CHEMICAL COMPOSITION AND EFFECTS OF DRIED *Irvingia gabonensis* (OGBONO) SEEDS AND FRESH GARDEN EGG LEAVES ON SELECTED BIOCHEMICAL INDICES OF DIABETIC ADULT MALE WISTAR RATS.

A PROJECT WORK

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN HUMAN NUTRITION

BY

IBEABUCHI, NGOZI VERONICA

PG/MSC/12/62014

DEPARTMENT OF HOME SCIENCE, NUTRITION AND DIETETICS

UNIVERSITY OF NIGERIA, NSUKKA

SEPTEMBER, 2015

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CERTIFICATION

IBEABUCHI, NGOZI VERONICA, a postgraduate student in the Department of Home Science, Nutrition and Dietetics has satisfactorily completed the requirements for the award of the degree of Master of Science (M.Sc) in Human Nutrition. The work embodied in her project is original and has not been submitted in part or full for any other diploma and degree of this or any other University.

Dr. J.U. Nwamarah
Supervisor

Prof. E.K. Ngwu
Head of Department

Prof. L.I. Salami
External Examiner

APPROVAL

This project has been approved for the award of M.Sc degree in Department of Home Science, Nutrition and Dietetics, University of Nigeria, Nsukka.

Dr. J.U. Nwamarah	Prof. E.K. Ngwu
Supervisor	Head of Department

DEDICATION

This work is dedicated to Almighty God for His unfailing love and strength in this journey. Also to my dearest husband and family you are most wonderful.

ACKNOWLEDGEMENT

The researcher is grateful to the Almighty God for the gift of life and for making this research possible. This project has helped her professionally and given her the privilege to work and interact with treasured individuals without whom this work would not have been accomplished successfully. She expresses her profound gratitude to her supervisor, Dr. (Mrs) J.U. Nwamarah a woman with a motherly heart. She thanks her for her advice, motherly love, guidance, patient, thorough supervision, suggestions and criticisms. Indeed your zeal and love made this possible. A special thanks to Prof. I.C. Obizoba for his wonderful contributions and fatherly love. They were always there to listen to her with no excuses. A special thanks to the Head of Department, Prof. E.K. Ngwu for her motherly advice.

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To the invisible, immortal and immaculate God, take all the GLORY.

IBEABUCHI, NGOZI VERONICA

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ABSTRACT

The study examined the chemical composition and the effects of *Solanum aethiopicum* leaves (garden egg) and *Irvingia gabonensis* seed (ogbono) on selected biochemical indices of alloxan induced adult wistar rats. Fresh *Solanum aethiopicum* leaves were plucked, sorted, washed with clean water and allowed to drain. The pulverized leaves were packaged in a plastic container and preserved in the refrigerator. Dried *Irvingia gabonensis* seeds were sorted washed and shade dried. The ground seeds were packaged in a plastic container and kept in the refrigerator. Proximate analysis was carried using standard methods. This analysis showed that *Solanum aethiopicum* leaves contained moisture (77.23%), fibre (2.61%), carbohydrate (10.88%) and protein (8.14%) while *Irvingia gabonensis* seeds had moisture (6.0%), fibre (2.27%), carbohydrate (56.07%), protein (10.52%) and fats (24.18%) content. Pro-vitamin A (2030 i.u), C (14.36mg/100g) and E (8.10mg/100g) were present in *Solanum aethiopicum* leaves. Vit.E (4.08mg) was found in *Irvingia gabonensis* seeds. Mineral level revealed Iron (2.04 mg/100g), magnesium (160.48 mg/100g), calcium (239.58mg/100g), potassium (40.25mg/100g) sodium (24.07mg/100g) in *Solanum aethiopicum* leaves. Mineral composition in the seed revealed Iron (5.27 mg/100g), magnesium (19.10 mg/100g), zinc (1.68 mg/100g), calcium (372.46 mg/100g), potassium (40.49 mg/100g), sodium (25.73 mg/100g) and copper (2.32 mg/100g). Phytochemical analysis was carried out using standard methods. The phytochemical and antinutrient analysis showed presence of tannin (20.93 mg/100g) in *Solanum aethiopicum* leaves while *Irvingia gabonensis* seeds contained tannin (2.32 mg/100g), alkaloids (5.15 mg/100g). There were seven groups of rats in this study. Six groups of rats were fed rat chow supplemented with *Solanum aethiopicum* leaves and *Irvingia gabonensis* seed. The experimental groups were diabetically induced with alloxan powder of 150mg/kg mixed with 10mls of diluted water. Group 1 was rats fed rat chow and water *ad libitum* only as control. Groups 2-4 were rats fed 5, 10 and 15g/kgBW of *Solanum aethiopicum* leaves and Groups 5-7 were rats fed 5, 10 and 15g/kgBW of *Irvingia gabonensis* seeds. Biochemical analyses (lipid profile and heamatological indices) were determined using standard methods. Serum cholesterol levels decreased in all the groups of rats fed the two test diets. The final result of serum cholesterol in the rats fed *Solanum aethiopicum* showed significant difference ($p<0.05$). There was also significant difference in cholesterol values of *Irvingia gabonensis.* The group fed 10g/kgBW *Solanum aethiopicum* decreased (18.52mmol/l) LDL of the rats. There were decreases of LDL in all the groups of rats fed 5g, 10g and 15g/kgBW of *Irvingia gabonensis* (21.74, 1.60 and 23.53mmol/l). The LDL tables differed significantly ($p<0.05$). The groups fed *Solanum aethiopicum* had increased HDL. The group fed diets containing 15g/kgBW *Irvingia gabonensis* reduced the (7.69mol/l) HDL of rats compared to the other groups. The HDL tables differed significantly. Triglycerides increased in all the groups of rats fed *Solanum aethiopicum.* There was 19.10mmol/l reduction of triglycerides in the group of rats fed 15g/kgBW *Irvingia gabonensis* seeds. The rats fed 5g/kgBW *Solanum aethiopicum* leaves decreased the (4.65) PCV value of the rats compared to other groups fed this diet. The groups fed *Irvingia gabonensis* seeds had increases of PCV in all the groups of rats. The PCV and triglycerides showed no significant difference ($P>0.05$). The groups fed 5g/kgBW *Solanum aethiopicum* leaves of each test diets decreased the RBC of the rats. The group fed diets containing 5g/kgBW *Solanum aethiopicum* reduced (12.71 $x_{10}^{6/L}$) WBC of rats compared to the other groups. There were increases of WBC of all the groups of rats fed *Irvingia gabonensis* seed. There were increases of

leucocytes of all the groups of rats fed these two diets. The result revealed that there was no significant difference ($p>0.05$) within the groups. The groups fed 5 and 15g/kgBW of the two test diets decreased the body weight of the rats. All the groups decreased in the blood sugar levels.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Vegetables play an important role in human nutrition, apart from the fact that we derive most of our recommended daily needs of mineral and vitamins from them. They are consumed in relatively small quantities as a side dish or a relish with staples. Vegetables can be leaves, root, stems and seeds (Gropper, Smith & Groff, 2005). They maintain alkaline reserve in the body. They have high vitamin, dietary fibre and mineral contents (Ball, 2006). The dark green leaves provide a high amount of carotene, ascorbic acid and micro minerals which play important roles in nutrient metabolism and delay the development of degenerative diseases (Yi-Fang, Jie, Xian-Hong & Rui-Hui, 2006). The wide variation in colour, shape, tastes and textures of various vegetables add an interesting touch to meals (Fasuyi, 2006). There is increasing epidemiological evidence in favour of an association between nutrition and susceptibility to infection. Health disorders such as heamorrhoids, gallstones, heart diseases, obesity and constipation could be corrected, or treated by copious consumption of vegetables (Whitney, 2002). Eating plenty of vegetables and fruits can help ward off heart disease and stroke, control blood pressure, prevent some types of cancer, avoids a painful intestinal ailment called diverticulitis, and guard against cataract and macular degeneration(two common causes of vision loss) (Joshipura, Hu & Manson, 2001).

Solanum aethiopicum, Ethiopian Eggplant or nakati is a fruiting plant of the genus Solanum mainly found in Asia and Tropical Africa. It is also known as Mock Tomato, Garden Eggs and Ethiopian Nightshade and locally called aghara in igbo language

(Lester & Seck, 2004). The leaves of *Solanum aethiopicum* are eaten as a leaf vegetable and are actually more nutritious than the fruit. Fruits of this variety are about two inches in diameter and turn bright orange-red when ripe, although they are usually eaten when still green (encyclopeadia, 2013).

Nuts are rich sources of multiple nutrients and their consumption is associated with health benefits and reduction of high body weight (Albert, Gaziano, Willett & Manson, 2002). This has prompted recommendations to increase their consumption. However, they are also high in fat (albeit largely unsaturated) and are energy dense. The associations between these properties, positive energy balance and body weight raise questions about its recommendations *(*Hu *et al.,* 1998). This issue is addressed through a review of the literature pertaining to the association between nut consumption and energy balance. Epidemiological studies document an inverse association between the frequency of nut consumption and Body Mass Index (BMI). Clinical trials reveal little or no weight change with inclusion of various types of nuts in the diet. Mechanistic studies indicate this is largely attributable to the high satiety property of nuts, leading to compensatory responses that account for 65ï 75% of the energy they provide (Traoret, 2008).

Irvingia gabonensis is a species of African trees in the genus Irvingia, sometimes known by the common names as wild mango, African mango, bush mango, dika or ogbono. They bear edible mango-like fruits, and are especially valued for their fat and protein rich nuts (Ngondi, Oben, Minka & Samuel, 2006). The geographical distribution of the species extends from the Casamance region (Senegal) to Angola and it is found in moist semi-deciduous forests. It does not exist in swampy areas. It is

found in most parts of Cameroon. The fruits are greenish yellow with fleshy fibrous pulp surrounding a large hard stone (Lamorde, 2010).

Unhealthy diet coupled with sedentary life style is known to be risk factors for life threatening chronic diseases and death: obesity, diabetes, hypertension, anaemia and some forms of cancers (Michel, Franco, Jeremy, Yong & Veronica, 2010). Broadly, it is agreed that diets that increase the risk of chronic diseases are relatively high in fats, saturated fats, sugar, salt, alcohol, refined grains and foods of animal origin, whereas diets that protect against chronic diseases are relatively high in minimally processed grains, legumes, fibre, vegetables, fruits and foods of plant origin (Popkin & Du, 2003). There is urgent need to develop methods to increase the availability of these important components of human diet available all year round.

Our ancestors whose diets consisted mainly of herbs, fruits, vegetables, nuts and starchy tubers (unlike many of the processed or refined foods we eat today) lived longer. They were not victims of many health problems man faces in present times (Dunn, 2012).

1.2 Statement of the Problem

Diabetes mellitus (DM) is a public health problem. The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 (Nyenwe, Odia, Ihekaba, Ojule & Babatunde, 2003). Oputa and Chinyere (2012) noted that, Nigeria has a population of about 150 million, of which 76 million are adults. This shows that diabetes mellitus is of public health importance in Nigeria.

Obesity has become a public health issue. The prevalence of obesity has doubled in adults and children and tripled in adolescents over the two decades. The speed with which obesity has become epidemic seems overwhelming (Mary and Sarah, 2004). Obesity is associated with a wide range of health problems (Bray, 2004).

Anemia is a widespread public health problem with major consequences for human health as well as social and economic development. It can be assumed that in resource poor areas significant proportions of young children and women of child bearing age are anemic. WHO (2013) estimates the number of anemia in people worldwide to be staggering two billion and that approximately 50% of all anemia can be attributed to iron deficiency.

Fruits, vegetables and nuts constitute an indispensable constituent of human diet (Suberu & Shinkafi, 2004). There is a precipitated change from indigenous food to various easy to cook foods such as pastas ï indomie, spaghetti, macroni etc (Leakey *et al.,* 2005). These changes in food habits in Nigeria and worldwide has caused negligence in consumption of nuts, fruits and vegetables.

Timothy (2000) reported that a major reason many of these foods were neglected and abandoned were due to ignorance and poverty. Many communities do not consume many indigenous foods to meet their micronutrient needs due to ignorance of their nutrient contributions to health.

Ngondi *et.al*.,(2005) reported that an experimental group received *Irvingia gabonensis* extract 1.05 grams 3 times a day (total 3.15 grams) for 30 days. The group experienced a decrease in total cholesterol, triglycerides and LDL cholesterol. HDL cholesterol increased.

Ngodi *et al.,* (2009) also reported improved blood glucose when fed African dikanuts.

Rural women, market women and others reported that the primary aim of *Solanum aethiopicum* leaf is to boost blood or combat anemia.

The cost of drugs given to the diabetic patients is also my major concern since there are traditional foods that can be used for the management of diabetes. A good number of medicinal plants are traditionally employed to allieviate diabetes. Some of these plants include *Solanum aethiopicum, Telfaira occidentalis, Combretum dilichopetalum, Irvingia gabonensis, Jatropha curcas* etc (Dina, Adedepo, Oyinloye & Saba, 2006).

It is therefore very necessary to scientifically find the chemical composition and effects of dried *Irvingia gabonensis* seeds (ogbono) and fresh *Solanum aethiopicum* leaves (garden egg) on selected biochemical indices of alloxan induced diabetic adult male wistar rats.

1.3 Objectives of the Study

1.31. Broad Objective:

The broad objective of the study were to determine the chemical composition and effects of dried *Irvingia gabonensis* seeds (ogbono) and fresh *Solanum aethiopicum* leaves (garden egg) on selected biochemical indices of alloxan induced diabetic adult male wistar rats.

1.32. The specific objectives of the study were to determine the:

i) proximate composition of *Irvingia gabonensis* seed and *Solanum aethiopicum* leaf;

ii) vitamins (pro.vit.A, vit.C and vit E) and minerals (iron, magnesium, zinc, calcium, potassium, sodium and copper) contents of the samples;

iii) phytochemical compositions (saponins, tannins and alkaloids) and antinutrient (oxalate and phytate) of the samples;

iv) effects of the sample*s* on blood glucose of alloxan induced diabetic adult male wistar rats;

v) effects of the samples on lipid profile of alloxan induced diabetic adult male wistar rats and

vi) effects of the samples on haematological indices of alloxan induced diabetic adult male wistar rats.s

1.4 Significance of the Study

The findings of this study if adequately published may provide information to nutritionist and community health workers by effective incorporation of the leaves and the nut in the management of some chronic diseases such as anemia, diabetes and obesity. The health professionals can use the result of this study to educate families on the importance of growing these plants in their home garden. The study will help the agricultural workers on the need to improve the production of these plants. The result will be essential for the researchers when incorporating food data base. It would help the health professionals, especially the nutritionists and dietitians in management, recommendation and counseling of patients in a hospital setting.s

OUTLINE OF LITERATURE REVIEW

1. Definition of Vegetables
2. Classification of Vegetables
3. Factors that Affect Consumption of Vegetables
4. *Solanum aethiopicum*
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CHAPTER TWO

LITERATURE REVIEW

2.1 Definition of Vegetables

Vegetable is any plant whose fruit, seeds, roots, tubers, bulbs, stems, leaves, or flower parts are used as food such as tomato, bean, beet, potato, onion, asparagus, spinach, or cauliflower. It is any part of a plant that is customarily eaten and not developed from a flower (Merchant, Hu & Spiegelman, 2003). A diet high in fruits and vegetables (F&V) has a significant protective effect against the risk of various cardiometabolic diseases including hypertension, stroke, diabetes and peripheral arterial diseases (He, Nowson & MacGregor, 2006). Due to the substantial health benefits gained from a diet high in fruits and vegetables, promoting their consumption remains a top priority for U.S. federal agencies. Recently, the My Pyramid food guidance system supported by the (United States Department of Agriculture) USDAôs Center for Nutrition Policy and Promotion, published new dietary guidelines (USDA, 2009). This Dietary Guidelines is based on the study from nutritional science which recommends more than five servings a day of fruits and vegetable (US Department of Health and Human Services) (US DHHS, 2011).

Vegetables are called ónegative calorie foodsôbecause it actually helps the body to lose weight by spending more amount of energy to digest the food than it actually adds to the overall caloric intake. The vibrant pigments in fruits and vegetables also have immense disease-fighting capabilities. They are rich in a host of useful nutrients, powerful antioxidants and should be included in a daily diet so as to improve healthy living (Joshipura, Hu & Manson, 2001)

2.11 Classification of Vegetables

Table 1: Classification of Vegetables

Vegetable	Description	Examples
1. flowers	The edible flowers of certain vegetables	cauliflower, broccoli.
2. fruits	Vegetable fruits are fleshy and contain seeds	egg plant, capsicum, okra,
3. fungi	When referring to vegetables, fungi are commonly known as mushrooms.	button, flats, shitake, oyster
4. leaves	The edible leaves of plants.	Cabbage, lettuce.
5. roots	Usually a long or round-shaped taproot	carrot,turnip, beetroot, swede
6. seeds	Also known as legumes, seeds are usually obtained from pods. The pod is sometimes eaten along with the seed	French bean, pea,snow snake beans, butter beans
7.stems	The edible stalks of plants when the stalk is the main part of the vegetable	asparagus, celery
8.tubers	Vegetables which grow underground on the root of a plant	potato, yam, Maori potato

Source: (Maynard &Hochmuth. 1997).

Under this classification, vegetables can be colour categorized into

- The dark green vegetables are rich in vitamins A and C, calcium and iron, which are essential for the production of sebum. Green foods are crucial to our health for a plethora of reasons including their role in strengthening the immune system, cancer prevention, improved blood circulation, blood purification, lowering cholesterol, promotion of healthy intestinal flora, increased energy, detoxification through improved liver, gall bladder functions, kidney function, and even clearing congestion.

- The red vegetables have plenty of lycopene. The vegetables usually contain anthocyanins. Anthocyanins have antioxidant properties that limit damage caused to cells by free radicals and may also lower risk for heart disease, stroke, cancer, muscular degeneration and memory problems. Lycopene may lower risk for cancer and heart disease.
- Orange and yellow vegetables: The compounds that give orange and yellow fruits and vegetables their colour are called carotenoids. Carotenoids may improve immune function and lower risk for heart disease, vision problems and cancer. Beta-carotene is a carotenoid that the body uses to produce vitamin A. Folate, potassium, bromium and vitamin C are also often found in orange and yellow fruits and vegetables (Jessica, 2008).

2.12 Factors that affect Consumption of Vegetables

2.12.1. Individual Factors

i) Sociodemographics: Johansson & Andersen (1998) suggests that age, gender, race/ethnicity, urban/rural residency, and socioeconomic status are important

demographic factors affecting fruits and vegetables consumption. Older adults, women and individuals with higher socioeconomic status consume more fruits and vegetables than younger people, men or those with lower socioeconomic status (Kiviniem, Orom & Giovino, 2011). Race/ethnicity can also affect dietary behavior because of cultural differences, group norms and psychological factors (Stewart, Harris & Guthrie, 2004). Black households have lower fruits and vegetables intakes (Guenther, Dodd & Reedy, 2006)

ii) Dietary Habits: Food/dietary habits are complex constructs reflecting numerous cultural, traditional and psychosocial factors affecting food choices (Rozin & Vollmecke, 1986). Distinct dietary differences exist around the world among different countries and cultures. For example, the norm in Chinese traditional cooking is to prepare dishes from fresh material every day. A combination of meat, vegetables and soup must be included. The Chinese meal is considered to be healthier than the western ones (Satia, Kristal & Patterson, 2002). Satia, Kristal and Patterson (2002) showed that Chinese women living in the U.S. and Canada were still influenced by their cultural food preferences for fruits and vegetables intake. Self-efficacy prevents overeating and promotes healthy dietary habits, has also been found to be an important psychosocial factor affecting fruits and vegetables intake (Richert, Reute & Wiedemann, 2010).

iii) Dietary Lifestyle: The primary lifestyle factors affecting food choices in the west are time constraints on food shopping and preparation, given the perishable nature of fruits & vegetables. Unlike processed foods, such as canned food, dry food, frozen food and other foods that have a long shelf life, fresh fruits and vegetables have a more limited storage time and require more frequent shopping to replenish supplies. A study determined that individuals believed that more visits to grocery stores were necessary to increase fruits & vegetables consumption (Anderson & Cox, 2000).

