamic store to the circulating transferrin pool comes from iron recovered from the processing of haemoglobin in red cells that have reached the end of their approximately four month life spans. Absorbed iron also enters this pool, but it amounts to only about 1 - 1.5mg a day. The release of iron into the circulation is tightly regulated in concert with requirements. It can be reduced or accelerated several fold. However, the saturation of transferrin with iron (the proportion

of the protein that is carrying iron at any one time) is held at approximately 35% in normal individuals with adequate iron reserves (Lynch, 2007).

2.10.2.2. Iron Absorption

With respect to the mechanism of absorption, there are two kinds of dietary iron: heme iron and non - heme iron. In human diet, the primary sources of heme iron are the hemoglobin and myoglobin from consumption of meat, poultry and fish, whereas, non - heme iron is obtained from cereals, pulses, legumes, fruits and vegetables. The average absorption of heme iron from meat — containing meals is about 25 percent (Hallberg, Hulthen and Gramatkovski, 1997). The absorption of heme iron can vary from about 40 percent during iron deficiency to about 10 percent during iron repletion (Hallberg, 1993). Heme iron can be degraded and converted to non - heme iron if foods are cooked at high temperature for too long. Calcium is the only dietary factor that negatively influences the absorption of heme iron and does so to the same extent that influences non - heme iron (Hallberg, 1993). Non - heme is the main form of dietary iron. The absorption of non heme iron is influenced by individual iron status and by several factors in the diet. If neither the diet nor body stores can supply the iron needed for hemoglobin synthesis, red blood cell synthesis is reduced. Eventually, the number of red blood cells falls so low that the amount of oxygen carried in the blood is decreased. Then a person exhibits anaemia (Wardlaw and Kessel, 2002).

2.10.2.3. Factors Influencing Dietary Iron Absorption

Phytates and Iron absorption: Phytates are found in all kinds of grains, seeds, nuts, vegetables, roots (e.g. potatoes) and fruits. Chemically, phytates are inositol hexaphosphate salts and are a storage form of phosphates and minerals. Other phosphates have not been shown to inhibit non - heme iron absorption. Phosphorus in unrefined cereals, legumes, nuts, seeds and tubers is mostly present as phytic acid (phytates) (Saha, Weaver and Mason, 1994). Since phytate is negatively charged, it complexes with positively charged Fe or Zn ions and in doing so, inhibits the uptake of these minerals (Saha *et al*., 1994). To overcome the “phytate factor”, there are some food processing methods that can be used such as fermentation, sprouting beans, candephytinize food

products, thus improving iron and zinc bioavailablity (Akpapunam and Sate, 1997). Additionally, vitamin c content is greatly increased with sprouting. Vitamin c level increases 17.5 times in germinated lentil and 8.5 times in germinated mung bean. Also l00g of germinated lentils abnd mung beans supply 90 percent and 96 percent of the RDA for iron in adult men and 41 percent and 43 percent of allowance for women (Kavas and Sedefhel, 1991). Sufficient amount of ascorbic acid can counteract this inhibition (Siegenberg, 1991). By contrast, non - phytate containing dietary fibre components have almost no influence on iron absorption. Almost all plants contain phenolic compounds as part of their defence system against insects, animals and humans. Only some of the phenolic compounds (manly those containing galloyl group) seem to be responsible for the inhibition of iron absorption. Tea, coffee and cocoa are common plant products that contain iron - binding polyphenols (Siegenberg, 1991). Many vegetables, especially green leafy vegetables (e.g. spinach) and herbs and spices (e.g. oregano) contain appreciable amounts of galloyl groups that strongly inhibit iron absorption. Consumption of betel leaves common in areas of Asia, also has a marked negative effect on iron absorption. Calcium, consumed as salt or in dairy products, interfere significantly with the absorption of both heme and non - heme iron (Gleerup, 1995). Because calcium and iron are both essential nutrients, calcium cannot be considered to be an inhibitor in the same way as phytates or phenolic compounds. The practical solution for this competition is to increase iron intake, increase its bioavailability or avoid the intake of foods rich in calcium and foods rich in iron at the same meal (Gleerap, 1995).

2.10.2.4. Enhancement of Iron Absorption

Ascorbic acid is the most potent enhancer of non - heme iron absorption (Siegenberg, 1991). Synthetic vitamin c increases the absorption of iron to the extent as the native ascorbic acid in fruits, vegetables, and juices. The effect of the ascorbic acid on iron absorption is so marked and essential that this effect could be considered as one of vitamin c's physiologic roles. Each meal should preferably contain at least 25mg of ascorbic acid and possibly more if the meal contains many inhibitors of iron absorption (Siegenberg, 1991). Therefore, a requirement of ascorbic acid for iron absorption should be taken into account when establishing the requirement for vitamin c, which is set only

to prevent vitamin c deficiency. Organic acids, such as citric acid, have in some studies, been found to enhance the absorption of non - heme iron. This effect is not observed as consistently as is the effect of ascorbic acid. Sawerkraut and other fermented vegetables and even some fermented soy sauces enhance iron absorption (Macfarlene, 1990).

2.10.2.5. Iron Absorption from Meal

The pool concept in iron absorption implies that there are two main pools in the gastrointestinal lumen - one pool of heme iron and another pool of non - heme iron and that iron absorption takes place independently from these two pools. The pool concept also implies that the absorption of iron from the non - heme iron pool results from all ligands present in the mixture of foods included in a meal (FAO, 2002). The absorption of non - heme iron from a certain meal not only depends on its iron content but also, and to a marked degree, on the composition of the meal (i.e. the balance among all factors enhancing and inhibiting the absorption of iron). The bioavailability can vary more than 10 - fold among meals with a similar content of iron, energy, protein, fat, etc (FAO, 2002). Just the addition of certain spices or a cup of tea may reduce the bioavailability by one - half or more. However, the addition of certain vegetables or fruits containing ascorbic acid may double or even triple iron absorption depending on the other properties of the meal and the amounts of ascorbic acid present (FAO, 2002). Hamlin and Latunde (2011) observed that iron and ascorbic acid administered jointly to anaemic mice significantly enhanced hemoglobin regeneration after 10 days. Consequently, the measurement of bioavailable iron will be influenced by the relative amount of enhancers and inhibitors interacting in the lumen of the gastrointestinal tract (Hamlin *et al.,* 2011). They also observed that ferrous sulphate was effective as an oral iron supplement in the treatment of anaemia. Ferrous sulphate is highly bioavailable when compared to other iron salts and is used as a standard.