However, low-income people facing the pressure of working two or three minimum wage jobs to survive do not have the luxury of shopping several times per week. Another barrier to increased consumption is preparation time. The increasing supply of processed vegetables may meet the increasing demand for convenient fruits and vegetables (Pollack, 2001).

iv) Health Status: Smoking, Body Mass Index (BMI), and self-rated health status are related to the food choices people make. An individualôs health status can be a consequence of his/her lifestyle, which also influences his/her food preferences. For example, people with lower awareness of hypertension take less fruits and vegetables than those have higher awareness, thus have higher risk of hypertension (Dickson, Blackledge & Hajjar, 2006). Female smokers consumed less fruits and vegetables which may indicate that they place less value on their long-term health status. Obese people tend to underestimate their food intake, including fruits and vegetables consumption (Heitmann & Lissner, 2000).

v) Sensory Appeal: Properties of fruits and vegetables, such as taste, smell and appearance provide an important way to satisfy consumers and these were among the most important factors that influence consumersô food choices (Glanz, Basil & Maibach, 2001). While the taste of fruits is generally perceived as pleasurable, the taste of certain vegetables, such as broccoli and spinach, is considered to be a barrier to their consumption. These appeal-related factors are especially important for young children who are more likely to be affected by the appearance and taste of food and less aware of its nutritional value. Coulthard and Blisset (2001) found that childrenôs sensitivity to food taste and smell moderated the positive relationship between a motherôs influence and childrenôs fruits and vegetables consumption. In other words, children who are

more sensitive to food taste and smell rely more on their own preferences rather than parents eating arrangements for them.

2.12.2 Household Factors

Limited studies have investigated the impact of family structure on fruits and vegetables consumption, especially how household factors affect childrenôs fruits and vegetables intake. Generally, married individuals with more family members, especially children, consumed significantly more fruits and vegetables (Pollack, 2001). Family influence is a key factor in childrenôs fruits and vegetables consumption. More than half of the variations in fruits and vegetables consumption among children could be attributed to family and home environment. Parenting, especially by non-working mothers, could make fruits and vegetables more available and accessible to children at home. Moreover, parenting practices are recommended as a significant way to increase the fruits and vegetables consumption of younger children, such as preschoolers. Such parenting practices include telling the children taking fruits and vegetables will make them strong and involving children in food preparation. In summary, household factors mainly contribute to childrenôs fruits and vegetables intake (OôConnor, Hughes & Watson, 2010).

2.12.3 Environmental Factors

i) Food Price: Food cost is a major factor, second only to taste, in determining food choices, especially for income-constrained groups (Glanz, Basil & Maibach, 2001). Lower energy density foods, which are to say healthier foods such as fruits & vegetables, are associated with higher costs. The relative cost disparity between fruits and vegetables and energy-dense foods has led to a higher percentage of low-income populations purchasing high density foods that are high in fat and sugar. Poverty combined with the high cost of fruits and vegetables may explain the disparity in

obesity, as well as other health disparities across socioeconomic status. Because of the strong relationship between food price and fruits and vegetables consumption, consumers are quite responsive to changes in fruits and vegetables prices, as reflected by Frenchôs study which found that a 50% reduction in price resulted in a four-fold increase in fruit sales and a two-fold increase in vegetable sales. Price sensitivity also varies by socioeconomic status. Young adults with lower income or education or with lower family socioeconomic status as measured by their mothersô education and parental income were more sensitive to the price of fruits and vegetables than their peers with higher socioeconomic status (Powell, Zhao & Wang, 2009).

ii) **Accessibility and Availability:** Location, access and food availability of grocery stores are all related to fruits and vegetables consumption. Low-income populations that lack adequate transportation are more likely to utilize high-cost convenience stores instead of accessing supermarkets that are further away but offer more choices and lower-priced fruits & vegetables. When employers provided an available fruit supply in workplaces, the fruits and vegetables intake will be increased for the low-income employees (Backman, Gonzaga & Sugerman, 2011).

iii) Social interaction: Social norms are considered to be significant factors in fruits and vegetables consumption because of how they affect peopleôs behavior patterns. People are influenced by the surrounding environment through social pressures, social norms and fashions (Sorensen, Stoddard & Dubowitz, 2007).

v) Seasons: The seasonal factor in fruits and vegetables consumption is especially influential in agricultural communities. The amount of fruit and vegetable supply, the quality of produce available and prices vary in different seasons, therefore fruits and vegetables consumption also changes seasonally (Pollard, Greenwood & Kirk, 2001).

2.13 *Solanum aethiopicum*

Solanum aethiopicum, Ethiopian Eggplant or nakati is a fruiting plant of the genus Solanum mainly found in Asia and Tropical Africa. It is also known as Mock Tomato, Garden Eggs and Ethiopian Nightshade. Currently there is a large movement towards increased cultivation of *Solanum aethiopicum* in West Africa. It grows all year long and can produce high fruit yields. The leaves of *Solanum aethiopicum* are eaten as a leaf vegetable and are actually more nutritious than the fruit. Fruits of this variety are about two inches in diameter and turn bright orange-red when ripe, although they are usually eaten when still green. These names are a result of its varied morphology, with ripe fruit often looking like a cross between an eggplant and a tomato, which are also from Solanum. It can produce fruit within just 75 days after planting. Infact, the Ethiopian eggplant was so much confused with the ordinary eggplant that this was considered by some a variety violaceum of *S. aethiopicum* (Lester & Seck, 2004).

Ethiopian Eggplant may have originated from the domestication of Solanum anguivi. The Scarlet Eggplant, also known as Gilo or jiló, was long held to be a distinct species (S. gilo) but is nowadays generally considered to be a cultivar group of *S. aethiopicum.* This traditional vegetable has high contents of protein, calcium, phosphorus, iron, potassium, carotene and vitamins A, B and C complementing the nutritional value of basic staple foods (Lester & Seck, 2004).

2.13.1 Uses of *Solanum aethiopicum*

The leaves of *Solanum aethiopicum* are eaten as a leaf vegetable and are actually more nutritious than the fruit. The highly variable fruit of the plant is eaten both raw and cooked and is becoming more popular as a cultivated crop. These fruits are usually

harvested while still green, before the skin becomes thick. The bitterness depends on the levels of saponin it contains, some with a sweet flavor and others very bitter. When the berries mature, they turn bright red because of high carotene content (encyclopeadia, 2013).

Solanum aethiopicum is one of the fibre diets. The most satisfactory prophylactic and therapeutic agent for functional constipation is a diet rich in fibre. Dietary fibre benefit patients who need to avoid straining at stool, patients with irritable bowel disease and diverticular disease of the colon (Selvendran, 1984). Dietary fibre increases the mass of stool, its water content and the rate of colonic transit. These effects are usually apparent within 24hours and with repeated administration, reach a maximum after several days. Lack of fibre in diets was established as a contributive factor in certain disorders of the bowel and other disease of man (Painter and Burkitt, 1975; Mendeleloff, 1977). The lignin and pectin in dietary fibre appear to lower plasma cholesterol by reducing the plasma low-density lipoprotein fraction (Anderson and Chen, 1979; Behall, 1984). The mechanism of action of dietary fibre as laxative has been described as essentially devoid of any systemic effect. There are reports suggesting that some vegetables have pharmacological effects underlining their purgative action on the gastrointestinal tract (Arowolo, 1989; Dina, 2001), and hypotensive effect on the circulatory system in the body (Arowolo, 1989).

Eating a plate-full of garden egg could be another way of beating glaucoma and heart diseases. Nigerian researchers have demonstrated how a meal of garden egg would be of benefit to patients suffering from raised intraocular pressure (glaucoma) and convergence insufficiency, as well as in diseases associated with hyperlipidemia such as ishcaemic heart diseases and arteriosclerosis (Chukwuma, 2009).

Fruits of bitter cultivars are used as medicine in many African countries. Medicinal applications include the use of roots and fruits as a carminative and sedative, and to treat colic and high blood pressure; leaf juice as a sedative to treat uterine complaints; an alcoholic extract of leaves as a sedative, anti-emetic and to treat tetanus after abortion; and crushed and macerated fruits as an enema. Igbo people in south-eastern Nigeria traditionally welcome visitors into the family house by offering fruits. *Solanum aethiopicum* is sometimes cultivated as an ornamental (Schippers, 2000).

A fresh, ripe, garden egg and Solanum gilo were investigated for their possible hypolidemic potentials in hypercholesterolemia induced in New Zealand white rabbits by feeding the animals with normal diet supplemented with one per cent cholesterol and groundnut oil for three weeks (Carasco, 1999). Many anti-inflammatory plants and agents modify inflammatory responses by accelerating the destruction or antagonising the action of the mediators of inflammatory reaction. Foods and fruits rich in flavonoids and other phenolic compounds have been associated with decreased risk of developing inflammatory and other related diseases (Sies, Schewe, Heiss & Kelm, 2005). These reports, thus, suggest that the flavonoids in garden egg plant might be a major anti-inflammatory constituent in the plant.

2.13.2 Nutritional Composition of *Solanum aethiopicum*

Chemical analyses done showed the nutritional and proximate of fresh fruits of *S. aethiopicum L.* (per 100 g) showed:

Table 2: Shows proximate analysis of *Solanum aethiopicum*

Nutrients	Quantity(g)
Moisture	89.27 ± 0.12 g
Protein	2.24 ± 0.03 g
Fat	0.52 ± 0.04 g
Ash	0.87 ± 0.03 g
Crude fibre	2.96 ± 0.08 g
Carbohydrate	4.14 ± 0.11 g
Calcium	498.47 ± 2.14 mg
Magnesium	1.98 ± 0.10 mg
Iron	1.02 ± 0.02 mg

Source: (Chinedu *et al.,* 2011).

A study carried to evaluate the proximate analysis of *Solanum aethiopicum, Lactuc taraxacifolia and Talinum triangulare* purchased from Sabo market in Ogbomoso, Nigeria. The potential cytotoxic effect of their aqueous extracts was also tested on Allium cepa (L). The proximate analysis revealed that the vegetable contained valuable nutrients that are needed for body development. The protein and moisture contents were significant in S. aethiopicum and T. triangulare respectively. The heavy metal evaluation showed the presence of iron, zinc, lead, copper and magnesium ions at various degrees in the vegetable extracts with significant difference observed in all except for lead.

Heavy metal analysis showed (ppm/mg/l) of solanum aethiopicum showed Fe 0.80 (±0.03) , zn 0.99 (±0.01), Pb 0.44 (0.01), cu 0.09 (±0.02) and mg 165.2 (±23.01)

(Yekeen, Adetiba, Azeez, Falodun, Akintaro and Yekeen, 2011). A determination of Vitamin A, C, E composition of *Solanum aethiopicum* leaves showed 94.66 ± 3.22 mg/100ml, 7.34 ±1.49 mg/100ml, 1.21± 0.05 mg/100ml respectively (Achikanu *et al.,* 2013).

2.2 Definition of Nuts

A nut in botany is a simple dry fruit with one seed (rarely two) in which the ovary wall becomes very hard (stony or woody) at maturity, and where the seed remains attached or fused with the ovary wall. In a culinary context, a wide variety of dried seeds are often called nuts, but in a botanical context, only ones that include the indehiscent fruit are considered true nuts(Alasalvar, Cesarettin, Shahidi & Fereidoon, 2006).

2.21 Uses of Nuts

Nuts are very often high in nutrients because they are the source of energy for the new plant. Most nuts contain a considerable quantity of fat and vitamins and are rich in essential amino acids. The high energy density makes nuts a very filling food. Nuts are an important source of nutrients for both humans and wildlife because nuts generally have high oil content; they are a highly prized food and energy source. A large number of seeds are edible by humans and used in cooking, eaten raw, sprouted, or roasted as a snack food, or pressed for oil that is used in cookery and cosmetics. Nuts (or seeds generally) are also a significant source of nutrition for wildlife. This is particularly true in temperate climates where animals such as jays and squirrels store acorns and other nuts during the autumn to keep from starving during the late autumn, all of winter, and early spring. Nuts used for food, whether true nut or not, are among the most common food allergens.

Several epidemiological studies have revealed that people who consume nuts regularly are less likely to suffer from coronary heart disease (CHD) (Kelly & Sabate, 2006). Nuts were first linked to protection against CHD in 1996 (Sabate *et al.*, 1993). Since then many clinical trials have found that consumption of various nuts such as almonds and walnuts can lower serum LDL cholesterol concentrations. Although nuts contain various substances thought to possess cardioprotective effects, scientists believe that their Omega 3 fatty acid profile is at least in part responsible for the hypolipidemic response observed in clinical trial (Rajaram, Hasso, Mejia & Sabaté, 2009).

In addition to possessing cardioprotective effects, nuts generally have a very low glycemic index (GI) (Mendosa, 2002). This is a result of their high fat and protein content and relatively low carbohydrate levels. Consequently, dietitians frequently recommend nuts be included in diets prescribed for patients with insulin resistance problems such as diabetes mellitus type 2 (Josse, Kendall, Augustin, Ellis & Jenkins, 2007).

One study found that people who eat nuts live two to three years longer than those who do not (Fraser & Shavlik, 2001) .However, this may be because people who eat nuts tend to eat less junk food. Nuts contain the essential fatty acids linoleic and linolenic acids, and the fats in nuts for the most part are unsaturated fats, including monounsaturated fats. Many nuts are good sources of vitamin E and B_2, and are rich in protein, folate, fiber, and essential minerals such as magnesium, phosphorus, potassium, copper, and selenium (Etherton, Poth, Sabate, Ratcliffe, Zhao, & Etherton, 1999). Nuts are most healthy in their raw form. The reason is that up to 15% of the healthy oils that naturally occur in nuts are lost during the roasting process (Ballie *et al.,* 2009). All grains, nuts, seeds and legumes, if not fermented, must also be cooked

long enough to break down their fibers, since human do not have digestive enzymes to break them down like herbivores (plant eating animals) i.e. cows.

2.22 Classification of Nuts

The Seeds, Peanuts, Corn and Chickpeas are referred to as Nuts.

i. Seeds: A wide variety of dried seeds are called nuts, but in a botanical context, only ones that include the indehiscent fruit are considered true nuts (Black, Michael, Halmer & Peter, 2008). Most seeds come from fruits that naturally free themselves from the shell, unlike nuts such as hazelnuts, chestnuts, and acorns, which have hard shell walls and originate from a compound ovary. Examples of seeds are Almonds edible seeds of drupe fruits, Brazil nut is the seed from a capsule, Candlenut (used for oil) is a seed, Cashew is the seed of an accessory fruit, Chilean hazelnut or Gevuina , Macadamia is a creamy white kernel of a follicle type fruit, Malabar chestnut, Pecan is the seed of a drupe fruit etc (Alasalvar, Cesarettin, Shahidi & Fereidoon, 2010).

ii. The Peanut or Groundnut (*Arachis hypogaea*): They are species in the legume or "bean" family (Fabaceae). The peanut were probably first domesticated and cultivated in the valleys of Paraguay (Michael, 2006). It is an annual herbaceous plant growing 30 to 50 cm (1.0 to 1.6 ft) tall. The leaves are opposite, pinnate with four leaflets (two opposite pairs; no terminal leaflet). A peanut is definitely both a nut and legume since the pods splits into two and contains edible seed that has both a nutshell and a nutmeat (Seijo *et. al.,* 2007).

2.23 Factors that affect the Nuts Consumption

Frequency and quantity of nut consumption have been documented to be higher in vegetarian than in non vegetarian populations. In a large, prospective epidemiologic

study of seventh-day Adventists in California was found that frequency of nut consumption had a substantial and highly significant inverse association with risk of myocardial infarction and death from IHD (ischemic heart disease risk). Nuts are not considered the most desirable food to protect against heart diseases because they are very high in fat (Sabate *et al.,* 1993).

2.24 *Irvingia gabonensis*

Irvingia gabonensis is specie of African trees in the genus Irvingia, sometimes known by the common names as wild mango, African mango, bush mango, dika or ogbono. They bear edible mango-like fruits, and are especially valued for their fat and protein rich nuts (Ngondi, Oben, Minka & Samuel, 2006). Irvingia gabonensis (bush mango) is a large evergreen tree commonly found in West and Central Africa. The geographical distribution of the species extends from the Casamance region (Senegal) to Angola and it is found in moist semi-deciduous forests. It does not exist in swampy areas. It is found in most parts of Cameroon. The fruits are greenish yellow with fleshy fibrous pulp surrounding a large hard stone.

Irvingia gabonensis is commonly referred to as Bush Mango or African Mango, and belongs to the Irvingiaceae plant family (Lamorde, 2010). It is a wild forest tree 15-40m with a bole slightly buttressed, possessing dark green foliage and yellow flowers. The fruits of *Irvingia gabonensis* is smooth yellow spheres with a hardened endocarp when ripe, possess seeds which are of interest for the usage of food products and especially as a soup thickener, pharmaceutical formulations, and cosmetics. These fruits are sometimes referred to as 'Mangoes' (the synonym of African Bush Mango). They are unrelated, because the true Mango fruits are borne from the plant Mangifera indica of the plant family Anacardiacea (Akubor, 1996). As mangoes sometimes they

grow in Africa, the differentiation between the two plants becomes important. *Irvingia gabonensis* is a plant, and the fruits it bears are sometimes referred to as the 'Mangoes' or 'African Bush Mangoes' despite being unrelated to the true Mango fruit (Sun & Chen, 2012). *Irvingia gabonensis* plays an important role in the rural Nutrition, Income and Traditional Medicine branches of western to southwestern tropical Africa from Nigeria to Angola (Lowe, Gillies, Wilson & Dawson, 2000).

2.24.1 Uses of *Irvingia gabonensis*

Through the assistance of the United Nation in Forestry and Agricultural Projects for Africa, a lot of researches have been made to enhance the production and commercial use of this plant as nutritional, income yielding and medicinal product for African countries. Many of these researches have been done contrary to the intentions of the United Nation to increase forestry and food in Africa by experiments with the fresh leaves and stem bark. The use of the fresh leaves and stem bark as commercial products rapidly lead to destruction of the plant and forest. This would cause scarcity of the fruits and seeds as nutritional products for these countries which is against the campaign of the United Nation to enhance forestry and food in Africa. That is why, it is important to advise all those interested in the functions and use of *Irvingia gabonensis* to avoid all products made from the fresh leaves and stem bark because most functions attributed to these products are functions derived from the fruit and seed products and even more as described in other topics of this article (Abdulrahman, 2006).

The fruits of this plant are eaten raw. The fruits are processed into jelly, jam, juice and sometimes wine. The juice produces a quality wine at 8% alcohol content after 28 days of fermentation that in a study was comparable in color, flavor, sweetness, and

acceptability to a German reference wine (Leakey *et al.,* 2005). The pulp is also used to prepare black dye for cloth colouration. Compared to the seed, the fruit is only a tiny resource. The episperm is cracked open to get to the seed. Seeds, also called dika nuts. They are eaten raw or roasted. Mostly however they are pounded to butter or a chocolate like block. Seeds are pressed to produce an edible oil (solid at ambient temperatures) or margarine which is used for cooking. The oil can be processed further to soap, cosmetics or pharmaceuticals. The press cake can be used as cattle feed or as thickening agent for soup (Leakey *et al.,* 2005). Seeds are ground or crushed and used as thickening and flavouring agent in soups and stews. The food thickening property is thought to be caused by mucilaginous polysaccharides which become more viscous with cooking and its drawbility. They can also be made into a cake called dika bread for preservation. The kernel is used in preparing sauces. The fruit pulp is eaten and the kernel is used for medicinal purposes and as a source of oil for making soap (Ngondi, Oben, Minka & Samuel, 2005)

2.24.2 Nutritional Composition of *Irvingia gabonensis*

The proximate analysis (moisture, crude protein, crude fat, mineral ash and total carbohydrates) in the kernels of African Bush Mango (*Irvingia gabonensis*) were investigated. The results revealed that the kernels contained moisture (2.5 g/100 g), crude protein (8.9 g/100 g), crude fat (68.4 g/100 g), mineral ash (2.3 g/100 g) and total carbohydrates (18.7 g/100 g) (Ogunsina, Bhatnagar, Indira & Radha, C, 2012).