2.10.2.6. Iron Requirements

Iron is found in almost all foods. Dietary iron intake is therefore related to energy intake. Iron requirements are highest in the second and third trimesters of pregnancy. This need is met utilizing the maternal stores accumulated prior to conception and during the first trimester owing to cessation of menstruation as well as markedly increased absorption during the second and third trimesters. Requirements are also high in young children

particularly between 6 and 18 months of age. Once birth iron reserves are exhausted, infants depend on weaning foods for iron because the iron content of human milk is low. Unfortunately, traditional weaning foods in many developing countries are poor sources of bioavailable iron. Children aged 6 to 18 months are therefore frequently iron deficient. Requirements are increased during the adolescent growth spurt and by the onset of menstruation in girls. Finally, women of childbearing age are at risk for iron deficiency because of their menstrual iron losses. Iron requirements are least in men and postmenopausal women (Lynch, 2007).

2.10.2.7. Iron Deficiency (Anaemia)

Nutritional anaemia remains common in many countries of the world and its eradication through effective interventions must be a priority for attention and action (Mannar, 2007). Iron deficiency anaemia occurs in three different stages. The first, depletion of iron stores, has no functional changes. When iron stores are exhausted, however and tissues begin to have insufficient iron, the resulting condition is iron deficiency. Negative effects amongst those who are iron deficient, but do not have outright anaemia, include cognitive impairment, decreased physical capacity and reduced immunity. Thus there are adverse consequences from iron deficiency even before iron deficiency anaemia is present. The final stage is iron deficiency anaemia which, when severe, can be fatal (Gleason and Scrimshaw, 2007).

Iron deficiency is probably the most frequent nutritional deficiency disorder in the world. A recent estimate based on the World Health Organization (WHO) criteria indicated that around 600 - 700 million people worldwide have a marked iron deficiency anaemia (FAO, 2002). The WHO identifies iron deficiency as being amongst the ten most serious risks in countries with a high adult mortality (Mannar, 2007). In industrialized countries, the prevalence of iron deficiency anaemia is much lower and usually varies between 2 percent and 8 percent. However, the prevalence of iron deficiency, including both anaemia and non - anaemic subjects is much higher in industrialized countries. For example, an absence of iron stores or subnormal serum ferritin values found in about 20 - 30 percent of women of fertile age. In adolescent girls, the prevalence is even higher (FAO, 2002). In developing countries, where the prevalence of iron deficiency is very high and severity of anaemia is marked, studies on the distribution of haemoglobin in

different population groups can provide important information as a valuable basis for action programmes (FAO, 2002). Iron deficiency could be preventing 40% - 60% of children in developing countries from growing to their full mental potential (Mannar, 2007). A more detailed analysis of sub samples may give excellent information for planning of more extensive programmes (FAO, 2002). A subject is considered iron deficient if >z test result were abnormal. Malaria, hookworm infection and nutritional deficiencies besides iron, may cause the anaemia that also affects men (Browman and Russel, 2001). Non - nutritional causes such as genetic abnormalities (e.g. Thalassemia) and chronic diseases also abound. Approximately 30 - 40% of young children and pre - menopausal women in developing countries are affected by iron deficiency (WHO, 1992). Iron deficiency in early childhood has a significant, negative effect on a child's physical and intellectual development. There has been an intensification of efforts in several countries and this gives encouragement that intervention can be successful and sustainable (Mannar, 2007)

Iron deficiency anaemia during pregnancy is prevalent because additional iron is needed to supply the mother's expanding blood volume and to support the needs of the growing foetus and placenta. Thus, during the second half of pregnancy (although pregnant women have been shown to absorb more iron from) even in healthy women, the iron requirement cannot be easily met by diet. Anaemia in pregnancy is associated with increased maternal and child morbidity and mortality and mortality and lower birth weight. Favourable pregnancy outcomes occur 30 - 45% less often in anaemic mothers, and infants of anaemic mothers are less likely to have normal iron reserves - so they start life at a disadvantage (Gleason and Scrimshaw, 2007).

2.10.2.8 Iron Depletion

This occurred when iron stored are reduced as indicated by lowered values of serum ferritin. Iron depletion erythropoesis is when iron stores are exhausted and there is decreased transferring saturation and increased eryhropoiesis. Iron deficiency anaemia (IDA) is characterized by low iron haemoglobin and is usually microcytic type of chronic anaemia. Some of iron deficiency anaemia include feeling tired and weak, decreased work and school performance, slow cognitive and social development during childhood,

difficulty maintaining body temperature and decreased immune function, which increases susceptibility to infection glossitis (an inflamed tongue) (National Academy Press, 2001). Considerable emphasis is placed on reducing iron anaemia in infancy and early childhood because of its association with impaired psychomotor performance as well as changes in behavior. Although some of the developmental deficits can be corrected with iron treatment, newer studies suggest that difference in cognitive and social adaptation remain and are likely to be permanent. The risk of iron deficiency is high during later infancy and young childhood, because the stores received at birth have been used to support normal functions and growth and only about 50% of the iron requirement of a 6 month old can be obtained from breast milk. Thus continued breastfeeding will supply only half the infant's iron needs and for many children, the other half (+4 mg/day) must come from fortified complementary foods or supplementation if anaemia is to be avoided. This further aggravated in infants who were of low birth weight, which is common when the mother is malnourished during pregnancy. Hence the WHO and UNICEF recommendation that low birth - weight infants in populations with high levels of anaemia receive supplementary iron beginning at 2 months and continuing up to 24 months (Gleason and Scrimshaw, 2007).

2.10.2.9 Economic Assessment of Addressing Nutritional Anaemia

The economic gains from addressing any micronutrient deficiency come from both cost reductions and from enhanced productivity. They include lower mortality, reduced healthcare costs, reduced morbidity, improved productivity and intergenerational benefits through improved health.

It is also important to assess the economic impact of interventions in terms of cost and benefits. In making economic assessments, there are a number of important issues that must be considered:

i. There may be more than a single outcome for the intervention (e.g. an intervention in pregnant women may both reduce low birth weights and maternal mortality via changes in maternal haemoglobin)

ii. Some interventions affect not only anaemia but also other health and economic outcomes (e.g. deworming can be effective in both improving haemoglobin levels as well as absorption of vitamin A)

iii. Measuring cost - effectiveness using the ultimate outcome of interest (e.g. mortality) is usually too costly and time consuming and so often only proximate indicators (e.g. proportion anaemic or haemoglobin) are used (Alderman and Horton, 2007).

2.10.2.10. Approaches to Assessing the Economic Benefit of Addressing Anaemia

There are two key approaches that are used:

i. Calculation of the expected gains in economic terms if a case of anaemia was prevented. This approach is useful for making comparisons of intervention costs.

ii. Estimation of the impact on GNP if anaemia rates could be reduced. This approach scales the individual gains and results in a stronger motivation for a change in political will.

Each approach has its advantages and limitations. It is important to recognize that placing precise figures on economic value involve a range of assumptions and requires the adaptation to a country's specific context (Alderman and Horton, 2007).