The approximately fatty acid composition is myristic acid 33-70%, laureic acid 20-59%, oleic acid 1-11%,palmitic acid 2% and stearic 1%. Works available have shown that, the seeds contain protein and fat. Fat is the most abundant component of kernels (70%) particularly two saturated fatty acids are 51.87% of myristic acid and 38.44% of

lauric acid. Its amino acids are reasonably adequate for human nutrition. Lysine, tryptopham, valine, threonine, isoleucine and phenylalanine have high concentrations in this seed. The role of dietary saturated fatty acids on the plasma level and low-density lipoprotein (LDL) metabolism was investigated mainly in animals and humans. Saturated fatty acids (myristic acid) are generally considered to induce the increase in plasma cholesterol, especially in the LDL-cholesterol concentration. In most studies which lead to this conclusion lauric, myristic and palmitic acids such as dairy fat and tropical oils, were considered the most noxious of fats. It is well known that this various fatty acids in the diet exert different effects on serum lipid and lipoprotein concentrations (Leakey *et al.,* 2005).

For its usage as a food, the nutritional breakdown of *Irvingia Gabonensis* seeds appears to be:

- 6.2mg Vitamin C per 100g reduced to 2.2+/-0.3 after heat treatment (Oboh & Ekperigin, 2004).
- 3.5-3.8mg iron per 100g
- 120-127mg calcium per 100g
- Magnesium (429+/-0.3ppm dry weight)
- Zinc (5.7+/-0.2ppm) (Onimawo, Oteno, Orokpo & Akubor, 2003).
- Potassium (587+/-0.4ppm) (Oboh & Ekperigin, 2004).

Besides these mentioned, Irvingia gabonensis contain traces of thiamin, riboflavin and niacin (Zoue, 2013). A study carried on the mean values of elemental concentrations in seed, seed coat and pulp of wild mango fruit.

Table 3: Shows the mean values of elemental concentrations in seed, seed coat and pulp of wild mango fruit

Elem.	Conc.	Pulp (Mesocarp)	Seed coat (Endocarp)	Seed
Al	ppm	28.13±1.33	45.51±1.37	35.67±1.39
As	ppm	BDL	10.45±0.34	8.22±0.98
Cl	ppm	507.79±26.61	1283.04±36.12	426.39±23.74
Co	ppm	0.323±0.022	0.739±0.023	0.836±0.032
Cu	ppm	44.12±3.97	11.93±0.55	57.22±4.20
Fe	ppm	64.58±5.23	1730.8±180.83	193.74±17.82
I	ppm	0.310±0.003	0.569±0.040	0.317±0.025
K	%	0.303±0.011	1.331±0.022	0.723±0.042
Mg	%	0.014±0.004	0.012±0.006	0.048±0.008
Mn	ppm	81.29±0.61	136.71±0.80	70.97±0.63
Na	ppm	74.71±1.23	81.39±1.22	43.83±1.06
Zn	ppm	6.72±0.62	63.54±5.99	57.86±5.50

Source: Ayivor, Debrah, Nuviadenu, and Forson, (2011).

2.3 Phytochemicals

The word phytochemical means plant chemicals. Many phytochemicals are believed to have a major positive impact on human health. They are non-nutritive plant chemicals that have protective or disease preventive properties against bacteria, viruses and fungi. They are non-essential nutrients, meaning that they are not required by the human

body for sustaining life. Plants produce these chemicals to protect themselves (Arts & Hollman, 2005). They also protect humans against diseases. Different types of phytochemicals are

i) Alkaloids

They are heterogenous group of naturally occurring compounds found in leaves, bark, roots or seeds of plants. Some alkaloids stimulate the nervous system. They act as pain relievers while some contain anti-microbial properties (Mcnaught & Wikinson, 1997).

ii) Saponins

They inhibit cancer cells and they are able to do it without killing normal cells in the process (www.aicr.org). It is also researched for cholesterol control. One of the prominent research programme on this subject was Rene Malinow, Orogen Regional primate center that demonstrated unequivocally the cholesterol lowering properties of saponins. Saponin causes a depletion of body cholesterol by preventing its reabsorption to its excretion, in much the same way as other cholesterol lowering drugs. Saponins are useful for treatment of hypercholesterolemia (Bodnar, 2007). They are plants active immune system.

iii. Tannins

Tannins have been implicated with various pharmaco-therapeutic effects (Ferreira, 2008). Tannins, in the form of proanthocyanidins, have a beneficial effect on vascular health. Topical applications of tannins drain out all irritants from the skin. They are useful as anti-inflammatory agent and in the treatment of burns and other wounds

based on their anti hemorrhagic and antiseptic potentials (Ketzis, 2006). They prevent urinary tract infection by preventing bacteria from adhering to the walls.

2.31 Phytochemical of *Irvingia gabonensis*

Fadare and Ajaiyeoba (2008) studied on Phytochemical and antimicrobial activities of the wild mango (*Irvingia gabonensis)* extracts and fractions. The phytochemical screening of the plant materials revealed the presence of tannins, saponins, alkaloids and the absence of cardiac glycosides. Thin layer chromatography indicated the presence of phenolic compounds. Irvingia gabonensis seeds also tend to contain:

- Ellagic acid, and methylated derivates thereof as well as their glycosides
- Ellagitannin structures
- Quercetin-3-O-rhamnoside
- Kaempferol-3-O-glucoside
- Possibly a diosmetin content

Sources: (Sun &Chen, 2012)

2.32 Phytochemicals of *Solanum aethiopicum*

Shalom *et al.,* (2011) reported that there was a significant presence of alkaloids, saponins, flavonoids, tannins and ascorbic acid in both fruits of solanum aethiopicum and solanum macrocarpon L. The terpenoids was found in trace amount, the steroids were present in S. aethiopicum L. and absent in S. macrocarpon L. These phytochemicals are of therapeutic importance and their presence in S. aethiopicum and S. macrocarpon fruits indicate the beneficial effects of the plants. Solanum

aethiopicum L. contained higher levels of the beneficial agents than S. macrocarpon L. The two indigenous eggplants are not only nutritionally and therapeutically valuable, but also have the potential providing precursors for the synthesis of useful drugs. Trease and Evans (2008) carried out a phytochemical analysis on the *Solanum aethiopicum* extract and its assayed includes alkaloid, glycosides, steroid, terpenoids, flavonoids, tannins, resins and saponins.

2.4 Anti-nutrients

They are natural compounds in plants which act to reduce nutrient utilization or food intake. It is capable of precipitating deleterious effect in man and animals. These substances disrupt certain physiological function in the body such as interference with digestion, depressed growth, pancreatic hypertrophy and interference with absorption of some micronutrients (Agbaire & Emoyan, 2011).

i) Phytate

They are naturally occurring organic complex found in plants. As much as 60-80% of phosphorus found in cereal grains and oil seeds exist as phytic acid (Simons & Verteegh, 1990).

ii) Oxalate

Oxalate in plants bind minerals and makes them less available to the body. Spinach, for example contains plenty of calcium, only about 5% of it is absorbed. This is because vegetable contains high concentration of oxalic acid (Liu, 2004).

2.41 Anti-nutrient of *Irvingia gabonensis*

The antinutrient composition of bush mango seed showed phytate, tannins and oxalate. Phytate (1043.6+/0.2mg/g)(Oboh & Ekperigin, 2004). Ekpe, Umoh & Eka (2007) also discovered that phytate and oxalate content reduced in *Irvingia gabonensis* after fermentation while tannin content increased. A wide range of microflora has been known to possess phytase activity which may be partly responsible for reduction in phytic acid content in the fermenting samples (Ojokoh, 2007).

2.42 Anti nutrient of *Solanum aethiopicum*

Eggplants (Solanum spp.) are important fruit vegetables that are mostly eaten in the unripe stage. The analyses of lycopene, glutathione and vitamin E contents of Solanum melongena and Solanum aethiopicum showed that these antioxidants increase from the unripe to the overripe stage during ripening. The overripe stage of the fruits was found to contain the highest level of lycopene. The overripe stages of these fruits are good sources of these important natural antioxidants that have nutritional and health benefits (Achikanu, Eze-Steven, Ude & Ugwuokolie, 2013). Richelle, Tavazz and Offord (2001) carried a study on a phytochemical Composition of *Solanum nigrum* L. leaves showed it contains phytic acid 0.82±0.01, Oxalate 78.65±0.04.

2.5 Concepts of Diabetes Mellitus

Diabetes mellitus is a group of metabolic diseases characterized by increased levels of glucose in the blood (hyperglycemia) resulting from defects in insulin secretion, insulin action or both (American Diabetes Association (ADA), 2004). Normally a

certain amount of glucose circulates in the blood. The major sources of glucose are absorption of ingested foods in the gastrointestinal tract and formation of glucose by the liver from food substances (Suzanne, Brenda, & Kerry, 2008).

According to Falase(1987), deficiency(absolute or relative), of insulin leads to diabetes mellitus, the most common endocrine disorder in the tropics and the world(Frazier & Dryzmkwoski, 2000) stated that Diabetes mellitus is a chronic disorder of carbohydrate, fat and protein metabolism caused by inadequate production of insulin by the pancreas or faulty utilization of insulin by the cells. Insulin lowers level of glucose in the blood by transporting glucose into the cells for use as energy and storage as glycogen. Cells therefore are deprived of fuel and they begin to metabolize fats and protein, this process allows wastes called ketone bodies to accumulate in the blood (Ketosis).

2.51 Classification of Diabetes Mellitus

Diabetes Mellitus can be broadly classified as follows:

- Type 1 or juvenile diabetes mellitus
- Type 11 or adult onset diabetes mellitus
- Gestational diabetes mellitus
- Others/secondary diabetes mellitus

i. Type 1 or Juvenile Diabetes Mellitus

This type is insulin dependent with an early onset, usually before 40yrs of age with little or no insulin being secreted by the patient and can be difficult to control(Stoller, Ahmad & Longworth 2002). Therefore individuals with this type of insulin depend totally on exogenous insulin. Due to absence of insulin production, persons with this

diabetes are prone to ketoses secondary to hyperglyceamia. In ketosis, the lack of insulin leads to the bodyôs inability to metabolise glucose for energy. As a result, fatty acids are incompletely oxidized leading to accumulation of ketones (Kneisi & Ames, 1986). Type 1 diabetes can affect children and adults but was traditional termed juvenile diabetes because it represents a majority of diabetes in children (Wikipedia free encyclopedia). It is less common type of diabetes (Tierney, Mcphee & Papadakis, 2002). In many cases of this type of diabetes, the individual appears to inherit a defect in which immune cells mistakenly attack and destroy insulin-producing pancreatic cells).

ii. Type 11 or Adult Onset Diabetes Mellitus

This type is also called non-insulin dependent diabetes mellitus (NIDDM) has a gradual onset in adults older than 40yrs of age. It is a common type of diabetes that develops gradually and is associated with insulin resistance. Type 11 diabetes mellitus accounts for approximately 85% of the diabetic population at present; Type 11 diabetes is believed to be the result of many years of insulin resistance, leading to disordered pancreatic insulin secretion and in turn fastens hypoglycemia (Stooler, Ahmad & Longworth, 2002).

Quite often, weight gain (particularly central obesity), physical inactivity, and a high fat exaggerate the insulin resistance and may accelerate the development of type 11 diabetes mellitus may require insulin therapy for improved control of glucose levels.

In most westernized countries, the risk for the development of type 11 diabetes mellitus continues to increase with age, resulting in an approximately 30% prevalence of type 11 diabetes mellitus in the geriatric population. This type of diabetes is

associated with family history of diabetes, older age, obesity and lack of exercise (Stooler *et al.,* 2002).

iii. Gestational Diabetes Mellitus (GDM)

Gestational diabetes is characterized by a diabetic state first detected during pregnancy (Stooler *et al.,* 2002). According to Iwueze (2007), gestational diabetes is any degree of glucose intolerance with its onset during pregnancy. Hyperglycemia develops during pregnancy because of secretion of placental hormones which cause insulin resistance. Gestational diabetes occurs in up to 14% of pregnant women and increases the risk of hypertensive disorders during pregnancy (Iwueze, 2007). Most western women experience deterioration in glucose tolerance during pregnancy. The metabolic changes which occur during pregnancy are accompanied by a reduction in sensitivity to insulin. The reduction in sensitivity to insulin can be up to 40% (Iwueze, 2007). The pancreas in most pregnant women is able to produce extra insulin to compensate for this with only 2 or 3% of all pregnant women developing gestational diabetes. It is usual for women to be screened for glucosuria at routine antenatal appointments. If glucosuria is detected, then the presence of gestational diabetes is confirmed by a glucose tolerance test. The symptoms of gestational diabetes are the same as for IDDM and NIDDM, in addition to which there is an increased risk of prenatal mortality and post partum hypoglycemia. Maternal hyperglycemia enhances the insulin produced by the fetus. The result of this is that the baby is likely to be larger. The blood glucose level returns to normal after delivery.

iv. Others/Secondary Diabetes

Other types of diabetes can occur as a consequence of genetic disorders, diseases of the exocrine pancreas, hormonal imbalances, drugs or chemicals, certain infections and immune system disorder (Whitney, 2001).

2.52 Causes of Diabetes Mellitus

The major cause of diabetes is still unclear. However, several contributory factors are considered important to it. These factors include hereditary, obesity, age and physical inactivity (Whitney, 2001) lack of insulin secretion in diabetes could be as a result of pancreatitis, pancreatectomy or any other disease of the pancreas. Destruction of the pancreas by tumor, trauma to the pituitary gland or other endocrine disorders can induce diabetes mellitus. Some genetic disorders render the bodyôs insulin receptors insensitive to insulin (Frazier & Drzymkwowski, 2000).

Drugs may also cause hyperglycemia these includes glucocorticoids, thiazide diuretics, phenytoin (Dilantin), interferon alpha, pentamidine(pentam 300 or Nebu pent and diazoxide (Proglycem or Hyperstat i.v) (Stoller *et al*., 2002)

2.53 Clinical Manifestation of Diabetes

Classical clinical manifestation of all diabetes includes the three Pôs: Polydipsia, Polyuria and polyphagia. Polyuria (increased urination) and Polydipsia(increased thirst) occur as a result excess loss of fluid associated with osmotic diuresis. Patients also experience polyphagia (increased appetite) resulting from the catabolic state induced by insulin deficiency and the breakdown of proteins and fats. Other symptoms include fatigue and weakness, sudden vision loss changes, tingling or numbness in hands and feet, dry skin, skin lesions or wounds that are slow to heal and recurrent

infections. The onset of type 1 diabetes may also be associated with sudden weight loss or nausea and vomiting or abdorminal pains if diabetic ketoacidosis(DKA) has developed (Suzanne *et al.*, 2008).

2.54 Prevalence of Diabetes

Diabetes mellitus is an epidermic of our time the disease affect the young as well as the old men and women, rich and poor. Report abound that the prevalence has been increasing over the past 30yrs (Akogu, 2009, Ngwu & Nwanbunze, 2008). The progressive increase in prevalence rate is associated with lifestyle changes and cigarette smoking. The prevalence of the disease for all the age groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030 (Nyenwe, Odia, Ihekaba, Ojule & Babatunde, 2003). In the United States it affects more than 7million people and as many as 8 million others may not be aware that they have diabetes. It has become a widespread disease affecting one of every 20persons of these, 15% are IDDM and NIDDM type (Paul, 2005). Diabetes complications have now become the fifth ranking cause of death in USA. Of the total 171 million diabetics in the world, India alone accounts for 33 million (Paul, 2005). According the Federal Ministry of Health and Social Services 1997, a national study of non-communicable diseases in Nigeria showed that 2.8% of the population had diabetes. The prevalence was higher in females and in those with increasing age.

2.55 Diagnosis of Diabetes

The diagnosis of diabetes is usually promoted by recent symptoms of Polyuria (excessive urination) and Polydipsia (excessive thirst) often accompanied by weight

loss. These symptoms typically worsen over days or weeks and some patients (especially type 1 diabetes) must have developed some degree of diabetic ketoacidosis by the time the diabetes is recognized.

According to the World Health Organization, Department of Non-communicable disease surveillance (WHO, 1998), diabetics Mellitus is characterized by recurrent or persistent hyperglycemia and is diagnosed as follows:

i. Fasting plasma glucose level at or above 126mg (7.0mmol/l). This test measures blood glucose in people who have not eaten for at least for at least 8 hours or after an overnight fast. Most physicians prefer to measure a fasting glucose level because of the ease of measurement and the considerable time commitment of the formal glucose tolerance test, which takes 2hrs to complete and offers no diagnostic advantage over the fasting state (Saydah *et al.*, 2001). (7.0mmol/l) is considered diagnostic for diabetic mellitus. People with fasting glucose level from 100 to 125mg/dl (6.1 and 7.0mmol/l) are considered to have impaired fasting glucose.

ii. Plasma glucose at or above 200mg/dl (11.1mmol/l) two hours postprandial or after a 75g oral glucose, which is a glucose tolerance test. This is usually confirmed by repeat of another test on a different day. Patients with plasma glucose at or above 140mg/dl or 7.8mmol/l but not over 200mg/dl two after a 75g oral glucose load are considered to have impaired glucose tolerance. Impaired glucose tolerance is a major factor for progression to full-blown diabetes mellitus as well as cardiovascular diseases (Santaguida *et al.,* 2008).

iii. Symptoms of hyperglycemia and casual plasma glucose at or above 200mg/dl (11.1mmol/l). This test is carried out any time of the day without regard to the time of last meal.

2.6 Concept of Anemia

Anemia is a condition in which the number of red blood cells (consequently their oxygen-carrying capacity) is insufficient to meet the bodyôs physiologic needs. Specific physiologic needs vary with a personôs age, gender, residential elevation above sea level (altitude), smoking behaviour, and different stages of pregnanc. Iron deficiency is thought to be the most common cause of anemia globally, but other nutritional deficiencies (including folate, vitamin B12 and vitamin A), acute and chronic inflammation, parasitic infections, and inherited or acquired disorders that affect haemoglobin synthesis, red blood cell production or red blood cell survival can all cause anemia (Eldridge, 2014). Anemia is a general term referring to the condition characterized by abnormally low levels of healthy red blood cells or hemoglobin. The most common inherited form of anemia is thalassemia. These forms of anemia primarily affect those of African, Southeast Asian, and Mediterranean heritage. Sickle cell anemia is another more serious inherited form of anemia which affects those of African and Mediterranean heritage and can lead to chronic fatigue and potentially life threatening sickle cell crises. Regarding dietary causes of anemia, the most common kind of anemia includes iron deficiency anemia, however, deficiencies of folic acid, B12, and Vitamin C can also lead to low levels of hemoglobin (Rolfes, Pina &

Whitney, 2009). These data reflect anemia as measured by hemoglobin status, of which the WHO estimates that 50% are caused by iron deficiency anemia (hereafter referred to as anemia). Anemia caused by dietary factors is a disease that is readily preventable and treatable. Concepts of disease are influenced by education, economics and most importantly by culture (WHO, 2001).