2.11. Animal Experimental Studies

Laboratory or model animals are recognized as important tools for investigating and understanding human and animal diseases and other biological processes (National Institute of Health, 1999; Microsoft Incorporation, 2002). Experimentation with laboratory animals forms the backbone of biomedical, bio - agricultural and bio - industrial research. Worldwide rodents are the major and most commonly used animals for biomedical research; the use of other animals is minimal (Microsoft Incorporation, 2002). Rats are the most widely studied experimental animal as demonstrated by the number of publications on studies using rats in the last decade - nearly 500, 000 public

medicine publications (National Institute of Health, 1999). The rat model with its enormous strengths and versatility of application has been found to be the most appropriate experimental model for the study of human disease and also for the study on bioavailability of nutrients in food (National Institute of Health, 1999).

2.12. Haemoglobin

The measurement of haemoglobin is essential for the diagnosis of nutritional anaemia and is one of the most common, easiest and least expensive methods. Kits are available from various manufacturers and there also small portable hemoglobinometers for use in the field. Unfortunately, the haemoglobin measurement is not very sensitive and specific for iron deficiency (only the third stage affects haemoglobin synthesis). Thus, to determine if iron deficiency is responsible for anaemia, it is usually necessary to include other indicators (Biesalski and Erhardt, 2007).

Abonormally low haemoglobin or anaemia is the test most commonly used to screen for iron deficiency. Anaemia occurs when haemoglobin production is sufficiently depressed to result in a haemoglobin concentration or hematocrit below the central 90% or 95% of range for a healthy reference sample of the same age and sex (Yip, 1994). A diagnosis of iron deficiency anaemia is made when anaemia is accomplished by other laboratory evidence of iron deficiency such as low serum ferritin, or when haemoglobin rises in response to iron treatment (Bowman and Russel, 2001).

Other biochemical indicators include:

1. Ferritin: It is currently considered the most important indicator of the iron status as even in the first stage of iron deficiency, its concentration decreases. Therefore it is the most sensitive indicator and the cost of ferritin ELISA kits or other methods of measurement of ferritin are relatively low. It is important to note that ferritin is increased by many factors, including infection and inflammation, thus a high value does not necessarily indicate a good iron status.

2. Soluble Transferrin Receptor: The measurement of this indicator is increasingly being used to determine iron deficiency in situations where infection is a factor, as it is

much less influenced by this condition. It is not as sensitive as ferritin, both more sensitive than hemoglobin (Biesalski and Erhardt, 2007).

2.13 Dietary Interaction

Foods constitute a complex chemical and biological mixture resulting from interaction of natural constituents, industrial processing and household preparations. The interaction cause marked change in the physio - chemical properties of a meal and thus, determine the amount and the bioavailability of nutrient. Furthermore, diet constituents continue to interact in gastrointestinal tract and at the level of intermediary metabolism. Since many recommendations for nutrient intake are based on studies using isolated nutrients or purified test meals, they do not necessarily reflect the requirement in terms of actual meals consumed by individuals (Caballero, 2006). Virtually any nutrient can cause adverse effects if ingested in excessive amounts. Such undesirable effects may depend on the inherent toxicity of the nutrient on the bioavailability of other dietary components. Likewise, non - nutritional substance, such as drug or natural contaminants, can interfere with nutrient utilization (Caballaro, 2006).

2.14 Nutrient - Nutrient Interaction

The term interaction denotes a bidirectional effect. Many interactions are unidirectional, i.e. one nutrient affects the biological disposition of another, which remains more or less passive. Bidirectional interactions are most common among nutrients with similar physio - chemical properties and sharing a common mechanism of absorption or metabolism. Some uni or bidirectional interactions are affected by the presence of a third dietary constituent. Nutrient interactions are not usually additive. For example, both heme iron and ascorbic acid taken separately increases the absorption of non - heme iron, however, simultaneous ingestion of both does not increase non - heme iron absorption (Caballero, 2006).

CHAPTER THREE

MATERIAL AND METHODS

3.1 Materials

Phyllantus nuriri and *Mucuna pruriens* leaves which were locally harvested in Awka South Local Government Area of Anambra
State, Nigeria.

3.2 Methods

The vegetables were separately plucked and sorted by removing extraneous material and then cleaned by washing with deionized water. The vegetables were pulverized using electric blender until the desired particle sizes were obtained (150-850 microns).

***Phyllantus nuriri* leaves**

↓

Picked

↓

Washed

↓

Pulverized

↓

Analyzed

Fig. 3.1: Processing *of Phyllantus nuriri* leaves.

***Mucuna nuriri* leaves**

↓

Picked

↓

Washed

↓

Pulverized

↓

Analyzed

Fig. 3.2: Processing of *Mucuna nuriri* leaves

3.3 Chemical Analysis

The proximate composition of fresh leaf from *Mucuna pruriens* and *Phyllantus nuriri* leaves were determined using standard methods of AOAC (2005). All analyses were done in triplicate.

3.4 Crude Protein

Crude protein was determined by automatic micro-kjeldahl method of AO AC (2005). Crude protein was estimated by multiplying nitrogen value with N conversion factor 6.25 (N x 6.25) protein.

3.5 Fat Determination (Soxhlet Method)

The fat contents of the samples were determined using the sohlet extraction method AOAC (2005).

The extraction flask was washed, dried, cooled and weighed prior to adding 2g of the sample. The sample was weighed into filter paper and introduced into thimble. Petroleum ether was added to the flask for extraction in the Soxhlet apparatus. After this, the extract was dried in an oven for 15 minutes at 100°C to remove any remaining solvent, cooled in the desiccators and reweighed.

Calculation:

$$\% \text{ fat} = \frac{\text{weighed of extract cup- weight of cup} \times 100}{\text{Original weight of sample.}}$$

3.6 Ash Determination

The ash contents of samples were determined using the method of AOAC (2005). Two grammes (2g) of each of the samples were weighed into crucibles heated in a furnace at 600°C for about three hours, cooled in desiccators and reweighed.

The percentage ash was calculated using the formula below

$$\% \text{ ash} = (\text{weight of crucible+ash}) = \frac{(\text{weight of crucible}) \times 100}{\text{Weight of sample}}$$

3.7 Crude Fibre

Two gramme samples were defatted with petroleum ether. The sample was boiled under reflux for 30 minutes with 20ml of a solution containing 1.25g of H_2SO4 per 100ml of solution. The solution was filtered through linen of several layers of cheese cloth on a fluted funnel and washed in boiling water until the washing was no longer acidic. The residue was transferred to the beaker and boiled for 30 minutes with 200ml of solution. The final residue was filtered through a thin but close pad of washed and ignited asbestos crucible. The residue was dried in electric oven, reweighed and then, incinerated, cooled and weighed. The loss in weight after incineration x 100 was the percentage of crude fibre.

3.8 Moisture Determination

The moisture contents of the samples were determined using AOAC (2005) procedure. Washed porcelain dishes were dried in a gallenkemp oven at 100°C for about 2 hours, cooked in desiccators and reweighed. Two grammes (2g) of each of the samples were weighed into the weighted dishes and placed in the oven at 100°C for 24 hours. The dishes containing the sample were cooled in desiccators, weighed and dried repeated similarly until a constant weight was obtained.

The percentage moisture was calculated using formula below:

$$\text{Moisture content} = \frac{\text{initial weight of dish + sample-final wt of dish + sample}}{\text{Weight of sample}}$$

$$\text{Percentage moisture} = \frac{\text{Moisture content}}{\text{Weight of sample}} \times \frac{100}{1}$$

3.9 Carbohydrate Contents

Total carbohydrate contents were determined by difference (subtracting crude protein, moisture, fat, fibre and ash content from 100%). The total carbohydrate of each sample was by difference.

Carbohydrate = 100 - (% protein + % fat + % ash + % Crude fibre + % moisture).

3.10 Mineral Determination

Mineral contents of the samples were analyzed using AOAC (2005) method. The samples were wet digested with concentrated nitrate and perchlorate.

Iron (Fe) copper (cu) and zinc (Zn) were determined by polarized Zeeman atomic absorption spectrophotometer.

The calcium, potassium and sodium contents were determined using the EDTA titration method. Stock solution was used. 25ml sample was diluted with 50ml of water. 2ml buffer solution was dissolved (16.9g NH4C1 in 143 ml NH40H), and 1.25 EDTA was added and diluted to 250 ml with water. 250 NaCN (PH 10.0) was added and 200mg indicator erichrome black T (mix 0.5 erichrome black T and l00g Nacl). One liter of water was used to dilute 0.01M EDTA by titration. End point was observed when the solution turned reddish.

$$\frac{A \times TCA \times V_1 \times 100}{W \times V_2}$$

A = ml EDTA
TCA = Titration factor of EDTA.
V_1 = Total volume
V_2 = Aliquot for determination
W = weight of sample

3.11 Vitamins Determination

Vitamin E and J3-carotene (provitamin A) were determined by High performance liquid chromatography (HPLC, model C030) of AO AC (2005).

The samples were digested and injected into the HPLC equipment. A pressure pump was used in moving the mobile phase and the components of the mixture through the stationary column. The pressure pump of different sizes was used and the detector mounted was capable of providing characteristic retention times for the sample components. The detector also takes care of the area counts which reflected the amount of each analyte passing through the detector.

Vitamin C was determined using AO AC (2005) method. Two grammes of each sample were dissolved with distilled water (2ml). Trichloroacetic acid (TCA) was added and colour was developed with 2, 6 − dichloroindophenol. The colour (pink) developed was read with spectrophotometer.

Calculation:

$$\frac{A \times (V_2 - V_1) \times 0.0052}{\text{Weight of sample}}$$

Where

a = volume of filtrate

V_1 = initial burette reading

V_2 = final burette reading

3.12 Anti - Nutrients

3.12.1 Phytate

Phytate was determined by photometric method adapted from the method of Latta and Eskin (1980). Five grammes (5g) of each sample were extracted with 2.46 HCL. One tenth sodium chloride was (0.1m NaCl) added to elude inorganic phosphorus and seven tenths mol of sodium chloride was added to elude phytate. One millimeter (l ml) of Wade reagent was added and read at 500nm in a spectrophotometer.

3.12.2 Oxalates

Oxalate was determined by AO AC (2005). About four (4) grams of the dried sample was extracted with 6N, HCL. The oxalates in this extract were precipitated with $CuCl_2$ and calcium salts. The precipitated oxalates were washed with 25% H2SO4 and dissolved in hot water before titrating against 0.05N KmN04, (1ml of 0.05N KMNO4) = 2.2mg.

3.13 Phytochemicals

3.13.1 Phenols

Determination of total phenols was by spectrophotometric method (AOAC, 2005). The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. 5 ml of the extract was pipetted into a 50ml flask, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amylalcohol were also added. The samples were made up to mark and left to react for 30 min for colour development. This was measures at 505 nm.

3.13.1 Alkaloid

This was determined using the Harborne (1983) method. About 5g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one - quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole situation was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

3.13.3 Saponin

The method used was that of Obadoni and Ochuko (2001). The samples were ground and 20g of each were put into a conical flask and 100 cm^3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigourously. The aqueous layer was recovered while the ether layer was

discarded. The purification process was repeated. 60 ml of n - butanol was added. The combined n - butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a waterbath. After evaporation, the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

3.13.4 Flavonoid

The flavonoid was determined by the method of Bohm and Kocipai - Abyazan (1994). About l0g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

3.13.5 Tannins

Tannin determination was by Van - Burden and Robinson (1981) method. About 500mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M FeCh in 0.1 NHC1 and 0.008 M potassium ferrocyanide. The absorbence was measured at 720 nm within 10 min.

3.13.6 Cyanogenic Glycoside

Estimation of cyanogeric glycoside content of samples was accomplished by determining the HCN (Hydrogen cyanide) released on hydrolysis. Extracts of each diet sample were obtained by homogenizing 30g of sample in 250ml of 0.1m orthophosphoric acid for 5 minutes and clear supernant was taken. An aliquot of the supernant was used for estimation of hydrogen cyanide using an auto analyzer Technicon AA11, according to the method of Rao and Hahn (1984).

3.14 Animal Experiment

3.14.1 Animal Housing

Twenty male adult rats were purchased from the department of Veterinary Medicine, University of Nigeria, Nsukka. Animals were divided into four groups of five rats each on the basis of body weight. The rats were housed individually in cages equipped to separate urine and faeces (metabolic cages). The study lasted for 28 days in which the first seven days was for acclimatization, second seven days for inducing of anaemia and 14 days for feeding trials. The extract was administered orally through drinking water bottles *ad libitum* for two weeks. Commercial hematinic (ferrous sulphate) was used as control. Group 1 was fed rat chow with FeSCU, Group 2 was fed rat chow with *Mucuna pruriens*, Group 3 rat chow with *Phyllanthus nuriri* and Group **4** rat chow aione. The weights of animals were recorded each day of analysis.

3.14.2 Diet Composition

Table 3.1: Composition of Experimental Diet

GROUP I	GROUP II	GROUP III	GROUP IV
Chow + Ferrous Sulphate	Chow + *Mucuna Pruriens* leaf extract	Chow + *Phyllanthus nuriri* leaf extract	Chow alone

3.14.3 Blood Sample Collection

Pre - hemorrhagic Sampling:

About 1ml of blood sample was obtained from each rat into a heparinized bottle to determine the initial blood pictures before bleeding. Blood collection was from ophthalmic venous plexus located in the orbited sinus of the rat using a heparinized capillary tube.

3.14.4 Anaemia Induction:

Anaemia was induced to the rats by a loss of about one - third of the total blood volume between the hours of 8.00 - 10.00am for the second seven days of the study. The blood was collected from ophthalmic venous plexus located in the orbital sinus of the rat using

a heparinized capillary tube. Blood was collected on day 0, 7, 14 of the period of feeding trial for hematological determination.