2.61 Classification of Anemia

The types of anemia we have are

i. Iron Deficiency Anemia (IDA)

The most common form of anemia is iron deficiency anemia which is usually due to chronic blood loss caused by excessive menstruation, increased demands for iron such as foetal growth in pregnancy and children undergoing rapid growth spurts in infancy and adolescence can also cause iron deficiency anemia. This condition is treated with iron supplementation as well as the treatment of the underlying cause of the iron deficiency (Adamson & Longo, 2008).

a. Causes of Iron Deficiency Anemia

Iron deficiency occurs when the rate of loss or use of iron is more than its rate of absorption and use (Kasper & Fauci, 2006). The reasons for this are

- Chronic blood loss: Most commonly due to excessive menstruation or bleeding into or from the gut as a result of a peptic ulcer, gastritis, haemorrhoids or in children, worm infestation.

- Increased use of iron: In pregnancy, due to the growth of the foetus or children undergoing rapid growth spurts in infancy and adolescence.
- Decreased absorption of iron
    - after a partial or total removal of the stomach;
    - lack of stomach acid;
    - chronic diarrhoea
    - malabsorption.

b. Signs and Symptoms of Iron Deficiency Anemia

The most common symptoms of chronic anemia include tiredness, weakness, shortness of breath and sometimes, a fast heartbeat. The tongue may also become smooth, shiny and inflamed and this is called glossitis. Angular stomatitis (erosion, tenderness and swelling at the corners of the mouth) may also occur. In some instances, the patient also suffers from pica, a craving for strange foods such as starch, ice and clay (Galloway *et. al.*, 2002).

ii. Aplastic Anemia

Aplastic anemia is a blood disorder in which the body's bone marrow doesn't make enough new blood cells. This may result in a number of health problems including arrhythmias, an enlarged heart, heart failure, infections and bleeding. Aplastic anemia is a rare but serious condition (Jarrah, Halabi, Bond & Abeggien, 2007). It can develop suddenly or slowly and tends to worsen with time, unless the cause is found and treated.

a. Causes of Aplastic Anemia

Damage to the bone marrow's stem cells causes aplastic anemia. In more than half of people who have aplastic anemia, the cause of the disorder is unknown (Pawloski & Moore, 2004).

A number of acquired diseases, conditions, and factors can cause aplastic anemia including

- Toxins, such as pesticides, arsenic, and benzene
- Radiation and chemotherapy
- Medicines such as chloramphenicol
- Infectious diseases such as hepatitis, Epstein-Barr virus, cytomegalovirus, parvovirus and HIV
- Autoimmune disorders such as lupus and rheumatoid arthritis

b. Signs and Symptoms Aplastic Anemia

The most common symptoms of aplastic anaemia are fatigue, shortness of breath, dizziness, headache, coldness in your hands or feet, pale skin, gums and nail beds and chest pains (Maxwell, 2005).

iii. Haemolytic Anemia

Haemolytic anemia is a condition in which red blood cells are destroyed and removed from the bloodstream before their normal lifespan is up. A number of diseases, conditions and factors can cause the body to destroy its red blood cells. Haemolytic anemia can lead to various health problems such as fatigue, pain, arrhythmias, an enlarged heart and heart failure (Young, 2005).

There are many types of haemolytic anemia, some of which are inherited and others that are acquired. Inherited haemolytic anemia include, Sickle cell anemia, Thalassaemias Hereditary spherocytosis, hereditary elliptocytosis, Glucose-6-phosphate dehydrogenase (G6PD) deficiency, Pyruvate kinase deficiency While acquired haemolytic anemia include Immune haemolytic anemia, Autoimmune haemolytic anemia, Alloimmune haemolytic anemia, Drug-induced haemolytic anemia, Mechanical haemolytic anemia, Paroxysmal nocturnal haemoglobinuria, Certain infections and substances can also damage red blood cells and lead to haemolytic anemia.

a. Causes of Haemolytic Anemia

The immediate cause of haemolytic anaemia is the early destruction of red blood cells. A number of diseases, conditions, and factors can cause the body to destroy its red blood cells. These causes can be inherited or acquired. Sometimes, the cause of haemolytic anaemia isn't known (Pierce, 2006).

- In inherited haemolytic anaemia, the genes that control how red blood cells are made are faulty. Different types of faulty genes account for the different types of inherited haemolytic anaemia. In each type of inherited haemolytic anaemia, the body makes abnormal red blood cells. The problem with the red blood cells may involve the haemoglobin, cell membrane, or enzymes that maintain healthy red blood cells.
- In acquired haemolytic anaemia, the body makes normal red blood cells; however, some disease, condition, or factor destroys the cells too early. Examples include immune disorders, infections and reactions to medicines or blood transfusions.

b. Signs and Symptoms of Haemolytic Anemia

The most common symptom of all types of anemia is fatigue. A low red blood cell count can also cause shortness of breath, dizziness, headache, coldness in your hands or feet, pale skin, gums and nail beds, as well as chest pain.

Symptoms of haemolytic anemia include jaundice, pain in the upper abdomen, leg ulcers and pain and a severe reaction to a blood transfusion.

iv. Sickle Cell Anaemia

Sickle cell anaemia is a serious disease in which the body makes sickle-shaped ("C"-shaped) red blood cells. Normal red blood cells are disk-shaped and move easily through your blood vessels. Red blood cells contain the protein haemoglobin (an iron-rich protein that gives blood its red colour and carries oxygen from the lungs to the rest of the body). Sickle cells contain abnormal haemoglobin that causes the cells to have a sickle shape, which donôt move easily through the blood vessels ï they are stiff and sticky and tend to form clumps and get stuck in the blood vessels (Pierce, 2006).

The clumps of sickle cells block blood flow in the blood vessels that lead to the limbs and organs. Blocked blood vessels can cause pain, serious infections, and organ damage. In sickle cell anaemia, a lower-than-normal number of red blood cells occur because sickle cells don't last very long. Sickle cells usually die after about 10 to 20 days and the body can't reproduce red blood cells fast enough to replace the dying ones, which causes anemia (Karanagh, Sprinz, Vinci, Baachner, Wang, 2011).

a. Causes of Sickle Cell Anemia

Sickle cell anemia is an inherited, lifelong disease. People who have the disease inherit two copies of the sickle cell gene one from each parent.

b. Signs and Symptoms of Sickle Cell Anemia

The most common symptoms of sickle cell anemia are linked to anemia and pain. Common symptoms for anemia include, Fatigue, Shortness of breath, Dizziness, Headache, Coldness in the hands and feet, Pale skin, Chest pain. Sudden pain throughout the body is a common symptom of sickle cell anemia. This pain is called a "sickle cell crisis", and often affects the bones, lungs, abdomen, and joints (Malowany & Butany, 2012).

v. Pernicious Anemia

Pernicious anemia is a condition in which the body can't make enough healthy red blood cells because it doesn't have enough vitamin B12 (a nutrient found in certain foods). People who have pernicious anemia can't absorb enough vitamin B12 due to a lack of intrinsic factor (a protein made in the stomach). However, other conditions and factors can also cause vitamin B12 deficiency.

a. Causes of Pernicious Anemia

- A lack of intrinsic factor is a common cause of pernicious anaemia as the body can't absorb enough vitamin B12.
- Some pernicious anemia occurs because the body's small intestine can't properly absorb vitamin B12 which may be due to the wrong bacteria in the small intestines; certain diseases that interfere with vitamin B12 absorption;

certain medicines; surgical removal of part of the small intestine; and tapeworm infection.

- Sometimes people develop pernicious anemia because they don't get enough vitamin B12 in their diets.

b. Signs and Symptoms of Pernicious Anemia

Apart from the symptoms of anemia (fatigue, dizziness, etc.), the vitamin B12 deficiency may also have some serious symptoms such as

- Nerve damage
- Neurological problems such as confusion, dementia, depression, and memory loss.
- Symptoms in the digestive tract include nausea and vomiting, heartburn, abdominal bloating and gas, constipation or diarrhoea, loss of appetite, and weight loss.
- An enlarged liver
- A smooth, beefy red tongue
- Infants who have vitamin B12 deficiency may have poor reflexes or unusual movements, such as face tremors (Ramani, James, Richaw & Pramila, 2007).

2.62 Prevalence of Anemia

Anemia is defined as haemoglobin concentration below established cut off levels, is a widespread public health problem with major consequences for human health as well as social and economic development. It can be assumed that in resource poor areas significant proportions of young children and women of child bearing age are anemic. WHO (2013) estimates the number of anemia people worldwide to be staggering two

billion and that approximately 50% of all anemia can be attributed to iron deficiency. Women, particularly pregnant women, and children are most at risk of anemia worldwide, and the WHO suggests that 52% of pregnant women, 42.3% of all women, and 48% of children are anemic in developing countries (WHO, 2001).

Table 4: Prevalence of Anemia

Population group	**Prevalence of anemia**		**Population affected**	
	Percentage	**95% CI**	**Number(millions)**	**95% CI**
Preschool-age children	47.4	45.7-49.1	293	283-303
School-age children	25.4	19.9-30.9	305	238-371
Pregnant women	41.8	39.9-43.8	56	54-59
Non-pregnant women	30.2	28.7-31.6	468	446-491
Men	12.7	8.6-16.9	260	175-345
Elderly	23.9	18.3-29.4	164	126-202
Total population	24.8	22.9-26.7	1620	1500-1740

Source: (Benoist, 2005). **CI:** (Confidence interval)

2.63 Diagnosis of Anemia

Haemoglobin concentration alone cannot be used to diagnose iron deficiency. However, the concentration of haemoglobin should be measured, even though not all anemia is caused by iron deficiency. Hemoglobin assessments are used widely to screen individuals for anemia, to draw inferences about the iron status of the persons, and to evaluate responses to nutritional interventions (Bridges, Parvin & Assendelft, 1987).

The methods of heamoglobin assessment are:

i) The blood Hb concentration is an important method/variable directing transfusion therapy in patients suffering major blood loss due to accidents, surgery, labour and many other critical conditions. Hb concentration is measured routinely using automated hematology analyzers such as those produced by the Sysmex Corporation. Although these counters are very accurate and reliable, they are expensive and transport of the samples to the laboratory delays the process which may delay treatment, resulting in preventable deaths (Marks, Habicht & Mueller, 1989).

ii) In resource poor settings where automated hematology analyzers are not available, the Cyanmethemoglobin method is often used. This method is cheaper than the automated method and takes more time (Neville, 1987*).*

iii) In blood donations, the semi-quantitative gravimetric copper sulphate method which is very easy and inexpensive may be used, but does not provide an acceptable degree of accuracy (James, Jones, Turner & Sokol, 2003).

v) The HemoCue Hb photometer has been widely used for these purposes in recent years (Cohen & Seidl, 1988) because it is portable, requires only a small sample of capillary/venous blood, is relatively inexpensive and simple to use, does not require access to refrigeration or even electricity, and gives immediate, digitally displayed results (Jahr, Lurie, Driessen, Gosselin & Gunther, 2002).

Ethylenediaminetetraacetic acid (EDTA) blood was collected from study participants drawn from five groups: pre-school children, school children, pregnant women, non-pregnant women and men. Blood collected was immediately processed to estimate the hemoglobin concentration using three different methods (HemoCue, Sysmex KX21N and Cyanmethaemoglobin). Agreement between the test methods was assessed by the method of Bland and Altman. The Intraclass correlation coefficient (ICC) was used to determine the within subject variability of measured haemoglobin. The result shows that haemoglobin determined by the HemoCue method is comparable to that determined by the other methods. The HemoCue photometer is therefore recommended for use as on-the-spot device for determining hemoglobin in resource poor setting (Bernard *et al.*, 2011).

In rats, simple method was used in determination of A1c-type glycated hemoglobin with high performance liquid chromatography (Kondo *et al.*, 1989).The hemoglobin was eluted by a two-step gradient, and the total assay time, including re-equilibration of the column, was 27 min. Two A1c type (A minor component of haemoglobin to which glucose is bound) and one pre-A1c type rat glycated haemoglobins were separated and measured. The change in major HbA1c of rats, in which diabetes was induced by streptozotocin and which were subsequently treated with insulin, was monitored. In diabetic rats (n = 10, average blood glucose greater than 300 mg/dL),

major HbA1c rose to 3.39 +/- 0.06% compared with controls (n = 10, 1.20 +/- 0.03%) during 5 wk. Insulin treatment decreased the HbA1c from 3.48 +/- 0.16% to 2.74 +/- 0.15% ($p < 0.01$) in 6 wk.

2.7 Lipid Profile

Lipid profile is a panel of blood tests that serves as an initial broad medical screening tool for abnormalities in lipids, such as cholesterol and triglycerides. The results of this test can identify certain genetic diseases and can determine approximate risks for cardiovascular disease, certain forms of pancreatitis, and other diseases (Sidhu & Naugler, 2012). This test is used to identify hyperlipidemia (various disturbances of cholesterol and triglyceride levels), many forms of which are recognized risk factors for cardiovascular disease and rarely pancreatitis. The lipid profile is used to guide providers in deciding how a person at risk should be treated. The results of the lipid profile are considered along with other known risk factors of heart disease to develop a plan of treatment and follow-up.

2.71 Types of Lipid Profile

The test includes four basic parameters: total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides. It is usually done in fasting blood specimen. Fasting refers to 12ï 14 h overnight complete dietary restriction with the exception of water and medication.

i. HDL (High-Density Lipoprotein)

High-density lipoprotein (HDL) is one of the five major groups of lipoproteins. Lipoprotein molecules enable the transportation of lipids (fats), such as cholesterol,

phospholipids, and triglycerides, within the water around cells (extracellular fluid), including the bloodstream.

Because of the high cost of directly measuring HDL and LDL protein particles, blood tests are commonly performed for the surrogate value, HDL-C, i.e. the cholesterol associated with ApoA-1/HDL particles. In healthy individuals, about 30% of blood cholesterol, along with other fats, is carried by HDL (Toth & Peter, 2005). This is often contrasted with the amount of cholesterol estimated to be carried within low-density lipoprotein particles, LDL, and called LDL-C. HDL particles remove fats and cholesterol from cells, including within artery wall atheroma, and transport it back to the liver for excretion or re-utilization; thus the cholesterol carried within HDL particles (HDL-C) is sometimes called "good cholesterol" (despite being the same as cholesterol in LDL particles). Those with higher levels of HDL-C tend to have fewer problems with cardiovascular diseases, while those with low HDL-C cholesterol levels (especially less than 40 mg/dL or about 1 mmol/L) have increased rates for heart disease (Toth & Peter, 2005). Higher native HDL levels are correlated with better cardiovascular health (Sirtori & Cesare, 2006). However, it does not appear that further increasing one's HDL improves cardiovascular outcomes (National Heart, Lung, and Blood Institute (NHLBI), 2011). Clinical laboratories formerly measured HDL cholesterol by separating other lipoprotein fractions using either ultracentrifugation or chemical precipitation with divalent ions such as Mg^{2+}, then coupling the products of a cholesterol oxidase reaction to an indicator reaction. The reference method still uses a combination of these techniques. Most laboratories now use automated homogeneous analytical methods in which lipoproteins containing apo B are blocked using antibodies to apo B, then a colorimetric enzyme reaction measures cholesterol in the non-blocked HDL particles. HPLC can also be used (Okazaki *et. al.*, 1997).

Subfractions (HDL-2C, HDL-3C) can be measured and have clinical significance. The measurement of apo-A reactive capacity can be used to measure HDL cholesterol but is thought to be less accurate (Hirano *et al.,* 2005).

ii. LDL (Low-Density Lipoprotein)

LDL is called low-density lipoprotein because LDL particles tend to be less dense than other kinds of cholesterol particles. An LDL particle is a microscopic blob consisting of an outer rim of lipoprotein surrounding a cholesterol center (Jennifer, 2014). In a world of good and bad cholesterol, LDL is the bad one. LDL collects in the walls of blood vessels causing the atherosclerosis. Higher LDL levels put you at greater risk for a heart attack from a sudden blood clot in an artery narrowed by atherosclerosis. LDL cholesterol helps determine the risk for heart disease. If LDL cholesterol is high, treatment can reduce the chance of having a heart attack (Mora, Rifai, Buring & Ridker, 2009).

Cholesterol is not all bad. Itôs an essential fat that your bodies' cells need. Some cholesterol comes from diet and some is made by the liver. Cholesterol cannot dissolve in blood, so proteins in the blood carry it where it needs to go. These carriers are called lipoproteins. Acting like a microscopic bus fleet, lipoproteins pick up and carry loads of cholesterol through the blood. Each form of lipoprotein has different preferences for cholesterol, and behaves differently with the cholesterol it carries.

LDL cholesterol leads to plaque growth and atherosclerosis (Campose, Khoo & Sacks, 2004).

- Some LDL cholesterol tends to deposit in the walls of arteries. This process starts as early as childhood or adolescence.

- White blood cells swallow and try to digest the LDL, possibly in an attempt to protect the blood vessels. In the process, the white blood cells convert the LDL to a toxic (oxidized) form.
- More white blood cells and other cells migrate to the area, creating steady low-grade inflammation in the artery wall.
- Over time, more LDL cholesterol and cells collect in the area. The process creates a bump in the artery wall called a plaque. Plaque is made of cholesterol, the body's cells, and debris.
- The process continues, growing the plaque and slowly blocking the artery.

iii. Triglycerides

The triglyceride test measures the level of triglycerides in the blood. They are chemical compounds digested by the body to provide it with the energy for metabolism. Triglycerides are the most common form of fat that we digest, and are the main ingredient in vegetable oils and animal fat (Nordestgaar, Benn & Schnohr, 2007). The triglyceride molecule is a form of the chemical glycerol (tri=three molecules of fatty acid + glyceride=glycerol) that contains three fatty acids. To be absorbed, these parts are broken apart in the small intestine, and afterwards are reassembled with cholesterol to form chylomicrons. This is the source of energy for cells in the body. Fat cells and liver cells are used as storage sites and release chylomicrons when the body needs the energy.

Elevated triglyceride levels are a risk factor for atherosclerosis, the narrowing of arteries with the buildup of fatty plaques that may lead to heart attack, stroke, and peripheral artery disease. Normal triglyceride levels in the blood are less than 150 mg per deciliter (mg/dl). It can be controlled to some extent by lifestyle modifications and

medications when necessary. Fasting for 9 to 12 hours before the test is required (National Heart, Lung and Blood Institute, 2014).

iv. Total Cholesterol

Cholesterol is a chemical compound the body requires as a building block for cell membranes and for hormones like estrogen and testosterone. The liver produces about 80% of the body's cholesterol and the rest comes from dietary sources like meat, poultry, eggs, fish, and dairy products. Plants sources contain no cholesterol. Cholesterol content in the bloodstream is regulated by the liver. Cholesterol is absorbed from the small intestine, metabolized and stored in the liver. When too much cholesterol is present in the body, it can build up in deposits called plaque along the inside walls of arteries, causing them to narrow (Friedewald, Levy & Fredrickson, 1990).

Elevated cholesterol levels are one of the risk factors for heart disease, stroke, and peripheral artery disease. The mechanism involving cholesterol in all three diseases is the same; plaque buildup within arteries decreases blood flow affecting the function of the cells and organs that these blood vessels supply. Some food groups may be beneficial in directly lowering cholesterol levels and include foods with plant sterol additives, high fiber foods like bran, oatmeal, and fruits like apples and pears, fish, nuts, and olive oil. Some of these foods like nuts and fruits are also high in calories, so moderation is always advisable.

2.8 Review of Studies done with these Plants

2.81 *Solanum aethiopicum* leaf

Saba, Dina, Adedapo & akhiromen (2003) investigated the pharmacological reactivity of guinea pig ileum to *Solanum aethopicum* extract that is commonly consumed in Nigeria. The leaves were cut into pieces and prepared for extraction as described. The final concentration of 1g/ml was obtained as extract which served as the stock solution for dilutions needed during the course of the work. The result of the extract of *Solanum aethiopicum* elicited dose dependent contractions of guinea pig ileum. The threshold contractile response was obtained at 225± 3.52mg of aqueous extract; maximum response was attained at 3200 ± 10.01mg of the extract. Atropine (5 x 10-5) mepyramine (5 x 10-5) and cimetidine (5 x 10-5) inhibited these responses. Log dose response curve plotted showed a shift to the right and was sub maximal in the presence of atropine, mepyramine and cimetidine respectively (Fig 1). The dose ratios of ED for the extract of the vegetable alone and in the combinations of atropine (0.8), mepyramine (0.7) or cimetidine (0.9) extrapolate`d from the curve approximated unity (1.0) (Fig 1), indicating pharmacological antagonism.

Anosike, Obidoa and Ezeanyika (2012) studied the effect of methanol extract of *Solanum aethiopicum* in experimentally induced inflammation using leukocyte mobilization and vascular permeability tests in rats and human red blood cell (HRBC) membrane stabilization. Twenty five (25) adult Wistar rats of either sex (120 g - 200 g) divided into five groups of five rats each were used for each of the animal models. Groups 2, 3 and 4 were administered varied doses of the *Solanum aethiopicum* extract (100, 200 and 400 mg/kg), while groups 1 (vehicle control) and 5 (treatment control) received normal saline and indomethacin (50 mg/kg) respectively. Vascular permeability was induced by the intra-peritoneal injection of 1 ml of acetic acid and monitored using 0.5 ml intravenous injection of 1% Evans blue solution. Leukocyte mobilization was induced by the intra-peritoneal injection of 0.5 ml of 3% agar

suspension in normal saline. Heat and hypotonicity induced heamolysis of HRBC membrane was used to assess membrane stabilization. These results showed that methanol extract of *Solanum aethiopicum* has anti-inflammatory properties and can reduce inflammatory injury and tissue damage (Anosike, Obidoa & Ezeanyika, 2012).

Chioma, Obiora and Chukwuemeka (2011) evaluated the possible antiulcer effect of the African garden egg, *Solanum aethiopicum (S. aethiopicum*) (a domestic vegetable) experimentally in rats. A methanol extract of the plant fruit was prepared by maceration. Twenty five overnight fasted rats for each model were divided randomly into five groups of five rats. Groups 1, 2, 3, 4 and 5 received normal saline, extract dose levels of 100, 200 and 400 mg/kg and 100 mg/kg of ranitidine respectively. All administrations were given orally. For the indomethacin and aspirin models, ulcerogenic agents (indomethacin, 50 mg/kg and aspirin 200 mg/kg) were given thirty minutes after extract treatments and animals sacrificed 8hr later. The acidified ethanol model (ethanol 60% + 0.1 mol/L HCl) was given 1 hr after extract treatment and animals sacrificed 1 h later. Ulcer index was checked and analysed with appropriate statistical tools. Extract of S. aethiopicum showed positive effect on all the models used. It produced higher ulcer inhibition than ranitidine in the indomethacin and acid-ethanol models. All the anti-ulcer effects of the extract at different doses were dose dependent but only in indomethacin model did it produce statistically significant ($P<0.05$) ulcer reduction in all doses compared to control(Chioma, Obiora & Chukwuemeka, 2011). Garden egg, a readily cultivated crop vegetable possesses ulcer protective properties against ulcers induced experimentally making it a cheap source of natural anti-ulcer remedy.

2.82 *Irvingia gabonensis* Seed

Ngodi (2005) investigated the effect of Irvingia gabonensis extract on body weight. A study divided 40 subjects into control and intervention groups, providing the intervention group with Irvingia gabonensis extract three times a day for one month. The control group received placebos. In just one month, the intervention group lost an average of 5.26 pounds, significantly more than the placebo group's loss of 1.32 pounds. The Irvingia gabonensis seed has recently been known to promote weight loss and greater fat burning. It was tested over time and through different products.

Ngodi (2005) also conducted a study in Cameroon which showed improvement in blood lipid or blood fat levels in subjects who received Irvingia gabonensis three times daily. This included significant drops in triglycerides, cholesterol and LDL cholesterol and blood levels. The levels of HDL often called good cholesterol increased. In comparison, the placebo group did not manifest any changes in blood lipid components. The results indicate that African mango could reduce blood cholesterol.

Ngondi expanded her research in (2009) by recruiting 100 overweight individuals for the study. An intervention group received daily doses of the dika nut extract. After 10weeks, subjects had improved blood glucose. Based on results of study with African mango for the treatment of diabetes, Ngondi proposed that Irvingia gabonensis use for curbing insulin resistance needs more detailed clinical studies.

Ngondi (2009) carried a study which showed that active extracts of African mango lowered blood cholesterol and triglyceride levels, which in turn lowered the blood pressure.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection and Identification of Sample

Fresh *Solanum aethiopicum* leaves and dried *Irvingia gabonensis* seed were used for this study. The samples were bought from Ogige market in Nsukka L.G.A of Enugu State. They were identified in the Department of Plant Science and Biotechnology, University of Nigeria Nsukka.

3.2 Processing of the Samples

Fresh *Solanum aethiopicum* leaves were plucked and sorted by removing extraneous materials, washed with clean water and allowed to drain for 15mins in a colander. The leafy vegetables were cut and ground using Warburg laboratory blender. The pulverized leaves were packaged in a plastic container and preserved in the refrigerator.

Dried *Irvingia gabonensis* seeds were sorted to remove extraneous particles, washed to remove dust and shade dried for easy grinding. The seeds were ground with a ken wood blender. The ground seeds were packaged in a plastic container and kept in the refrigerator.

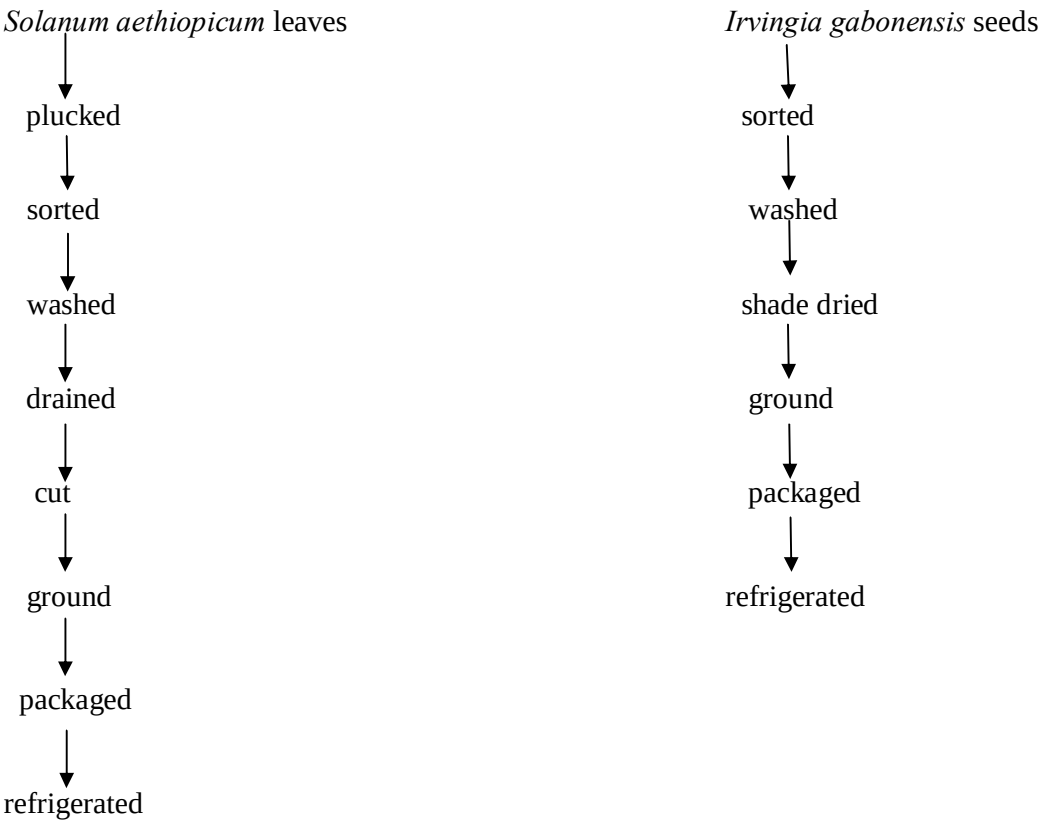

Fig 1: flow chart for the processing of the two Samples

3.3 Proximate Analysis

3.3.1 Moisture Content Determination

The standard method of AOAC (2005) was used to determine the moisture content of the samples.

i. Procedure

Clean aluminum dishes were labeled, dried in an oven at 100°C for 30minutes, cooled in a desiccator containing reignited silica gel as desiccant, and weighed to a constant mass. Two grams each powdered samples was accurately weighed into the labeled aluminum dishes. The dishes and samples were weighed again before drying in the oven at 100°C for 5 hours in the first instance. Next quickly transfer into a desiccator containing silica gel to cool. After cooling, the dishes containing the samples were quickly weighed with minimum exposure to atmosphere. The procedure was repeated, but dried for 3 hours for each subsequent drying to constant mass.

ii. Calculation

The moisture content of each sample was calculated as the difference in masses before and after drying to constant mass. Values were expressed as percentage moisture. Each dried sample were transferred into clean dry labeled airtight sample container and kept until required for other analysis.

3.3.2 Ash Determination

The ash content was determined by the method of AOAC (2005).

i. Procedure

Porcelain crucibles with lids were dried for 15 minutes in a hot air oven at 105°C, cooled in a desiccator and weighed. Two grammess of each sample were separately weighed into the appropriately labeled crucible and weighed again. Crucibles and contents were ignited in muffle furnace (Model M-525) at 550°C for 10 hours to light gray ash. Thereafter, they were removed and placed immediately in a dessicator to cool and is weighed.

ii. Calculation

The difference in mass or loss in mass of the crucible and samples before ashing is the organic matter content of each sample. The difference between the mass of the

crucibles alone and crucible plus ash will give the mass of ash of each sample. Values for ash will be calculated and expressed in percentages.

3.3.3 Crude Fat Estimation

Crude fat was estimated by repeated extraction of the samples with petroleum ether (preferably 60 ï 80°C). The method of Pearson (1976) will be employed. The method is based on the principle that non-polar components of the samples are easily extracted into organic solvents.

i. Procedure

One gramme, (moisture ï free) of each sample, was placed into labeled fat-free thimbles. These were weighed, plugged with glass wool and introduced into the soxhlet extractors containing 50ml petroleum ether (b.p 60 ï 80°C). Clean dry receiver flasks were weighed and fitted to the extractors. The extraction units were assembled, cold water was allowed to circulate, and the temperature of the water bath was maintained at 60°C. Extraction was carried out for 45 minutes. At the end of this time, the thimbles containing the samples were removed and placed in an oven at 70°C for three hours and dried to constant mass.

ii. Calculation

The crude lipid was obtained as the difference in mass before and after the extraction.

3.3.4 Determination of Protein Content

The protein content of the samples was determined by using (AOAC) (2005) method Micro Kjeldahl technique.

i. Digestion of the Sample

One gram of the sample was weighed into the digestion flasks and anhydrous sodium sulphate of 4g will be added. This was followed by the addition of 0.75g of copper sulphate and a pinch of selenium as catalyst. Finally, 20ml sulphuric acid were added

with a few boiling chips. The content of the flask were heated on a heating mantle in the fume hood until solution becomes clear. After digestion, the solution was cooled and diluted appropriately with distilled water.

ii. Distillation of Sample

Five millimetres aliquot of the digest were pipetted into the Markham (distillation) apparatus after steaming the apparatus out. This was followed by dropwise addition of 10ml 50% NaOH solution. Ten millimetres and 4% boric acid mixed indicator was pipetted into a 100ml conical flask and placed at the receiving end of the Markham apparatus for the collection of the distillate. Distillation was carried out until about 50ml distillate is obtained.

iii. Titration of Distillate

The distillate was titrated against 0.01N HCl. Titre will be obtained at the first appearance of a pink colour. The protein content was then calculated as follows:

$$N\% = \frac{DF \times Titre \times Molarity\ of\ acid\ titrant \times Molecular\ weight\ of\ Nitrogen \times 100}{Weight\ of\ sample\ to\ be\ used\ (1000)}$$

$$P\% = N\% \times 6.25$$

3.3.5 Determination of Crude Fibre Content

The crude fibre content of the extracts was determined using Gravimetric method as described by AOAC (1984). This method is based on the recognition that both souble and insoluble fibres are beneficial to human health. They produce different physiological responses. Both make up the total dietary fibre content of the samples,

total dietary fibre content of the samples was used to determine the crude fibre content of the extracts.

The sample was enzymatically digested with alpha-amylase, amyloglucosinase and protease. the insoluble fibre was collected by filtration. Soluble fibre was precipitated by bringing the filterate to 78percent ethanol and collected by filteration. The filtered fibre residues were washed with ethanol acetone, oven dried and weighed. One duplicate will be used to determine nitrogen content by kjeldahl procedure (to obtain content). The other duplicate were incinerated to determine the ash content.

i Procedure

Each representative sample (1g) was mixed separately with a buffer (40ml) with heat stable enzyme (alpha-amylase) and the pH adjusted to 8.2. This was incubated for 15minutes at 100^0C and cooled to 60^0C. The heat stable enzyme (amylase) was added to digest and to remove starch from the sample. The enzyme (protease) was added and incubated for 30mins at 60^0C. This will remove protein from the samples. The pH was adjusted to 4.0 and the enzyme (amyloglucosinase) was added. This was incubated for 30mins at 60^0 to gelatinize (solubilize) the starch granules in the samples. This digest was filtered using Whatman (no.1) filter paper. Wash the filtered residue with 10ml distilled water (twice) and use for the determination of protein and ash contents.

The filtered residue was washed twice with 95% ethanol (10ml). After, it was washed twice with acetone (10ml) and collected into the already dried and weighed crucible. This was dried with air oven and weighed to obtain the weight of the residue. The fibre residue was used for residual protein and ash determination by dividing the residue into two equal parts. One part was analyzed for protein. The fibre content was determined using the formula given by Bennink in Nelson (2002):

Fibre = residual weight ï (weight of protein + ash)

3.3.6 Determination of Total Carbohydrate

The total carbohydrate content of the diet samples was obtained by difference as described by AOAC (2005). This involved subtracting the sum of percentage of protein, fat, ash, fibre and moisture from 100.

3.4 Vitamin determination

3.4.1 Provitamin A (carotenoid)

Pro-vitamin A (ɓ- carotene) was determined using the method adopted from International vitamin A Consultative Group (IVACG) (1992). The sample was washed with volatile organic solvent (chloroform). The absorbance of the filtrate was read with UV- spectrophotometer at 328nm.

3.4.2 Vitamin C

This was determined using AOAC (2010) method. About two grammes of each of the sample were dissolved with distilled water. Two milliliters of trichloroacetic acid (TCA) was added and colour were developed with 2, 6-dichloroinodophenol and read with spectrophotometer.

3.4.3. Vitamin E

The AOAC (2010) method was used to determine the vitamin E content. One gramme of the sample was weighed into a 100ml flask. Ten (10) ml of absolute alcohol and 20ml of alcoholic tetraoxosulphate IV and (H_2SO_4) was added. Ten (10) ml of the clear solution was pipetted into a test tube and heated in a water bath at $90^{O}C$ for 30mins. It was allowed to cool and the absorbance read in a spectrophotometer at wavelength of 470nm. The blank and the standard were also prepared and the absorbance will be taken at 470nm wavelength.

Vitamin E in mg/100g = $\frac{a-b \times c}{s-bw}$

Where, a = absorbance of test sample

b = absorbance of the standard solution

c = concentration of standard in mg/100g

w = wavelength of the sample used

3.4 Determination of Phytochemicals

3.4.1 Determination of Alkaloids

This was done by the alkaline precipitation-gravimetric method described by Harborne (1973). 5g of the sample was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added. The beaker was covered and allowed to stand for 4hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was washed with dilute ammonium hydroxide after which it was filtered with a pre-weighed paper. The residue after the filtration is the alkaloid which was dried and weighed. Percentage alkaloid was calculated thereafter.

3.4.2 Determination of Saponins

Saponin was determined according to the Ochuko and Obadoni (2002) method. 10g of the sample was weighed into a 250ml conical flask and 100ml of 20% ethanol added to it. The sample was heated over a water bath for 4hours at 55 °C. The mixture was filtered and the residue re-extracted with 200ml of 20% ethanol. The combined extracts were reduced to 40ml over water bath set at 90°C. The concentrate was

transferred into a 250ml separating funnel and 20ml of diethyl ether was added and the solution shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60ml of n-butanol was added. The combined n-butanol extracts was washed twice with 10ml 5% sodium chloride solution. The remaining solution was heated in a water bath and evaporated to dryness after which it was dried to constant mass and saponin content calculated as percentage.

3.4.3 Determination of Tannins

The method of AOAC (1980) was also used in this determination.

i. Procedure

Two grams residues of petroleum ether extracts as earlier described were boiled with 300ml distilled water for 2hours, cooled and diluted to 500ml with more water and then filtered. 25ml of the filtrates were transferred separately into 2L porcelain dish. 20ml indigo solution and 750ml distilled water added, followed by 1ml of KMnO4 (earlier standardized with 0.1N oxalic acid) at a time until the blue solution turned green; then few drops until solution becomes golden yellow. Triplicate analyses making up to 100ml with distilled water. It was vigorously shaken several times and allowed to stand for 15mins each time. Suspensions were filtered through No. 1 Whatman filter paper.

ii. Spectrophotometric Measurement

Five millimeter of each sample extract was pipetted into labeled test tubes and 5ml of oxidation solution (potassium ferricyanide: NaOH mixture 1.9v/v) was added. Each

mixture was shaken and then allowed to stand for 1min. Three drops of hydrogen peroxide solution was added to each test tube and shaken again. Absorbance of each preparation was determined at 369nm against a blank prepared in the same manner, but 5ml of water was added instead of sample extract. Triplicate determinations were carried out on each sample extract.

iii. Calculation

The thiamine content in mg per 100g samples will be obtained as follows:

$$\text{Mg Vit. } B_1 = \frac{\text{Abs.} \times 100 \times 110 \times 1000}{5}$$

Whesre 100 is the volume to which extract was made up to. 110 is a conversion factor. 5 is the weight of sample that was taken for extraction.

3.5 Anti Nutrient Analysis

3.5.1 Determination of phytate

This was achieved using the method of AOAC (2000)

Phytate was extracted using dilute HCL and extract mixed with sodium EDTA-NaOH solution and placed in an ion-exchange column. The extracted phytate was diluted with 0.7ml NaCl solution and wet-digested with H_2S0_4/HNO_3 mixture to release phosphate, which was measured colorimetrically after reacting with ammonium molybdate solution. The amount of the phytate in original sample was obtained as hexaphosphate equivalent.

i. Procedure

Two grammes of each sample were separately weighed into labeled 150ml Erlenmeyer flasks. Exactly 40ml of 2.4% HCL was added to each sample, covered and shaken vigorously on an MSE orbital shaker for 3 hours at room temperature. Meanwhile, a column was prepared by adding 3ml of distilled H_2O to the slurry of 0.5 anion exchanger resin AG 1-x-4chlorides obtained from BIO-Rad laboratories. This was allowed to settle and washed with 15ml of 0.7M NaCl solutions, followed by 15ml of distilled water.