3.14.5 Biochemical Indices

The following biochemical indices were carried out - the hemoglobin level, red blood cell count, serum iron and serum beta carotene.

3.14.6 Determination of Haemoglobin Content (Sahli Method)

Material - Sahli hemoglobinometre (Comparator box), sahli tube, 0.1NHC1, Blood pipette, stirrer.

Procedure:

The dilution tube (sahli tube) was filled to 10 mark of the calibration with 0.1NHC1. With the blood pipette, the blood was sucked (collected from the ocular puncture) exactly to the mark in the pipette, 20ml. The blood at the outside of the pipette was dried and adjusted to the mark .It short beyond the mark. The blood was discharged into the tube containing 0.1NHC1 by sucking up and down. The mixture was allowed to stand for 5 minutes for acid - haemation to form. Distilled water was dropped, drop by drop, into the test tube and stirred after each drop till the colour of the mixture is exactly the same with the standard colour. The reading of the colour was taken in the sahli tube and applied into the formular - y(14)/100. Where, y = reading of the mixture colour (Akah, Okoli & Ndu, 2001).

3.14.7 Procedure for the Determination of Red Blood Cell Count

Using the red blood cell (RBC) pipette of the improved Neubar haemocytometer, blood samples were collected up to the 0.5 mark of the pipette from the puncture at the retroocular plexus at the eye of the rat. The blood was blown into the bulb of the pipette and immediately taken to the 101 mark, where it was mixed with red blood cell diluting fluid (hayem solution) to prevent haemolysis, coagulation and rouleaux formations. Hayem's solution consists of (0.5g $HgCl_2$, 0.5g NaCl and 2.5g Na_2S04 + H_20). The pipette was rubbed in between the palms for a homogenous mix of the blood solution (*Akah et al*., 2007).

3.14.8 Preparation of film and counting of RBCs

The counting chamber was moistened with the tip of the finger and the cover slip pressed down firmly on the counting chamber support, until a series of concentrically - arranged Newton's rings are seen. The above process called charging of the counting chamber is done to allow for a firm adherence. The tip of the pipette containing the blood solution was touched at the edged joint of the charged chamber and the blood fluid, through capillary tension drawn into the counting area of the chamber. The counting chamber was then placed on a microscope and scanned with (x4) objective lens, while the counting of the five designated grids (top right, top left, center, back left, back right) was done using (xl0) objective lens.

The total number of red cells is equal to (the number of ceils in the five grid section x dilution factor x volume correction factor), (*Akah et al*., 2007).

3.14.9 Procedure for Serum Iron Determination

About 0.1ml of serum was placed in test tube and 3ml of water added; about 1ml of 2.5% hydroquinone was added and the pH adjusted to 4.5, following by 1.5ml of acetate buffer (pH - 4.5). 1ml of 2, 2 - dipyridyl in ethanol was added, made up to 10ml, and the absorbance taken at 520nm.

Standard Serum Iron; y = 0.166x; x - y/0.166 (mg/ml).

3.14.10 Procedure for the Determination of Serum Beta Carotene

About 0.5ml of serum was placed in 9.5ml of acetone - petroleum ether (1:1) and allowed to stand for about 5 minutes. The mixture was transferred to a separating funnel and the upper layer was collected. The absorbance was taken at 450nm against petroleum ether.

Serum Beta Carotene Standard; y = 1.856x; x = y/1.856 (mg/l00ml).

3.15 Statistical Analysis

All the data obtained from the chemical and phytochemical analysis were subjected to various statistical analysis using mean and standard deviation. (Steel and Torie, 2000).

CHAPTER FOUR

RESULTS

Table 4.1: Proximate composition of fresh *Mucuna pruriens* and *Phyllantus nuriri* leaves (wet weight basis).

Nutrients (%)	*Mucuna pruriens*	*Phyllantus nuriri*
Moisture	79.67± 3.51	73.36±6.56
Crude protein	3.14 ±0.27	3.87 ±0.31
Fat	0.65 ±0.15	1.08 ±0.10
Ash	1.25 ±0.16	3.02 ±0.61
Crude fibre	3.82 ±0.21	3.20 ±0.28
Carbohydrate	11.47± 0.64	15.47± 0.47

Mean ± SD of three determinations

Table 4.1 presents the proximate composition of *Mucuna pruriens* and *Phyllantus nuriri* on wet weight basis. *Mucuna pruriens* had 79.67% moisture, 3.14% protein, 0.65% fat, 1.25% ash, 3.82% crude fibre and 11.47% carbohydrate. *Phyllantus nuriri* contained 73.36% moisture, 3.87% crude protein, 1.08% fat, 3.02% ash and 3.20% ash, 3.20% crude fibre and 15.41% carbohydrate.

Table 4.2: Micronutrient contents of fresh *Mucuna pruriens* and *Phyllantus nuriri* leaves (wet weight basis).

Nutrients (mg/100 g)	*Mucuna pruriens*	*Phyllantus nuriri*
Zinc	2.86 ±0.19	1.83 ±0.34
Iron	7.45 ±1.19	11.27±0.71
Calcium	23.48 ± 0.59	34.71 ±2.23
Copper	0.64±0.30	1.13±0.24
Magnesium	17.80 ±0.36	60.31 ± 1.36
Sodium	9.86 ±0.19	1.86 ±0.24
Potassium	93.96 ±1.94	54.05 ± 2.06
Vitamin C	26.16 ±1.34	20.13 ±0.91
Vitamin E	3.06 ±0.29	2.89 ±0.16
P - Carotene	3.62 ±0.33	3.65 ±0.36

Means ± SD of three determinations

Table 4.2 presents micronutrient content of fresh *Mucuna pruriens* and *Phyllantus nuriri* leaves on wet weight basis. *Mucuna pruriens* contained 2.86mg zinc, 7.45mg iron, 23.48mg calcium, 0.64mg copper, 17.80mg magnesium, 9.86mg sodium, 93.86mg potassium, 26.16mg vitamin C, 3.06mg vitamin E and 3.62mg β - carotene. *Phyllanthus nuriri* had 1.83mg zinc, 11.27mg iron, 34.71 mg calcium, 1.13mg copper, 60.3lmg magnesium, 1.86mg sodium, 54.05mg potassium, 20.13mg vitamin C, 2.89mg vitamin E and 3.65mg β - carotene.

Table 4.3: Phytochemical content of fresh *Mucuna pruriens* and *Phyllantus nuriri* leaves (wet weight basis).