Sample was removed from the shakers and filtered through Whatman filter paper No. 42. One millimetre of each separately mixed with 0.1ml Na_2 EDTAï NaOH reagent in a 25ml volumetric flask. Mixtures was diluted to the mark with diluted water, mixed and transferred quantitatively to the prepared column. The first eluent from the column was discarded. The Column was washed with 15ml 0.7M NaCl and discarded. The column was then washed with 15ml 0.7M NaCl and fraction collected into a digestion flask. Concentrated H_2So_4 (0.5ml) and 3.0ml concentrated HNO_3 was added to the flask. Before the next sample is added to the column, 15ml of distilled water was passed through it. Mixtures in flasks were digested on micro-Kjeldahl rack at 50^0C until active boiling cease and thick yellow vapour were given out. Heating will continue for another 10mins before burns burner were turned off. Flask was allowed to cool to room temperature. Exactly 10ml water was added to the flask and swirled to dissolve the digests. All solution was separated transferred quantitatively to be labeled 50ml volumetric flasks. 2.0ml molybdate solution was added to each sample, mixed thoroughly, then followed by 1.0ml sulfonic acid reagent and mixed again. The solutions were then diluted to volume with more distilled water. Mixtures were allowed to stand for 15minutes and absorbance read at 640nm. A blank solution were

prepared by mixing 1ml 2.4% HCL with 1.0ml Na2EDTA-NaOH reagent, and diluted to 25ml with distilled water before pouring into column and treated as samples.

ii. Preparation of Standard Curves

A standard curve will be prepared 1.0, 3.0, 5.0 and 7.0ml phosphate standard solution containing 80, 240, 400, and 560ug phosphorus respectively, into labeled 50ml volumetric flasks. 20ml distilled water will be added to each to each flask, mixed again. Absorbance of the solutions will be read at 640nm. A standard curve will be generated (Appendix 1). Triplicate determination will be carried out on all the samples.

iii. Calculations

Phytate concentration in the diet was extrapolated from the generated standard curve, and expressed as mg/100mg sample.

3.5.2 Determination of Total Oxalate

Total oxalate in the diet samples was assayed using the method of AOAC (1990). It was however slightly modified where powdered sample was used instead of canned vegetable juice.

Oxalate is precipitated as insoluble calcium oxalate, which was collected by centrifuging. The centrifuge was dissolved in an excess of hot dilute H_2So_4 and the oxalate titrated (in hot) with standardized $KMnO_4$.

i. Procedures

Two gram of the powdered diet samples was separately weighed into labeled 250ml beaker and 150ml distilled water and 55ml 6M HCL added. Two drops of alcohol was

added and mixture boiled for 15minutes, cooled and transferred quantitatively into 500ml volumetric flask, diluted to volume with distilled water and mixed again. Mixture was allowed to stand overnight, mixed thoroughly, and then filtered through No. 42 Whatman filter paper.

Exactly 25ml of the filtrates was separately pipette into labeled 50ml flasks and then tungtophosphoric acid added, mixed and let to stand for 5 hours. Mixtures were filtered through the whatman filter paper and 20ml of the filtrate were pipetted again into centrifuge tubes followed by ammonium hydroxide solution drop wise until a pH of 4.5 was achieved by using indicator paper. 5ml acetate buffer (pH 4.5) was then added to maintain a constant pH.

Mixtures were allowed to stand overnight at room temperature, after they were centrifuged for 15mins at 1700rpm to compact the precipitate. Supernatants were carefully decanted and calcium oxalate precipitates washed three times with centrifugation and decantation using cold washing liquid (12.5ml HoAc, diluted to 250ml with distilled water). Precipitates was re-dissolved in 5ml dilute H_2So_4 (1:9v/v). The dilute H_2So_4 also will serve as the blank solution. All mixtures were then heated in boiling water bath 15mins and the hot solutions will be titrated with 0.01N $KMnO_4$ until a persistent pink colour is obtained. Triplicate titrations were carried out on each sample.

ii. Calculation

The volume of $KMnO_4$ used to titrate the hot solution of each sample was used to calculate the oxalate content of each sample as follows:

Mg oxalate/100g sample = ml of 0.01N $KMno_4$ x 1350

Weight of sample taken

Where 1350=0.45 (mg oxalic acid equivalent to 0.01N $KMNo_4$ x [(30/20) x (50/25) dilution factors] x 100 (to convert to 100g sample).

3.6 Preparation of Samples

3.6.1 *Solanum aethiopicum* Leaves

One kilogramme(1kg) of the freshly crushed *Solanum aethiopicum* leaves was weighed out, two hundred milliliter(200ml) of water was added prior to production of a paste sample. The solution of the sample was heated in constant water bath for 30mins at 45^0C. The sample was stored in the refrigerator at 4^0C for later use.

3.6.2 *Irvingia gabonensis* Seeds

Irvingia gabonensis seeds weighing one kilogram (1kg) was ground and placed in marceratin flask. Five hundred milliliter (500ml) of distilled water was poured into this marceratin flask and constantly shaken for 30mins to get semi-liquid solution. The sample was poured in a glass cork and stored in the refrigerator at 4^0C for later use.

3.6.3 Determination of Concentration

The concentration samples of *Irvingia gabonensis* seeds and *Solanum aethiopicum* leaves were determined by placing two milliliter (2ml) in a crucible and heat to dryness. The difference in the weight of fresh and dry sample will give the concentration of active ingredient in the sample.

3.7Study design

Experimental study design was employed for this study. The study was conducted for 21 days consisting of 5 days acclamatisation, 1 day for inducement of diabetes, 2 days for establishment of diabetes and 14 days on experimental diets.

3.8 Rat study

3.81 Procurement of Rats

A total of forty rats with no prior drug treatment were used for this study. The rats were healthy adult male wistar rats weighing between 150-200g. The rats were purchased from the animal house of the Department of Veterinary Pathology, University of Nigeria Nsukka.

3.82 Housing

These rats were distributed in metabolism cages and maintained under standard environmental conditions (temperature, humidity, separation of feaces and urine) during the study period. The rats were housed in a temperate laboratory animal house at ambient temperature ($\pm 25^0$C). The grower top feed was purchased from the vital feed shop in Nsukka town Enugu state and the study was carried out in the Department of Home Science, Nutrition and Dietetics University of Nigeria, Nsukka.

3.83 Induction of Diabetes

Forty adult male rats weighing between 150-200g were used. Alloxan powder of 150mg/kg was mixed with 10mls of diluted water and used for induction of diabetes through the intra peritoneal route of administration. After 48hrs of induction, the blood glucose level of the rats were determined using accu check glucometer and its test strips. Rats that record up to 200mg/dl and above were considered diabetic. Rats that are diabetic were grouped and treated with the concentration of the two plants.

3.84 Feeding of the Animals

The rats were fed commercial pellet diet (rat chow) and water *ad libitum* for 5days to acclimatize them to laboratory hygienic conditions and diet. The individual weights of the rats were taken at the beginning of the experiment during and after the experiment to determine the weight gain. The rats were divided into seven groups of five rats each made up of six (6) experimental groups and one control group. The experimental groups were induced with alloxan to become diabetic. After 48hrs of induction, the blood glucose levels of the rats were determined using accu check glucometer. All the rats received rat chow and water *ad libitum.* Group 1 was the control group. Groups 2-4 received 5g/Kg Bw, 10g/Kg Bw and 15g/Kg Bw respectively of *Solanum aethiopicum* leaf sample. Groups 5-7 received 5g/Kg Bw, 10g/Kg Bw and 15g/Kg Bw respectively of *Irvingia gabonensis* seed sample. The samples were administered using canola after dissolving in water for easy passage through the canola. The six groups continued with the acclamitization diet and the two samples (*Solanum aethiopicum* and *Irvingia gabonensis*) for the duration of the experiment (14days). The experiment lasted for 21days.The administration of the diets was oral route. Blood was withdrawn from the rats every 7 days of the administration of the samples to obtain the values of the blood constituents.

Table 5: Shows the feeding of the rats

Group	Diet	No. of rats
1	control group (rat chow with distilled water)	5
2	diabetic group with 5g/kg Bw of Sa leaves sample	5
3	diabetic group with 10g/kg Bw of Sa leaves sample	5
4	diabetic group with 15g/kg Bw of Sa leaves sample	5
5	diabetic group with 5g/kg Bw of Ig seeds sample	5
6	diabetic group with 10g/kg Bw of Ig seed sample	5
7	diabetic group with 15g/kg Bw of Ig seeds sample	5

Note: Sa = *Solanum aethiopicum*
Ig = *Irvingia gabonensis*
Bw= Body weight

3.9 Lipid Profile Test

Blood sample from the rats were collected through the retro-plexus of the rats eye and analysed for lipid profile i.e cholesterol, HDL(high density lipoprotein), LDL(low density lipoprotein ,VLDL(very low density lipoprotein , triglycerides) at day 1, day 5 and day 10.

3.9.1 Determination of Cholesterol

The method developed by Aliain (1974) was adopted

Assay principle:

The enzymatic reaction sequence employed in the assay of cholesterol

$$\text{Cholesterol} + H_2O \longrightarrow \text{cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \longrightarrow \text{Cholesterol-3-one} + H_2O$$

$$2H_2O_2 + \text{4-Aminoantipyrine} + \text{p-hydroxy benzene sulfonic acid} \longrightarrow$$

$$\text{Quinoneimine(Red eye)} + 2\,H_2O$$

Cholesterol esters were hydrolysed to produce cholesterol. Hydrogen peroxide was produced from the oxidation of cholesterol oxidase. In a coupled reaction catalysed by speroxidase, quinoneimine dye coloured red was formed from 4-aminoantipyrine, p-hyroxy sulfonic acid and hydrogen peroxide. The absorption at 520nm of the solution of this dye was proportional to the concentration of cholesterol in the sample.

i. Procedure of cholesterol

Test tubes were collected and labeled blank, standard and sample corresponding to each group and week. 1.0ml of reagent will be pipetted to all test tubes and pre-warm at 37^0C for at least 2mins. 0.01ml of sample was added to their respective tubes, mixed and returned to 37^0C. All tubes were incubated at 37^0C for ten minutes.

The spectrophotometer was zeroed with the reagent blank at 520nm.

3.9.2. Determination of HDL (High Density Lipoprotein)

The methods that were used for the determination of HDL-cholesterol were that described by Lopes-Virella et al. (1977).

i. Principle of HDL

Low density lipoproteins (LDL and VLDL) and chylomicrons fractions were precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol in the high density lipoprotein fraction which remains in the supernatant was determined.

ii. Procedure of HDL

200ul of standard was pipetted into a test tube and 500ul of diluted precipitant RI was added to it. 200ul of sample from each group in different weeks were precipitated into different test tube corresponding to each group. 500ul of diluted precipitant was added to the test tubes and incubated at 37^0C for five minutes. The absorbance for the standard and samples were taken at 546nm and recorded.

3.9.3 Determination of LDL (low density lipoprotein) using Polyvinyl Sulphate Method 0.7g/l

EDTA Na ⟶ 5.0mM

Polyethylene-glycol monomethyl ether ⟶ 170g/l

ii. Procedure of LDL

To 0.2ml of the sample (serum) in a test tube 3 drops of 0.1ml of the precipitant solution was added. It will be mixed well and let to stand for 15mins approximately at room temperature (20-25^{0}C). They were centrifuged at 2000xg/15 minutes. The cholesterol concentration was determined in the supernatant.

3.9.4. Determination of Triglycerides Using Glycerol Phosphate Oxidase/Peroxide

The method will be described by Bucolo and David (1973) and Fosatti and Prencipe (1982)

i. Principle of Triglycerides

Triglycerides in the sample was originated by means of the coupled reactions described below, a colored complex that can be measured by spectrophotometry.

Triglycerides H_20 ⟶ lipase ⟶ Glycerol + fatty acids

Glycerol + ATP ⟶ glycerol kinase ⟶ glycerol-3-phosphate + ADPs

Glycerol-3-phosphate +4-chlorophenol + O_2 ⟶ dihydroxyacetone-P + H_2O_2

$2H_2O_2$ + 4-Aminoantipyrine + 4-chlorophenol ⟶ Quinoneimine + $4H_20$

ii. Procedure of Triglycerides

Preparation Standard

These reagents were brought to room temperature. 10ul of triglycerides standard was pipette into a labeled test tubes. 0.1ml of reagent A was added to the test tubes the whole preparations i.e. the standard, blank and samples were mixed thoroughly and incubated for five minutes at 37^0C. The absorbance of the standard and sample was measured against the blank at 500nm.

3.9.5 Determination for Hematological Indices.

Blood samples were obtained from the optical plexus of the rats using a heparinized (plain) haematocrit capillary. Blood sample collected as in lipid profile was subjected to haemalogical analysis at day 1, day 5, day 10 and day 15. The determination of the differential leucocyte count, packed cell volume, haemaglobin concentration, red blood cell count, and white blood cell count was done according to Aka, Okoli & Ndu (2007).

i. Differential Leucocyte Count: A thick blood film was made on a grease free microscope slide and will be allowed to dry. The dry blood film was stained with leishman stain and was washed off after ten minutes and was allowed to air-dry. The prepared slide was viewed in the microscope, while the neutrophils and the lymphocytes were counted and their percentage composition calculated.

ii. Packed Cell Volume: Haematocrit capillary tube was filled with blood by placing one of the open ends of the tube in the blood bottle and tilting it at an angle about 30^{o}. One end of the filled capillary tube was sealed with a plastercine and the tube centrifuged for 20minutes at 300 rpm in a haematocrit centrifuge. A haematocrit reader was used to read off the length of the packed cell in percentage.

iii. Haemaglobin Concentration: Sahli haemoglobinometre was used for the determination of haemoglobin concentration. Up to the ten (10) mark of the sahli tube was 0.1N Hcl placed. With the Sahli blood pipette, 20µl of blood was placed into the Sahli tube and was sucked up and down. The mixture was allowed to stand for 5minutes for the formation of acid haematin. The dark mixture formed was diluted gradually with distilled water till the colour when compared with that in the haemoglobinometre is slightly darker than the standard. The dilution continued till the colour turns exactly and slightly paler than the standard. The volumes of the noted

colour change was taken and the average of that of the slightly darker and paler were compared and was ensured that the variation is not more than ±5 for the average value to be adopted. The average value was applied in the formular below:

X (Y)/100 where X is the 14g Hb in 100ml of blood, y is the average value of dilutions.

iv. Red blood Cell Count:

The following equipment was used for the experiment; the microscope, haemocytometre (counting chamber), red cell pipette, ringer solution, cover slip, the apparatus were set up and procedure according to David (2009) will be followed strictly.

Using the dilution pipette with RED mixer from hemacytometer kit, blood was drawn up to the 0.5 mark. Continuing to hold the pipette as horizontal as possible, draw Ringer's solution diluent up to the 101 mark. (Dilution of 1 to 200)

The tip of the pipette was sealed with the finger and shaked well to mix. Half of the content of the pipette was emptied into a waste container and a small amount of the diluted blood was placed into one chamber of the hemacytometer to just fill the chamber of the hemacytometer. The preparation was allowed to sit for a minute (for cells to settle).

The center of the grid was focused with 100x objectives, and was counted with 400x objectives. The count of each five fields (each with 16 smallest squares) with a clicker (fields: top R & L, bottom R & L, center) was noted. Include in the count all cells touching left and bottom sides, ignore cells touching top and right sides. The

RBCs/cmm was calculated by adding the cells in the 5 groups and multiplying by 10,000 (i.e., add four zeros).

v. White Blood Cell Count:

Same as in RBC except that the diluting fluid is 1.5% acetic acid tinted with methyl violet. The pipette is similar but with different graduation. Unlike the RBC, the leucocytes cells in the entire 9 big grid was counted and applied in the formular n X 200/9

3.10 Statistical Analysis

All the data was expressed in mean and standard deviation. Statistical analysis was carried out using Statical Package for Social Science (SPSS) version 21. Duncanôs studentized new multiple range test was adopted to separate and compare means at 5% probability.

CHAPTER FOUR

RESULTS

Table 4.1 presents the proximate composition of fresh *Solanum aethiopicum* (Sa) and dried *Irvingia gabonensis* (Ig) (percent wet and dry weight) respectively. The moisture values for Sa and Ig were 77.23% and 6.04%, ash 0.13% and 0.73%, fibre 2.61% and 2.27%, carbohydrate 10.88% and 56.07%, protein 8.14% and 10.52% and fats 0.51% and 24.18%, respectively.

Table 4.1: Proximate composition of *Solanum aethiopicum* (Sa) and *Irvingia gabonensis* (Ig) (wet and dry weight (%)

Sample	Moisture	Ash	Fibre	CHO	Protein	Fats
Sa	77.23±0.33	0.13±0.01	2.61±0.29	10.88±0.11	8.14±0.01	0.51±0.01
Ig	6.04±0.06	0.73±0.02	2.27±0.02	56.07±1.51	10.52±0.09	24.18±1.41

Mean ± standard deviation of two determinations
Key:
Sa -*Solanum aethiopicum*
Ig -*Irvingia gabonensis*

Table 4.2 presents the vitamin compositions of the two food samples. The pro.vit A, vit.C and vit.E of Sa were 2.03i.u, 14.36mg and 8.10mg respectively and the Ig had 0.00i.u, 0.00mg and 4.08mg of Pro.vit A, vit.C and vit.E respectively.

Table 4.2: Vitamin composition of *Solanum aethiopicum* (Sa) and *Irvingia gabonensis*(Ig) (mg/100g)

Sample	Pro.vit A(i.u)	Vit C(mg)	Vit E(mg)

Sa	2030±42.45	14.36±0.50	8.10±0.13
Ig	0.00±0.00	0.00±0.00	4.08±0.25

Key:
Sa -*Solanum aethiopicum*
Ig -*Irvingia gabonensis*

Table 4.3 contains mineral composition of the two foods. The Sa had iron(fe) 2.04mg, magnesium(mg) 1.60mg, zinc(Zn) 0.42mg, calcium(Ca) 2.40mg, potassium(k) 40.25mg, sodium(Na) 24.07mg and copper(cu) 0.08mg and the Ig had iron(fe) 5.27mg, magnesium(mg) 19.10mg, zinc(Zn) 1.68mg, calcium(Ca) 3.72mg, potassium(k) 40.49mg, sodium(Na) 25.73mg and copper(cu) 2.32mg.

Table 4.3: Mineral composition of *Solanum aethiopicum* (Sa) and *Irvingia gabonensis*(Ig) (mg/100g)

Sample	**Iron**	**Mg**	**Zn**	**Ca**	**K**	**Na**	**Cu**
SA	2.04±0.06	160.48±2.09	0.42±0.11	239.58±0.83	40.25±0.35	24.07±1.32	0.08±0.04
1V	5.27±0.10	19.10±0.01	1.68±0.11	372.46±3.48	40.49±0.54	25.73±0.32	2.32±0.03

Key:
SA -*Solanum aethiopicum*
IG -*Irvingia gabonensis*

Table 4.4 contains the antinutrient and phytochemical contents of the foods. The tannin for Sa was 20.93mg, alkaloid 0.63mg, saponin 0.01mg, phytate 0.28mg and oxalate 0.06mg. Ig had tannin 2.32mg, alkaloid 5.15mg, saponin 0.49mg, phytate 0.26mg and oxalate 0.13mg.

Table 4.4: Antinutrient and phytochemical of *Solanum aethiopicum* (Sa) and *Irvingia gabonensis*(Ig) (mg/100g)

Samples	**Tannins**	**Alkaloids**	**Saponins**	**Phytate**	**Oxalate**

SA	20.93±0.10	0.63±0.04	0.01±0.00	0.28±0.01	0.06±0.01
Ig	2.32±0.25	5.15±0.21	0.49±0.01	0.26±0.00	0.13±0.02

Key:
Sa -*Solanum aethiopicum*
Ig -*Irvingia gabonensis*

Table 4.5 presents the initial and final cholesterol values of the rats fed Sa diet. The group of rats fed diets containing 10g/kgBW Sa had the highest decrease (67.25%) cholesterol value more than the rats fed 5g/kgBW and 15g/kgBW Sa diet (25.49 and 41.55%) respectively. In the baseline result, the groups of rats fed Sa diet did not differ significantly ($p>0.05$) but final result showed that they differed significantly ($p<0.05$).