Phytochemical (%)	*Mucuna pruriens*	*Phyllantus nuriri*
Alkaloids	0.003±0.00	1.68±0.12
Tannins	0.54±0.04	0.68±0.64
Saponins	7.01±0.23	3.95±0.29
Flavonoids	3.16±0.35	6.22±0.01
Cyanogenic glycosides	12.47±0.42	1.17±0.49
Phenols	1.06*0.49	0.21±0.00

Table 4.3 presents the phytochemical constituents of fresh *Mucuna pruriens and Phyllantus nuriri* leaves. *Mucuna pruriens* contained 0.003 alkaloids, 0.54 tannins, 7.01 saponins, 3.16 flavonoids, 12.47 cyanogenic glycosides and phenols 1.06%. *Phyllantus nuriri* leaves had alkanoids 1.68, tannins 0.68, saponins 3.95, flavonoids 6.22, cyanogenic glycosides 1.17 and phenol 0.21%, each.

Table 4.4: Anti - nutrient constituent and food toxicant content of fresh *Mucuna pruriens* and *Phyllantus nuriri* leaves (wet weight basis)

Anti - nutrient (mg)	*Mucuna pruriens*	*Phyllantus nuriri*
Phytate	0.004 ± 0.00	1.28 ±0.24
Oxalate	0.62 ±0.14	1.31 ±0.009

Table 4.4 presents the anti - nutrient and food toxicant content of fresh *Mucuna pruriens* and *Phyllantus nuriri* leaves on wet weight basis. *Mucuna pruriens* contained phytate 0.004mg, and oxalate 0.62mg. *Phyllantus nuriri* leaves contained 1.28mg phytates and 1.31 mg oxalate.

Table 4.5: Haemoglobin (HB) (mg/dl) levels of rats fed rat chow, rat chow with FeSC>4, rat chow with *Mucuna pruriens* and rat chow with *Phyllantus nuriri*

Days	**Rat Chow**	**Rat Chow + $FeS0_4$**	**Rat Chow + MP**	**Rat Chow + PN**
Day 7	10.02	10.41	11.10	10.20
Day 14	10.13 (1.10%)	14.02 (34.68%)	12.94 (16.57%)	13.62 (23.73%)

Day 7: The day anaemia was confirmed

Day 14: Test of recovery

FeS04 - Ferrous Sulphate

MP - *Mucuna pruriens*

PN - *Phyllantus nuriri*

Table 4.5 presents haemoglobin (Hb) levels of rats fed rat chow and various supplements — ferrous sulphate, *Mucuna pruriens* and *Phyllantus nuriri*. There were decreases in haemoglobin levels of the rats confirming anaemia on day 7 after induction anaemia. Rats fed rat chow had 10.02mg/dl, rat chow and $FeSO_4$ 10.41 mg/dl, rat chow and *Mucuna pruriens,* 11.10mg/dl and rat chow and *Phyllantus nuriri*, 10.20mg/dl. There were increases in haemoglobin for the rats fed rat chow and ferrous sulphate (34.68%), *Phyllantus nuriri* (23.73%) and *Mucuna pruriens* (16.57%) during the 14 days test of recovery. There was a slight increase of the haemoglobin for the rats fed rat chow alone (1.10%) from 10.02 to 10.13mg/dl.

Table 4.6: Serum iron level (mg/dl) levels of rats fed rat chow, rat chow with $FeSO_4$, rat chow with *Mucuna pruriens* and rat chow with *Phyllantus nuriri*

Days	**Rat Chow**	**Rat Chow + $FeS0_4$**	**Rat Chow + MP**	**Rat Chow + PN**
Day 7	0.06	0.04	0.06	0.05
Day 14	0.05 (-16.67%)	0.11 (175%)	0.07(16.67%)	0.09 (80%)

Day 7: The day anaemia was confirmed

Day 14; Test of recovery

FeS04 - Ferrous Sulphate

MP - *Mucuna Pruriens*

PN - *Phyllantus Nuriri*

Table 4.6 shows the mean serum iron levels for rats fed rat chow diet supplemented with ferrous sulphate, *Mucuna pruriens* and *Phyllantus nuriri.* There were decreases for all the groups after 7 days of anaemia induction, confirming anaemia. The group fed rat chow had 0.06mg/dl, rat chow and $FeSO_4$ 0.04mg/dl, rat chow and *Mucuna pruriens* 0.06mg/dl, and rat chow and *Phyllantus nuriri*, 0.05mg/dl. There were increases in serum iron level for the rats fed chow and ferrous sulphate (175%), *Phyllantus nuriri* (80%) and *Mucuna pruriens* (16.67%) during the 14 - day recovery test. There was decrease in serum iron level of the rats fed rat chow alone from day 7 to day 14 (-16.67%).

Table 4.7: Serum Beta-carotene (fig/g) levels of rats fed rat chow, rat chow with FeSC>4, rat chow with *Mucuna pruriens* and rat chow with *Phyllantus nuriri*

Days	Rat Chow	Rat Chow + $FeS0_4$	Rat Chow + MP	Rat Chow + PN
Day 7	1.20	1.10	1.15	1.18
Day 14	1.17(2.50%)	2.61(137.27%)	1.98(72.17%)	2.29 (94.07%)

Day 7: The day anaemia was confirmed

Day 14: Test of recovery

FeS04 - Ferrous Sulphate

MP - *Mucuna Pruriens*

PN - *Phyllantus Nuriri*

Table 4.7 shows the mean serum beta carotene values for all the groups of rats. There were decreases in serum beta carotene values confirming anaemia on day 7 after induction of anaemia. Rats fed rat chow had 1.20µg/dl, rat chow and $FeSO_4$ 1.10µg/dl, rat chow and *Mucuna pruriens* 1.45µg/dl and rat chow and *Phyllantus nuriri* 1.18µ/dl. There were increases in serum beta carotene level for the rats fed rat chow and ferrous sulphate (137.27%), *Mucuna pruriens* (72.17%) *and Phyllantus nuriri* (94.07%) during the 14 - day treatment. There was decrease in serum beta carotene level of the rats fed rat chow alone from day 7 to day 14. It decreased from 1.20µg/dl to 1.17µ/dl (2.05% decrease).

Table 4.8: Red blood cell count 10^6 cell/µg of blood levels of rats fed rat chow, rat chow with $FeSCO_4$, rat chow with *Mucuna pruriens* and rat chow with *Phyllantus nuriri*

Days	**Rat Chow**	**Rat Chow + $FeS0_4$**	**Rat Chow +** MP	**Rat Chow +** PN
Day 7	5.33	5.21	5.27	5.28
Day 14	5.28(0.94%)	7.06 (35.51%)	6.25(18.60%)	6.69 (26.70%)

Day 7: The day anaemia was confirmed

Day 14: Test of recovery

FeS04 - Ferrous Sulphate

MP - *Mucuna Pruriens*

PN - *Phyllantus Nuriri*

Table 4.8 shows the mean red blood cell count values for all the groups of rats. The red blood cell count of rats fed rat chow diet had a decrease of5.33 x 10^6 cell/µg of blood, rat chow and $FeS0_4$ 5.21 x 10^6 cell/µg of blood, rat chow and *Mucuna pruriens* 5.27 x 10^6 cell/µg of blood and rat chow and *Phyllantus nuriri* 5.28 x 10^6 cell/µg of blood on day 7 after induction of anaemia. There were increases in red blood cell count for the rats fed rat chow and ferrous sulphate (35.51%), *Mucuna pruriens* (18.60%) and *Phyllantus nuriri* (26.70%) during the 14 — day treatment. There were decreases in red blood cell count of rats fed rat chow from day 7 to day 14 (0.94%).

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.0 Proximate Composition

The moisture content of *Mucuna pruriens* and *Phyllantus nuriri* were 79.67% and 73.36% respectively. In line with the observation of Olaiya and Adebisi (2010) of the vegetables they studied and the value reported by Sheela *et al* (2004) for some under - utilized green leafy vegetables in Southern Karnataka. Moisture is the most abundant constituent of green leafy vegetables. The high moisture content of vegetables facilitates digestion of food (Olaiya and Adebisi, 2010).

The range of protein values obtained for these two vegetables, *Mucuna pruriens* and *Phyllantus nuriri* are in line with literature reports of Sheela *et al* (2004) who observed protein content of vegetables to be between 0.80 - 3.60% per l00g. Fresh vegetables are not good sources of protein. The values for crude protein content of fresh vegetables according to Aleter and Adegun, (1995) ranges from 1.5 to 1.7%. Some workers obtained a mean of 4.2% for 17 vegetables Aleter and Adegun, 1995). The fat content of these vegetables *Mucuna pruriens* and *Phyllantus* are in line with the values (0.20 - 2.60%) reported by Sheela *et al* (2004). Consumption of these vegetables will be of immense advantage especially to the elderly, since consumption of these vegetables will reduce high incidence of obesity, diabetes, cardiovascular diseases, high blood pressure, which are associated with high intake of fatty foods.

The carbohydrate values are in line with those Sheela *et al* (2004) reported for some vegetables. The value for carbohydrates for these vegetables was not a surprise because it is known that fresh vegetables are not good sources of carbohydrates. The little carbohydrate content in these vegetables is capable of supplying part of the daily requirement for carbohydrate for the individual. The end - product of carbohydrate digestion (glucose) provides energy to cells in the body, particularly the central nervous system (Effiong, Ibia and Udofia, 2009). The fairly high level of ash in both vegetables (1.25 and 2.02%) confirms the high concentration of mineral in the vegetables.

5.1 Mineral Composition

Potassium values of these vegetables, were *Mucuna pruriens* and *Phyllantus nuriri*, were lower than the values (214.42 - 591.0mg per l00g) observed by Tayie and Asiebey - Berko (2001) and higher than the values (1.34 - 3.34mg per l00g) observed by Akindahusi *et al* (2006) for vegetables. This meant that protein and nitrogen retention of consumers of these vegetables would be high. Potassium is also required in the muscle and nerve function.

The result of this study shows a lower concentration of sodium (9.86 and 1.86 mg per 100 sample) for *Mucuna pruriens* and *Phyllantus nuriri.* This is far lower than the RDA > 200mg/day (Nelson and Cox, 2000). Sodium is a very common electrolyte not generally found in dietary supplements (Nelson *et al,* 2000).

The calcium contents of these vegetables (23.48mg for *Mucuna pruriens* and 34.71mg for *Phyllantus nuriri)* suggest that these vegetables would be more advantageous to the body in functions associated with minerals. Calcium is required for bone and teeth formation and proper functioning of the nervous system.

The result of this study shows that these vegetables could provide some health benefits as dietary components because they contain significant quantities of magnesium (*Mucuna pruriens* 17.80mg and *Phyllantus nuriri* 60.1mg/100g sample). Magnesium is an obligate co - factor for DNA synthesis and the proportion supplied by vegetables is used to supplement low magnesium based staple foods such as cassava in Nigeria (Olaiya *et al,* 2000). The magnesium values obtained in this study is higher than the values (0.04 - 8.20 mg/l00g) reported by Okafor *et al* (1996) but was lower than the value 0.38 reported by Ujowundu *et al* (2010) for a vegetable.

The zinc contents of these vegetables studied are' lower than the values reported by Akindahusi *et al.* (2006) for egg plant and agree with the value reported by Okafor *et al.* (1998). Ngwa (2010) observed for indigenous vegetables, 2.05 - 6.80 mg/l00g and 23.00 and 3.00mg/100g. Zinc protects the skin and improves resistance to infectious diseases, inflammation and allergies (Nnam *et al.*, 2009). Lippard and Berg (1994) stated that zinc is a trace mineral element because they play a catalytic role in enzymes and the RDA < 200mg/day.

The results of the study show that *Mucuna pruriens* and *Phyllantus nuriri* are good sources of micronutrients. Iron content of these vegetables, *Mucuna pruriens* 7.45 and *Phyllantus nuriri* 11.27, were in line with the range of values 3.68 - 7.34mg/100g, 4.17 and 11.04mg/100g as reported by Nnamani *et al* (2009) on the underutilized vegetables studied, respectively. The highest value was obtained form *Phyllantus nuriri.* Hence, including these iron rich vegetables in daily diet would help to prevent iron deficiency anemia, since RDA for iron is 12.00mg/day (FAO, 2002).

5.2 Vitamins

The concentration of beta - carotene (pro - vitamin A), 3.62 mg/lOOg and 3.65mg/100g for *Mucuna pruriens* and *Phyllantus nuriri,* respectivel. Carotenoid is a component of rhodopsin, the visual pigment in the mammalian eye, as such, the consumption of these vegetables would be beneficial for good vision. The two vegetables did not meet the RDI for vitamin A.

The two vegetables were high in ascorbate. This result is in line with the result of Sheela *et al.* (2004) for some vegetables. Vitamin C maintains blood vessel flexibility and improves circulation in the arteries of smokers. The most important benefit claimed for vitamin A and C is their role as antioxidants (scavenger for oxygen - free radicals). These chemically active particles are by - products of many of the body's normal chemical processes. Their numbers are increased by environmental assaults, such as smoking, chemicals, toxins and stress (Ujowundu *et al.,* 2010).

The vitamin E in *Mucuna pruriens* and *Phyllantus nuriri* obtained in the present study is as high as the value in some vegetables such as asparagus and raw spinach. This means that these vegetable are high anti - oxidant sources to precipitate a healthy circulatory system, proper blood clotting and improved wound healing. Though the values obtained in the study were lower than the RDI for vitamin E (Aaron 2009).