Table 4.5 Serum cholesterol values of rats (mmol/l) (Sa diet)

Groups & exp. diets	Baseline	Final	Percentage difference
1 control group	3.25 ± 0.19^{b}	3.67 ± 0.29^{abc}	12.92ŷ
2 5g/kgBW of Sa	3.53 ± 0.13^{b}	2.63 ± 0.12^{d}	25.49Ź
3 10g/kgBW of Sa	3.45 ± 0.17^{b}	1.13 ± 0.03^{cd}	67.25Ź
4 15g/kgBW of Sa	3.08 ± 0.18^{b}	1.80 ± 0.12^{bc}	41.55Ź

Sa: *Solanum aethiopicum*, Bw: Body weight, Exp. Diets: Experimental diets, Means in the column with different superscript letters differ ($p<0.05$). SEM± =Standard error of mean

Table 4.5.1 presents the initial and final cholesterol values of the rats fed Ig diet. The rats fed 10g/kgBW Ig diet had highest decrease (39.15%) cholesterol value compared to 5 and 15g/kgBW (9.01 and 23.39%). The baseline and final result showed that the rats fed Ig diet differed significantly ($p<0.05$).

Table 4.5.1 Serum cholesterol values of rats (mmol/l) (Ig diet)

Groups & exp. diets	Baseline	Final	Percentage difference
1 control group	3.25 ± 0.19^{b}	3.67 ± 0.29^{abc}	12.92ŷ

5	5g/kgBW of Ig	3.33±0.11[b]	3.03±0.03[bc]	9.01Ź
6	10g/kgBW of Ig	2.58±0.15[a]	1.57±0.09[ab]	39.15Ź
7	15g/kgBW of Ig	2.18±0.19[a]	1.67±0.27[a]	23.39Ź

Table 4.6 presents low density lipoprotein (LDL) values for the groups of rats fed Sa diets. The groups of rats fed 10g/kgBW Sa diet decreased its LDL value by 18.52%, other groups (5 and 15g /kgBW) increased the LDL value (5.88 and 11.82%) respectively. The baseline result showed that there was significant difference ($p<0.05$) between the rats fed Sa diet. The same in final result, the significant difference ($p<0.05$) occurred between rats fed 15g vs 5 and 10g Sa diet.

Ig: *Ongna gabonaya*, Bw: Body weight, Exp. Diets: Experimental diets, Means in the column with different superscript letters differ ($p<0.05$). SEM± =Standard error of mean

Table 4.6 Low lipoprotein(LDL) values of rats (mmol/l) (Sa diet)

Groups &exp. Diets	Baseline	Final	Percentage difference
1 control group	1.48±0.11[bc]	1.50±0.21[b]	1.35ŷ
2 5g/kgBW of Sa	0.85±0.10[a]	0.90±0.12 [a]	5.88ŷ
3 10g/kgBW of Sa	1.35±0.10[bc]	1.10±0.06[ab]	18.52Ź
4 15g/kgBW of Sa	1.10±0.13[ab]	1.23±0.03[b]	11.82ŷ

Sa: *Solanum aethiopicum*, .Bw: Body weight, Exp. Diets: Experimental diets, Means in the column with different superscript letters differ ($p<0.05$). SEM± =Standard error of mean

Table 4.6.1 presents low density lipoprotein (LDL) values for the groups of rats fed Ig diets. There were decreases (21.74, 1.60 and 23.53%) in LDL of all the groups of rats fed 5, 10 and 15g/kgBW of Ig diet. The (baseline and final) result showed that they differed significantly ($p<0.05$).

Table 4.6.1 Low lipoprotein(LDL) values of rats (mmol/l) (Ig diet)

Groups &exp. Diets	Baseline	Final	Percentage difference
1 control group	1.48±0.11[bc]	1.50±0.21[b]	1.35ŷ
5 5g/kgBW of Ig	1.15±0.24[ab]	0.90±0.06[a]	21.74Ź

6 10g/kgBW of Ig	1.25±0.10^{ab}	1.23±0.03^{b}	1.60Ź
7 15g/kgBW of Ig	1.70±0.08^{c}	1.30±0.06^{b}	23.53Ź

Ig: *Irvingia gabonensis*, Bw: Body weight, Exp. Diets: Experimental diets, Means in the column with different superscript letters differ ($p<0.05$). SEM± =Standard error of mean

Table 4.7 contains the high density lipoprotein (HDL) for rats fed Sa diets. The rats fed diets containing 15g/kgBW Sa diet had the highest increase (94.55%) HDL value, than those fed 5 and 10g/kgBW Sa diet (12.32 and 7.00%) respectively. They differed significantly ($p<0.05$).

Table 4.7 High density lipoprotein (HDL) contents of rats (mmol/l) (Sa diet)

Groups & exp. Diets	Baseline	Final	Percentage difference
1 control group	1.89±0.19^{ab}	1.94±0.25^{ab}	2.65ŷ
2 5g/kgBW of Sa	2.84±0.18^{d}	3.19±0.22^{d}	12.32ŷ
3 10g/kgBW of Sa	2.43±0.06^{bc}	2.60±0.05^{cd}	7.00ŷ
4 15g/kgBW of Sa	1.10±0.04^{abc}	2.14±0.12^{bc}	94.55ŷ

Sa: *Solanum aethiopicum*, .Bw: Body weight, Exp. Diets: Experimental diets, Means in the column with different superscript letters differ ($p<0.05$). SEM± =Standard error of mean

Table 4.7.1 contains the high density lipoprotein (HDL) for rats fed Ig diets. The groups of rats fed 5 and 10g/kgBW Ig diet increased (12.13 and 3.30%) HDL value respectively and those fed 15g/kgBW had decrease (7.69%) HDL value. The rats fed Ig diet differed significantly ($p<0.05$).

Table 4.7.1 High density lipoprotein (HDL) contents of rats (mmol/l) (Ig diet)

Groups & exp. Diets	Baseline	Final	Percentage difference
1 control group	1.89±0.19^{ab}	1.94±0.25^{a}	2.65ŷ
5 5g/kgBW of Ig	2.39±0.13^{abc}	2.68±0.06^{cd}	12.13ŷ
6 10g/kgBW of Ig	1.82±0.12^{a}	1.88±0.11^{ab}	3.30ŷ
7 15g/kgBW of Ig	1.56±0.29^{a}	1.44±0.31^{a}	7.69Ź

Ig: *Irvingia gabonensis,* Bw: Body weight, Exp. Diets: Experimental diets Means in the column with different superscript letters differ ($p<0.05$). SEM± =Standard error of mean

Table 4.8 presents the triglycerides values of rats fed Sa diet. The group of rats fed diets containing 5 and 15g/kgBW Sa had higher increase (35.43 and 21.71%) triglycerides respectively compared to those fed 10g Sa diet and they are not significantly different (p>0.05).

Table 4.8 Triglycerides contents of rats (mmol/l) (Sa diet)

Groups & exp. Diets	Baseline	Final	Percentage difference
1 control group	1.78±0.29[a]	2.23±0.09[b]	25.28ŷ
2 5g/kgBW of Sa	1.75±0.10[a]	2.37±0.15[b]	35.43ŷ
3 10g/kgBW of Sa	1.88±0.09[a]	2.17±0.18[b]	15.43ŷ
4 15g/kgBW of Sa	1.75±0.06[a]	2.13±0.03[b]	21.71ŷ

Sa: *Solanum aethiopicum,* Bw: Body weight, Exp. Diets: Experimental diets, Means in the column with different superscript letters differ (p<0.05). SEM± =Standard error of mean

Table 4.8 presents the triglycerides values of rats fed Ig diet. The groups of rats fed 10g/kgBW Ig diet had the highest (26.11%) triglycerides compared to other groups of rats followed by those fed 5g/kgBW Ig diet (21.21%). The group fed 15g/kgBW Ig diet had decrease (19.10%) triglycerides value. They baseline result showed no significant difference (p>0.05) but the final result showed that the rats fed 15g/kgBW showed significant difference (p<0.05) to those groups fed 5 and 15g/kgBW.

Table 4.81 Triglycerides contents of rats (mmol/l) (Ig diet)

Groups & exp. Diets	Baseline	Final	Percentage difference
1 control group	1.78±0.30[a]	2.23±0.09[b]	25.28ŷ
5 5g/kgBW of Ig	1.98±0.09[a]	2.40±0.06[b]	21.21ŷ
6 10g/kgBW of Ig	1.80±0.08[a]	2.27±0.03[b]	26.11ŷ
7 15g/kgBW of Ig	1.78±0.09[a]	1.44±0.31[a]	19.10Ź

Ig: *Irvingia gabonensis.* Bw: Body weight, Exp. Diets: Experimental diets, Means in the column with different superscript letters differ (p<0.05). SEM± =Standard error of mean

Table 4.9 presents packed cell volume values for the rats fed Sa diet. The rats fed 10 and 15g/kgBW Sa diet had increase (8.24 and 15.72%) PCV value compared to those fed 5gkgBW Sa diet that had decrease (4.65%) PCV value. They were not significantly different (p>0.05).

Table 4.9 Packed cell volume (PCV) contents of rats (mg/100g) (Sa diet)

Groups	Baseline	Final	Percentage difference
1 control group	39.00±2.04[a]	44.33±2.33[a]	13.67ŷ
2 5g/kgBW of Sa	41.25±1.49[a]	39.33±5.04[a]	4.65Ź
3 10g/kgBW of Sa	42.50±2.63[a]	46.00±3.21[a]	8.24ŷ
4 15g/kgBW of Sa	39.75±0.63[a]	46.00±2.08[a]	15.72ŷ

Sa: *Solanum aethiopicum* ,Body weight, Exp. Diets: Experimental diets, Means in the column with different superscript letters differ (p<0.05). SEM± =Standard error of mean

Table 4.9.1 presents packed cell volume values for the rats fed Ig diet. The rats fed 10g/kgBW Ig diet had the highest increase compared to those fed 5 and 15g/kgBW Ig diet (8.33 and 9.86%) and they are not significantly different (p>0.05).

Table 4.9.1 Packed cell volume (PCV) contents of rats (mg/100g) (Ig diet)

Groups	Baseline	Final	Percentage difference
1 control group	39.00±2.04[a]	44.33±2.33[a]	13.67ŷ
5 5g/kgBW of Ig	40.00±0.82[a]	43.33±0.88[a]	8.33ŷ
6 10g/kgBW of Ig	38.50±1.19[a]	43.67±0.33[a]	13.43ŷ
7 15g/kgBW of Ig	39.75±0.63[a]	43.67±0.88[a]	9.86ŷ

Ig: *Irvingia gabonensis*.Bw: Body weight, Exp. Diets: Experiment, Means in the column with different superscript letters differ (p<0.05). SEM± =Standard error of mean

Table 4.10 presents the RBC values for the rats fed Sa diets. The rats fed 15g/kgBW Sa had the highest increase (13.04%) RBC followed by 10g/kgBW (2.89%). The rats

fed 5g/kgBW Sa diet had decrease (1.20%) RBC value. The baseline result showed significant difference ($p<0.05$). The final result showed that rats fed of 5g/kgBW Sa diet had significant difference ($p<0.05$) to those fed 10 and 15g/kgBW Ig diet.

Table 4.10 Red blood cell (RBC) contents of rats (mm^3) (Sa diet)

Groups	Baseline	Final	Percentage difference
1 control group	11.00 ± 3.54^{ab}	11.33 ± 3.33^{a}	3.02ŷ
2 5g/kgBW of Sa	10.43 ± 4.25^{a}	10.30 ± 4.16^{a}	1.20Ź
3 10g/kgBW of Sa	11.83 ± 3.84^{b}	12.17 ± 4.41^{b}	2.89ŷ
4 15g/kgBW of Sa	11.45 ± 3.33^{ab}	13.00 ± 5.77^{b}	13.54ŷ

Sa: *Solanum aethiopicum* „.Bw: Body weight, Exp. Diets: Experimental diets, Means in the column with different superscript letters differ ($p<0.05$). SEM± =Standard error of mean

Table 4.10.1 presents the RBC values for the rats fed Ig diets. There were increases (5.75 and 3.02%) RBC value in the rats fed 10 and 15g/kgBW Ig diet respectively. The group fed 5g/kgBW Ig diet decreased the RBC value of the rats. The rats fed 5g/kgBW Ig diet differed significantly ($p<0.05$). to those of 10 and 15g/kgBW Ig diet in the baseline result but there was no significant difference ($p>0.05$) in the final result.

Table 4.10.1 Red blood cell (RBC) contents of rats (mm^3) (Ig diet)

Groups	Baseline	Final	Percentage difference
1 control group	11.00 ± 3.54^{a}	11.33 ± 3.33^{a}	3.02ŷ
5 5g/kgBW of Ig	13.13 ± 3.15^{c}	12.00 ± 2.89^{ab}	8.60Ź
6 10g/kgBW of Ig	11.13 ± 6.88^{ab}	11.70 ± 7.23^{ab}	5.12ŷ
7 15g/kgBW of Ig	11.25 ± 3.23^{ab}	11.60 ± 8.33^{ab}	3.11ŷ

Ig: *Irvingia gabonensis.*Bw: Body weight, Exp. Diets: Experimental diets Means in the column with different superscript letters differ ($p<0.05$). SEM± =Standard error of mean

Table 4.11 presents the WBC of the rats fed Sa diet. The rats fed 10g/kgBW Sa diet had the highest increase (8.99%) WBC value followed by those fed 5g/kgBW Sa diet (1.81%). The rats fed 5g/kgBW Sa decreased WBC value of the groups of rats. There was significant difference (p0.05) in the result.

Table 4.11 White blood cell (WBC) contents of rats ($x_{10}^{6/L}$) (Sa diet)

Groups & exp. diets	Baseline	Final	Percentage difference
1 control group	835.00.±464.58^{bc}	866.67±581.19^{ab}	3.79ŷ
2 5g/kgBW of Sa	970.00±946.93^{c}	1093.30±135.32^{c}	12.71Ź
3 10g/kgBW of Sa	685.00±170.78^{ab}	746.60±176.38^{a}	8.99ŷ
4 15g/kgBW of Sa	1290.00±645.49^{d}	1313.30±290.59^{d}	1.81ŷ

Sa:*Solanum aethiopicum*, Bw: Body weight, Exp. Diets: Experimental diets Means in the column with different superscript letters differ (p<0.05). SEM± =Standard error of mean

Table 4.11.1 presents the WBC of the rats fed Ig diet. The groups fed 5g/kgBW Ig diet had the highest increase (15.19%) WBC value, followed by those fed 15g/kgBW (11.83%), then those fed 10g/kgBW Ig diet (6.50%). There was significant difference between those fed 15g/kgBW vs 5 and 10g/kgBW Ig diet in the baseline result. In the final result, there was significant difference (p<0.05) between them.

Table 4.11.1 White blood cell (WBC) contents of rats ($x_{10}^{6/L}$) (Ig diet)

Groups & exp. diets	Baseline	Final	Percentage difference
1 control group	835.00±464.58^{bc}	866.67±581.19^{ab}	3.79ŷ
5 5g/kgBW of Ig	845.00±505.80^{bc}	973.33±581.19^{bc}	15.19ŷ
6 10g/kgBW of Ig	820.00±571.55^{bc}	873.33±240.37^{ab}	6.50ŷ
7 15g/kgBW of Ig	620.00±141.42^{a}	693.33±133.33^{a}	11.83ŷ

Ig: *Irvingia gabonensis.* Bw: Body weight, Exp. Diets: Experimental diets, Means in the column with different superscript letters differ (p<0.05). SEM± =Standard error of mean

Table 4.12 presents the leucocytes values for the rats fed Sa diet. The rats fed 10g Sa diet had the highest increase (19.78%) leucocytes value compared to those fed 5 and 15g SA diet (14.89 and 7.22%). There was no significant different ($p<0.05$) between them.

Table 4.12 Leucocytes contents of rats (mg/100g) (SA diet)

Groups & exp. Diets	Baseline	Final	Percentage difference
1 control group	33.50 ± 2.21^{ab}	37.00 ± 1.15^{a}	10.45ŷ
2 5g/kgBW of Sa	30.75 ± 1.97^{ab}	35.33 ± 2.91^{a}	14.89ŷ
3 10g/kgBW of Sa	32.00 ± 2.16^{ab}	38.33 ± 2.33^{a}	19.78ŷ
4 15g/kgBW of Sa	35.75 ± 1.93^{ab}	38.33 ± 1.86^{a}	7.22ŷ

Sa: *Solanum aethiopicum*, Bw: Body weight, Exp. Diets: Experimental diets, Means in the column with different superscript letters differ ($p<0.05$). SEM± =Standard error of mean

Table 4.12.1 presents the leucocytes values for the rats fed Ig diet. The group fed 15g Ig diet had the highest increase (11.47%), followed by 10g (6.90%) and then those fed 5g Ig diet (4.51%). The baseline result showed significant difference ($p<0.05$). In the final result, there was no significant difference ($p<0.05$) between them.

Table 4.12.1 Leucocytes contents of rats (mg/100g) (Ig diet)

Groups & exp. Diets	Baseline	Final	Percentage difference
1 control group	33.50 ± 2.22^{a}	37.00 ± 1.15^{a}	10.45ŷ
5 5g/kgBW of Ig	37.00 ± 1.22^{b}	38.67 ± 0.67^{a}	4.51ŷ
6 10g/kgBW of Ig	29.00 ± 3.08^{a}	31.00 ± 1.53^{a}	6.90ŷ
7 15g/kgBW of Ig	32.00 ± 2.85^{ab}	35.67 ± 4.48^{a}	11.47ŷ

Ig: *Irvingia gabonensis* .Bw: Body weight, Exp. Diets: Experimental diets, Means in the column with different superscript letters differ ($p<0.05$). SEM± =Standard error of mean

Table 4.13 presents the body weights of the rats fed Sa diet. The groups fed 5 and 15g Sa diet decreased (5.75 and 2.52%) body weights of the rats. The rats fed 10g Sa diet had increase (9.76%) the body weight of the rats. The baseline and the final day of the result differed significantly ($p<0.05$).

4.13 Mean Body weight of rats (g) (Sa diet)

Groups	Baseline	day 4	day 8	day 12	day 14	Perc. Diff.
1 control grp	119.18±3.34[a]	141.25±3.27[a]	155.45±4.21[a]	167.28±5.27[a]	172.85±6.63[bc]	45.03 ŷ
2 5g/kgBW of Sa	171.33±4.42[d]	515.60±7.73[c]	169.85±15.46[a]	156.85±14.75[ab]	161.68±11.12[ab]	5.75Ź
3 10g/kgBWof Sa	182.15±23.34[b]	156.33±24.52[a]	185.60±15.41[a]	190.60±14.23[ab]	199.93±12.52[c]	9.76ŷ
4 15g/kgBWof Sa	139.56±4.10[ab]	119.08±9.40[b]	123.05±14.25[a]	134.33±12.96[ab]	136.03±12.51[a]	2.52Ź

Sa: *Solanum aethiopicum* .Bw: Body weight, Exp. Diets: Experimental diets, Means in the column with different superscript letters differ ($p<0.05$). SEM± =Standard error of mean

Table 4.13.1presents the body weights of the rats fed Ig diet. The group of rats fed 5g Ig diet had the highest increase (17.17%), followed by 15g (7.65%). The rats fed 10g Ig diet increased (0.52%) the body weight of the rats. The baseline and the final day of the result differed significantly ($p<0.05$).