5.3 Phytochemicals

The results show that these vegetables studied contained appreciable amount of pbytochemicals. The result showed that alkaloid level in these vegetables were lower than the values reported for alkaloid in the leafy vegetable like *V. amygdalina* (1.78%)

(Nnam, Onyechi and Madukwe, 2012). Tannin level of these vegetables, *Mucuna pruriens* and *Phyllantus nuriri* (0.54% and 0.68%) were higher than the safe level of tannins (0.15 - 0.20%) as recommended by Schiavone *et al.* (2007). Flavonoids were detected in both vegetables, *Mucuna pruriens* and *Phyllantus nuriri* (3.16% and 6.22% respectively), which is higher than the report of Nnam *et al.* (2012) for some leafy vegetables, *V. amygdalina*, *O. gratissimum, G. latifolium* and *G. africanum.* Flavonoids have been reported to function as pigments and antioxidants (Icumar, 1991). Flavonoids lower high blood pressure as well as cholesterol in animal studies and have strong anti - inflammatory. Flavonoids and alkaloids are known to protect the body by decreasing the risk of heart diseases, stroke and some types of cancer (Nnam *et al*, 2009).

Saponins are bitter and reduce the palatability of food. They have increased potential to lower cholesterol levels in humans due to the hypocholesterolenic effect of saponins (Nnam *et al*, 2009). These phytochemicals, either alone or in combination with others, are powerful tools to combat various ailments, especially cancers.

5.4 Anti-nutrients (Phytate and Oxalate)

The phytate values for *Mucuna pruriens* (0.004mg/100g) and *Phyllantus nuriri* (1.28mg/100g) were below the limit for phytate (5.00mg/100g) (Munro and Bassir, 1969).

Oxalate levels for these two vegetables were lower than the limit for oxalate; *Mucuna pruriens* 0.62mg and *Phyllantus nuriri* 1.31 mg. Phytic acid and oxalic acid usually form insoluble salts with mineral elements such as zinc, calcium and iron to prevent their utilization. The lower level of these anti - nutrients would therefore permit the absorption of these elements (Sarkiyayi *et al.* ,2010).

Hotz and Gibson (2007) suggested that many traditional methods of food preparation such as fermentation, cooking and malting increase the nutritive quality of plant foods through reducing certain anti - nutrients such as phytic acid, polyphenols and oxalic acid. Subjecting these vegetables to these processes will reduce the toxic level and at the same time boost the phytochemical properties of the vegetables (Schiavone *et al*, 2007). This suggests that consumption of these vegetables may help in cancer prevention due to the anti - oxidant effect of the substance (Nnam *et al*, 2009).

5.4.1 Hemoglobin

The rats fed ferrous sulphate in combination with rat chow had a significant increase in hemoglobin (Hb) (34.68%) when compared with the control group (1.10%). The finding is in line with the result obtained by Hamlin and Latunde Dada (2011) which shows that ferrous sulphate was effective as an oral iron supplement in the treatment of anaemia. Ferrous sulphate is highly bioavailable when compared to other iron salts and is used as a standard. The increase observed in rats fed rat chow in combination with *Phyllantus nuriri* and *Mucuna pruriens* extract (23.73% and 16.57%), respectively may be attributed to the high iron contents (11.27mg and 7.45mg) and ascorbic acid levels (20.13mg and 26.16mg) of the leaves. This is in line with the observation of Hamlin and Latunde - Dada (2011). They observed that iron and ascorbic acid administered jointly to anaemic mice significantly enhanced heamoglobin regeneration after l0days.

Phyllantus nuriri and *Mucuna pruriens* extracts have been used in some traditional setting to treat anaemia. The result of this work shows a modest response in regenerating heamoglobin level in anaemic rats after 14 days treatment. The leaves could be included in meals for the treatment of anaemia. These increases were presumably attributable to the supply of increased level of iron in a moderately bioavailable form by these vegetables.

5.4.2 Serum iron

A high increase (80%) was observed in the serum iron level of the rats fed rat chow in combination with *Phyllantus nuriri* extract. This may be due to inter - relationship of nutrients such as high iron, ascorbic acid and beta carotene (Siegenberg, 1991). Siegenberg (1991) observed that ascorbic acid is the most potent enhancer of non - heme iron. The lower increase (16.67%) in serum iron observed for rats fed rat chow in combination with *Mucuna pruriens* may be due to lower level of iron observed in the leaf extract (Table 4.2). Serum iron levels for both vegetable extracts *(Mucuna pruriens* and *Phyllantus nuriri)* tend to increase in both treatment groups but was not as efficacious as ferrous sulphate judging by the parameter of heamoglobin level.

Ferrous sulphate had 175% increase for serum iron. Consequently, the measurement of bioavailable iron will be influenced by the relative amount of enhancers and inhibitors interacting in the lumen of the gastrointestinal tract (Hamlin and Latunde - Dada, 2011).

5.4.3 Serum beta carotene

The increase in serum beta carotene values for the two vegetables *Phyllantus nuriri* (94.07%) and *Mucuna pruriens* (72.17%) may be due to high level of iron, ascorbic acid and beta carotene (pro - vitamin A), (Table 4.2). This is because of the interrelationship that exists between iron and vitamin A. Iron mediates the conversion of pro-vitamin A to vitamin A (Siegenberg, 1991). This also agrees with Haskell *et al.* (2005) who reported a positive impact on vitamin A pool size in population at risk of Vitamin A deficiency (VAD) after daily consumption of cooked leafy vegetable. Ascorbic acid is known to increase both iron and vitamin A utilization. This shows that these two vegetables are good and cheaper source of pro-vitamin A and can be included in meals.

5.5 Red blood cell count

There were increases in red blood cell count for rats fed rat chow and *Phyllantus nuriri* (26.70%) and *Mucuna pruriens* (18.60%). This increase may be attributed to the iron level of the diets - *Mucuna pruriens* and *Phyllantus nuriri* (7.45 and 11.27mg, respectively). The vegetables, *Phyllantus nuriri* and *Mucuna pruriens* could prevent anaemia, since they were able to improve the iron status of the rats in these groups. Iron played a significant role in the synthesis of heamoglobm, thereby increasing the synthesis of red blood cells.

CONCLUSION

The results of the study showed that *Phyllantus nuriri* and *Mucuna pruriens* leaves have a lot of potentials as human food and for the prevention of iron deficiency anaemia. These vegetables could make significant nutritional contribution to the diet of the populace because of their high nutrient and phytochemical contents. *Phyllantus nuriri* and *Mucuna pruriens* are good sources of Beta carotene, iron and ascorbic acid. Feeding rats with rat

chow supplemented with *Phyllantus nuriri* and *Mucuna pruriens* extracts improved the levels of heamoglobin, serum iron, serum beta carotene and red blood cell of the rats.

RECOMMENDATIONS

Based on the results of the study, the following recommendations were made:

1. Nutrition educators should use the information from this study to counsel families on the consumption of these lesser - known vegetables.
2. Students should also be assisted to carryout the same study on humans to see the nutritional effect.
3. Organoleptic evaluation of these vegetables should be carried out to evaluate acceptability.

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