4.13.1 Mean Body weight of rats (g) (Ig diet)

Groups	Baseline	day 4	Day 8	day 12	day 14	Perc.Diff.
1 control grp	119.18±3.34[a]	141.25±3.27[a]	155.45±4.21[b]	167.28±5.72[a]	172.85±6.63[a]	45.03ŷ
5 5g/kgBW of Ig	209.60±3.48[ab]	191.08±5.07 [d]	173.68±8.71[c]	167.73±6.05[a]	173.60±4.54[a]	17.17Ź
6 10g/kgBW of Ig	162.00±16.6[a]	114.60±11.17[a]	150.76±12.14[a]	154.60±10.06[a]	162.85±9.27[b]	0.52ŷ
7 15g/kgBW of Ig	140.18±3.27[b]	118.30±7.18[d]	120.03±5.93[a]	124.93±3.73[b]	129.45±4.55[ab]	7.65Ź

Ig: *Irvingia gabonensis* .Bw: Body weight, Exp. Diets: Experimental diets, Means in the column with different superscript letters differ ($p<0.05$). SEM± =Standard error of mean

Table 4.14 presents the blood sugar values of the rats fed Sa diet. The groups fed Sa diets decreased blood sugar of the rats. The rats fed 10g Sa diet had the highest decrease (65.14%), followed by those fed 15g (53.81%), then 5g (43.94%) Sa diet. The baseline and the final day of the result showed no significant difference ($p>0.05$).

4.14 Blood sugar of rats (mg/dl) (Sa diet)

Groups	Baseline	day 0 (after inductn)	day 5	day 10	day 14	Perc.Diff
1 control grp	66.50±1.71[a]	69.50±2.22[ab]	68.25±0.85[a]	105.25±8.66[b]	91.75±7.22[bc]	32.01ŷ
2 5g/kgBW of Sa	74.50±9.07[a]	447.75±43.49[a]	469.50±35.40[c]	432.50±49.66[b]	251.00±12.12[ab]	43.94Ź
3 10g/kgBWofSa	73.00±4.92[a]	479.75±47.52[a]	367.25±26.6[ab]	248.25±42.53[ab]	167.25±18.75[ab]	65.14Ź
4 15g/kgBWofSa	66.00±3.85[a]	390.25±48.77[a]	335.00±35.24[b]	265.25±55.69[c]	180.25±26.21[ab]	53.81Ź

Sa: *Solanum aethiopicum*, Bw: Body weight, Exp. Diets: Experimental diets, Means in the column with different superscript letters differ ($p<0.05$). SEM± =Standard error of mean

Table 4.14.1 presents the blood sugar values of the rats fed Ig diet. The rats fed 10g IG diet had the highest decrease (61.68%) compared to those of 5 and 15g Ig diet (41.38 and 51.83%) respectively. The baseline had no significant difference ($p>0.05$) between them and but final result showed significant difference ($p<0.05$).

4.14.1 Blood sugar of rats (mg/dl) (Ig diet)

Groups	Baseline	day 0 (after inductn)	day 5	day 10	day 14	Perc. Diff
1 control grp	66.50±1.71[a]	69.50±2.22[ab]	68.25±0.85[a]	105.25±8.66[cd]	91.75±7.22[ab]	32.01ŷ
5 5g/kgBW of Ig	62.50±3.30[a]	336.50±28.22[c]	436.75±24.6[ab]	235.00±21.36[a]	197.25±17.33[d]	41.38Ź
6 10g/kgBWof Ig	70.00±2.58[a]	508.25±40.43[ab]	404.50±89.30[ab]	297.50±61.97[a]	194.75±29.90[a]	61.68Ź

					b	
7 15g/kgBWof Ig	68.25±6.0 [a]	403.25±60.83[bc]	507.50±9.46[ab]	350.50±12.37[a]	194.25±8.62[cd]	51.83Ź

Ig: *Irvingia gabonensis.* Bw: Body weight, Exp. Diets: Experimental diets, Means in the column with different superscript letters differ ($p<0.05$). SEM± =Standard error of mean

CHAPTER FIVE

DISCUSSION

Proximate composition of *Solanum aethiopicum* leaves diet and *Irvingia gabonensis* seeds

Solanum aethiopicum (Sa) leaves

The high moisture value for Sa 77.23% was not a surprise because vegetables contain more moisture on wet weight basis. Water makes up about 60% of the body weight, for the body to function properly we need two quarts of water or (liquid) per day. This can be derived from the consumption of fruits and vegetables. Fruits and vegetables contain large quantities of water in proportion to their weight (Guest contributor, 2012). The low ash 0.13% for Sa is a commonly observed phenomenon. The fibre value for Sa (2.61%) shows that is a better source of fibre. *Solanum aethiopicum* is one of the fibre diets. The most satisfactory prophylactic and therapeutic agent for functional constipation is a diet rich in fibre. Fibre is a type of complex carbohydrate found in foods that come from plants ï fruits and vegetables, nuts, seeds and whole grains. Eating foods with fibre can prevent stomach and intestinal problems such as constipation, cholesterol and blood sugar (National institute on aging, 2013). The Sa

had 10.88, 8.14 and 0.51% for carbohydrate, protein and fat respectively and these results are in line with earlier studies carried out by Shalom, Abayomie, Okwuchukwu, Opeyemi and Olajumoke (2011) on proximate composition of *Solanum aethiopicum*.

***Irvingia gabonensis* (Ig) seeds**

The low moisture value for Ig 6.04% was expected because it extends the shelf life and sensory quality of nuts, as low moisture helps reduce microbial growth (Cesarethin and Feredoon, 2000). The high fat content (24.18%) of Ig was expected because it is an oil seed which stores its energy in form of fat and it is the most abundant component of Ig kernel particularly two saturated fatty acids: 51.87% of myristic acid (C14:0) and 38.44% of lauric acid (Womeni, Ndjouenkeu, Kapseu, Tchouanguep, Parmentier & Fanni, 2006).

The high fat for Ig as well as carbohydrate and protein were also expected because itôs a nut. Nuts and seeds are good sources of proteins (Silou, Biyoko, Heron, Tchapla & Maloumbi, 2004). Most nuts contain a considerable quantity of fat and are rich in essential amino acids (Lester and Seck, 2004). Proteins are bodyôs building blocks and they are used to build and repair tissues. Proteins from plant sources tend to lower cholesterol and provide other health promoting nutrients. The body uses extra protein and carbohydrate for energy. The Ig had (2.27%) fibre which showed that it is a good source of fibre. All grains, nuts, seeds and legumes, if not fermented, must also be cooked long enough to break down their fibers, since human do not have digestive enzymes to break them down like herbivores (plant eating animals) i.e. cows. This finding is in agreement with the result of Ogunsina, Bhatnagar, Indira and Radha (2012) who reported high carbohydrate, protein, fat and low moisture in *Irvingia gabonensis* seed.

Vitamins levels of *Solanum aethiopicum* leaves and *Irvingia gabonensis* seeds

Sa diet

The result of this study showed that Sa had rich sources of vit.A, C and E. Generally, vegetables are rich in vitamins, minerals, trace elements, dietary fibre (Mellow, 2003). This finding is in agreement with a study carried out by Achikanu *et al* (2013) who reported that *Solanum aethiopicum* are rich sources Vit. A, C and E. Shalom *et al.* (2011) reported that there was a significant presence of ascorbic acid in both solanum aethiopicum and solanum macrocarpon L.

Ig diet

The zero value of vit.A and C for Ig indicates that it is not a good source of these nutrients to meet its requirements. The result of *Irvingia gabonensis* in this study on vit.C did not agree with Oboh and Ekperigin (2004) who reported that dried *Irvingia gabonensis* contgains about 6.2mg of vit.C. The *Irvingia gabonensis* had (4.08mg) vit.E value which goes with a finding which says that nuts are good sources of vitamin E (Etherton, Poth, Sabate, Ratcliffe & Zhao, 1999).

Mineral composition of *Solanum aethiopicum* leaves and *Irvingia gabonensis* seeds

Sa diet

The values in the mineral composition of Sa in this finding agreed with a study carried by Ngondi, Oben & Mina (2005) on the determination of proximate analysis in *S. aethiopicum* and *T. triangulare* who reported traces of iron, zinc, lead and copper. The traditional vegetable has high contents of protein, calcium, phosphorus, iron, potassium, carotene and vitamins A, B and C complementing the nutritional value of basic staple foods (Lester & Seck, 2004).

Ig diet

The higher values of Iron, magnesium, zinc, calcium, potassium and sodium in Ig was not a surprise because nuts (*Irvingia gabonensis)* are often high in nutrients and are good source of energy for the new plant. They are also important source of these nutrients due to its high oil content (Kelly & Sabate, 2006). Literature reports that Ig is a good source of fibre and essential minerals such as mg, ca, fe and k (Etherton, Poth, Sabate, Ratcliffe, Zhao & Etherton, 1999).

Phytochemical and antinutrient composition of *Solanum aethiopicum* leaves and *Irvingia gabonensis* seeds.

Sa diet

There was low level of alkaloids, saponins and high level of tannin in Sa of this result. This may confer on the vegetable more therapeutic and nutritional benefits. Tannins are of wide occurrence in plants and are usually found in greatest quantity in dead and dying cells. They exert an inhibitory effect on many enzymes due to ability to denature protein (Trease & Evans, 2002). This result is not in agreement with earlier work done by Shalom *et al.* (2011) who reported that there was a significant presence of alkaloids, saponins, flavonoids and tannin in both *solanum aethiopicum* and *solanum macrocarpon* L. There was low level of phytate (0.28mg) and oxalate (0.06mg) from the result. This finding is not in line with a study carried by Richelle, Tavazz and Offord (2001) who reported phytic acid 0.82±0.01mg, Oxalate 78.65±0.04mg on the phytochemical composition of *Solanum nigrum* L. leaves.

Ig diet

There was presence of saponin, alkaloid, tannin in Ig from this study. This result is in line with a study carried by Fadare and Ajaiyeoba (2008) who revealed the presence of tannins, saponins, alkaloids and the absence of cardiac glycosides in *Irvingia gabonensis*. The Ig had low level of phytate and oxalate in the result (0.26 and 0.13mg) and this did not agree with Oboh & Ekperigin (2004) who reported significant phytate, tannins and oxalate in antinutrient composition of bush mango. Ekpe, Umoh & Eka (2007) also discovered that phytate and oxalate content reduced in *Irvingia gabonensis* after fermentation

Lipid profile of *Solanum aethiopicum* and *Irvingia gabonensis* seeds leaves fed rats

Sa diet

There were decreases in Cholesterol level of the rats fed Sa diet but the group of rats fed 10g/kgBW Sa diet had the highest decrease which showed that the level had more efficacies in reducing the cholesterol level. It was not a surprise because the lignin and pectin in dietary fibre of *Solanum aethiopicum* appear to lower plasma cholesterol by reducing the plasma low-density lipoprotein fraction (Anderson and Chen, 1979; Behall, 1984). The rats fed 10g/kgBW Sa only decreased the LDL value; other levels (5 and 15g/kgBW) increased the LDL value. This increase in LDL of the rats may be as a result of some medications example diabetics. There were increases in HDL of all the groups of rats fed Sadiet but the rats fed 15g/kgBW had more efficacies in increasing the HDL of the rats. These results are in agreement with a study carried by (Chinedu *et al*., 2013) on effects of *Solanum aethiopicum* on plasma lipid profile in rats. This is also in line with a study carried out on the hypercholesterolemia rabbits treated with Solanum which elicited reduction in cholesterol, LDL and increased HDL (Odetola, Iranloye, Akinloye & Akintola, 2015). There were increases in triglycerides

of all the groups of rats fed Sa diet. This shows that these levels have much the efficacy to increase triglycerides. This may be due to some medical condition like diabetes because most diabetes usually has high triglycerides as a result of some metabolic processes. This is also is not in line with a study carried out on the hypercholesterolemia rabbits treated with Solanum which elicited reduction in serum triglycerides (Odetola, Iranloye, Akinloye & Akintola, 2015).

Ig diet

There were decreases in cholesterol of all the groups of rats fed Ig diet. This is in line with an earlier study carried out by Ngodi (2009) who reported that active extracts of *Irvingia gabonensis* lowered blood cholesterol. The groups of rats fed Ig diet decreased the LDL of the rats but the rats fed 15g/kgBW Ig had more efficacies in decreasing the LDL value of the rats. There were increases (12.13 and 3.30%) in HDL of the rats fed 5 and 10g/kgBW respectively except those fed 15g/kgBW Ig diet (7.69%). The increase in LDL and HDL agreed with a study carried by Ngodi (2005) who conducted a study in Cameroon on African dikanut, reported significant drops in triglycerides, cholesterol and LDL cholesterol and increased HDL. Only the 15g/kgBW Ig diet decreased the triglycerides of the rats, others increased the triglycerides of the rats. The increase in triglycerides of some groups of rats do not agree with Nangue *et al.* (2011) who reported that the increasing amount of dika nut fat significantly alter triglyceride at high dose diet.

Haematological indices in rats fed *Irvingia gabonensis* seeds and *Solanum aethiopicum* leaves

Sa diet

Haematological parameters have been associated with health indices and are diagnostic significance in routine clinical evaluation of the state of health (Saliu, 2012). It gives the physiological information on the blood and its main function is to detect blood disorders such as aneamia and leukaemia (Baker *et al.*, 2010). The various increases in WBC for groups of rats fed Sa diet suggest that these diets except those of 5g/kgBW Sa diet at this level were able to support synthesis of new WBC to fight any foreign body or infection. This observed increase in WBC might be an indication of activation of the immune system; hence, a normal cell-mediated immune response (El-Demerdash, 2004). On the other hand, the increases in PCV of the groups of rats fed Sa diet except those of 5g/kgBW demonstrated that this level was not beneficial to prevent anemia in rats. The highest increase in percentage of 15g/kgBW Sa showed that the diet had more efficacies in increasing the PCV and RBC of the rats. There appeared an increase in leucocytes regardless the level of the Sa diets. This suggests that these diets at this level were able to counteract foreign substances and diseases in these rats. The increases in this finding are in line with a study carried out on the hypercholesterolemia rabbits treated with Solanum which elicited improved blood levels.

Ig diet

The increases in PCV of the groups of rats fed Ig diet demonstrated that these levels were beneficial to prevent anemia in rats. The decrease in percentage of 5g/kgBW Ig showed that the diet at this level do not have the efficacy in increasing the RBC of the rat. The various increases in WBC for groups of rats fed Ig diet suggest that these diets were able to support synthesis of new WBC to fight any foreign body or infection. There appeared to be an increase in leucocytes regardless the level of the Ig diet. This

suggests that this diet at this level were able to counteract foreign substances and diseases in these rats.

Mean Body weight of the groups of rats

Sa diet

There were decreases in body weights of the rats fed each experimental diet at doses 5/kgBW and 15g/kgBW Sa diet. This showed that there is efficacy in reducing weight in this level of supplementation. Vegetables are called negative calorie foods because it actually helps the body to lose weight by spending more amount of energy to digest than it actually adds to the overall caloric intake (Joshipura & Manson, 2001). The result agreed with a study carried by Anosike & Ezeanyika (2012) who reported that *Solanum aethiopicum* has a wide range of utilization form weight reduction to treatment of several ailments including swollen joint pains, constipation, excessive weight gain etc. The decrease is also in agreement with a study on anti-inflammatory activity of the methanol extract of *Solanum aethiopicum* on experimentally induced paw oedema and granuloma tissue formation in rats (Chioma, Onyechi & Lawrence, 2012).

Ig diet

From this study, it was observed that the 5g/kgBW Ig diet had higher decrease compared to the other levels of the supplementation. This shows that 5g/kgBW at this level had the efficacy in reducing the body weight of the rats. This decrease with Ig experimental diet was not a surprise because itôs a good fiber. Ngodi (2005) have proven that it helps lower cholesterol thereby reducing the subjectôs body weight. The crude fiber found in the *Irvingia* seed also works as a bulk-forming laxative which also works to suppress appetites (Abdulrahman, 2004). It agreed with a study carried by Ngodi (2005) who that divided 40 subjects into control and intervention groups, providing the intervention group with *Irvingia gabonensis* extract three times a day for one month and control group received placebos. In just one month, the intervention group lost an average of 5.26 pounds, significantly more than the placebo group's loss of 1.32 pounds. Its high fiber content also allows the body to eliminate all toxins, fats and bacteria that have been accumulated in the colon. *Irvingia gabonensis* is said to promote weight loss and one of the methods is by inhibition of the enzyme Amylase (responsible for absorption of sugar in the body), others are adiponectin and glycerol-3-phosphate dehydrogenase (Punch newspaper, 2013). This result agreed with a study carried by Ngodi (2005) who reported that *Irvingia gabonensis* seed helps to promote weight loss and greater fat burning. A study published by Chukwuma (2009) reported that an extract derived from the seed may help overweight people reduce body weight and lower their cholesterol.

Blood sugar of the groups of rats

Sa diet

There were general decreases in blood sugar of all the groups of rats fed Sa diets. The highest decrease (65.14%) in those fed 10g/kgBW Sa dose, followed by 15g/kgBW

(53.81%) and then 5g/kgBW Sa diet (43.94%) indicates that Sa had higher anti-diabetic effect. *Solanum aethiopicum* is a fibre diet that helps to prevent absorption of fats and sugars in the body (Anderson, Baid, Davis *et al.,* 2009). It also helps to keep blood sugar levels under control (Emebu & Anyika, 2011).

Ig diet

There were decreases in blood sugar of the rats fed Ig diet. Consequently, dietitians frequently recommend nuts be included in diets prescribed for patients with insulin resistance like diabetes (Josse, Kendall, Augustin, Ellis & Jenkins, 2007). The result of this study is in agreement with Ngondi (2009) who reported that an intervention group of 100 overweight individuals who received daily doses of the dika nut extract, after 10weeks had reduced blood glucose.

CONCLUSION

In conclusion, this study showed that *Solanum aethiopicum* and *Irvingia gabonensis* contains moisture, fibre, carbohydrate and protein. *Irvingia gabonensis* had high fat content. *Solanum aethiopicum* is a better source of vitamins A, C and E. *Irvingia gabonensis* contains vit.E. The tannin was high in *Solanum aethiopicum*. *Irvingia gabonensis* contains low level of tannin and alkaloid. *Irvingia gabonensis* had high levels of beneficial micronutrients iron, magnesium, calcium, sodium and potassium. *Solanum aethiopicum* also contains iron, magnesium, calcium, potassium, sodium and copper. The two test diets had the potential of reducing the cholesterol for all the groups of rats. The group fed 5g/kgBW *Solanum aethiopicum* diets only decreased the LDL of the rats. The groups fed *Irvingia gabonensis* generally reduced the LDL of the rats. The rats fed *Solanum aethiopicum* had increases in HDL and triglycerides. The rats fed 15g/kgBW *Irvingia gabonensis* reduced the HDL and triglycerides of the rats. The rats fed 5g/kgBW *Solanum aethiopicum* had decreased PCV. There were increases in PCV for all the groups of rats fed *Irvingia gabonensis.* The groups fed 5g/kgBW of *Solanum aethiopicum* and *Irvingia gabonensis* decreased the RBC of the rats. The groups fed 5g/kgBW of *Solanum aethiopicum* reduced the WBC of the rats. There

were increases in WBC for all the groups of rats fed *Irvingia gabonensis.* The rats had increases in both leucocytes and heamoglobin when fed the two test diets. The rats fed 5 and 15g/kgBW test diets had decreases in body weight. There were general decreases in all the groups of rats fed *Solanum aethiopicum* and *Irvingia gabonensis* diets.

In conclusion, *Solanum aethiopicum* and *Irvingia gabonensis* had high nutritive, therapeutic values and its continuous consumption could improve some health challenges.

RECOMMENDATIONS

1. Cultivation of *Irvingia gabonensis* seed and *Solanum aethiopicum* leaves should be encouraged to prevent their extinction.
2. Nutritional awareness based on their cholesterol, vitamins, minerals and nutrients should be created for more clarification.
3. There is need for the dietitians and nutritionist to incorporate these food supplements in recommendation of diets to patients.
4. There is need to incorporate these supplements when planning food menu.

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