NUTRIENTS, PHYTOCHEMICAL COMPOSITIONS OF *HIBISCUS CANNABINUS*, *ADANSONIA DIGITATA*, *SESAMUM INDICUM*, *CASSIA TORA* LEAVES, THEIR HYPOGLYCEMIC ACTIVITY AND LIPID PROFILE IN ALLOXAN-INDUCED DIABETIC RATS

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NOVEMBER, 2014

TITLE PAGE

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RESEARCH WORK SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIRMENTS FOR THE AWARD OF MASTERS DEGREE IN HUMAN NUTRITION

DEPARTMENT OF HOME SCIENCE, NUTRITION AND DIETETICS

UNIVERSITY OF NIGERIA, NSUKKA

CERTIFICATION

Nwankwo, Rita Ngozi, a post graduate student in the Department of Home science, Nutrition and Dietetics with registration number PG/MSc/ 11/ 58350 has satisfactorily completed the research work for the award of degree of M.Sc in Human Nutrition. The work embodied in this research work is original and has not been submitted in part or full for any other diploma or degree of this or any other university.

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DEDICATION

This research is dedicated to the wits and struggles of my dear husband Sir I.C. Nwankwo

ACKNOWLEDGEMENTS

My gratitude and thanks go first to God almighty who started this good work in me and has ever been faithful. To him be all glory, honour and majesty forever and ever Amen.

The researcher will ever remain grateful and indeed indebted to her supervisors, Professor N.M. Nnam, and Dr. U.S Onoja for their meticulous guidance during this study. She cannot thank them enough for all their contributions towards the successful completion of this work. Your interest and concern for my wellbeing and welfare, your encouragement, openness and prompt response to her problems, ever useful suggestions and most of all the free access and use of your office and books.

Her gratitude also goes to all the lecturers and members of staff of the department of Home Science, Nutrition and Dietetics, University of Nigeria, Nsukka especially Prof.I.C.Obizoba, Prof. E.K Ngwu, Dr (Mrs) V. Ibeanu, Dr (Mrs) O. Onuoha, Ms Lizzy Udenta for their encouragement, friendly disposition towards her and the various contributions and help rendered to her during the course of this work.

The reseachersô thanks go to Prof. Madusolum and the laboratory Technologists of Biochemistry Department, Modibo Adama University Yola for their technical assistance. She is indebted to the department of Home Science, Nutrition and Dietetics, University of Nigeria, Nsukka for providing the metabolic unit used in conducting the rat study.

Her immense gratitude also goes to the Management of Federal Polytechnic Mubi especially Mr. S.O. Onyemuchi (former Rector), David Gbenyi and Hajiya Fadimatu Maigeri who gave fillip to the next level of her career.

To her Husband late Sir. I.C. Nwankwo (KSJI) and children, your love, care, encouragement support, prayers and thoughtfulness made it all worthwhile. Thank you for being there.

She is greatly indebted to all others who supported her morally and spiritually during this work especially Mrs. Ngozi Chukwuemeka, Mrs. Agnes Ngura and Rev. Fr. Jude Nwankwo.

Nwankwo, Rita Ngozi (Mrs).

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ABSTRACT

The study investigated the nutrients and phytochemical compositions of some leafy vegetables in Nigeria (*Hibiscus cannabinus, Adansonia digitata, Sesamum indicum and Cassia tora* leaves) and the effects of their extracts on blood glucose and lipid profile of alloxan induced diabetic rats. Two kilogrammes of each of the vegetables were bought fresh, sorted by removing extraneous material, washed with deionized water and separately pulverized using Gallenkamp mixer Kenwood ï MPR 201. A half of the vegetables was used for chemical analysis and a half for methanol extract production. Standard methods were used to determine in triplicate the proximate, some minerals, vitamins, antinutrients, food toxicants, and phytochemical constituents of the fresh leaves and their methanol extracts. Animal study was carried out to ascertain the effect of the nutrients on blood glucose and lipid profile of alloxan- induced diabetic rats. Forty five male adult albino rats (150-200g) divided into nine groups of five rats each on basis of body weights were used for the study. The group of rats fed rat chow and glibanclamide drug served as standard control. The other groups were fed rat chow and graded doses of each vegetable extract (500mg and 1000mg/kg bodyweight) daily for twelve days. Water was given *ad libitum.* The proximate principles were lower in the fresh leaves than in the extract except for crude fibre. The leaves had 80.20% - 95.09% moisture, 1.62% ï 3. 89% protein, 0.05% ï 0.06% fat, 0.06% ï 1.35% ash, 1.56% ï 4.16 crude fibre and 1.04% ï 13.71% carbohydrate. Mineral values were 236.68 ï 437.11mg sodium, 0.87 ï 2.67mg potassium, 0.63 - 4.97mg calcium, 172.50 ï 235.70mg phosphorus, 0.51 ï 0.59mg zinc, 0.26 ï 0.59mg iron, 3.37 ï 3.44mg copper and 0.24 ï 0.28mg magnesium. The leaves contained 11.57 - 22.28 µg beta carotene, 1.25 - 2.88mg thiamin, 0.87 - 2.82mg riboflavin, 15.60 - 29.37mg vitamin C, niacin 0.74 - 1.61mg and 25.89 -31.43mg vitamin E. All the vegetables had traces of oxalate, 0.01mg - 0.05mg phytate, 0.37mg - 0.43mg tannins. Hydrocyanides levels of the vegetables were low (0.01 - 0.02mg). Food toxicants (cadmium and lead) levels of the leaves were (0.01 - 0.03mg and 0.02 - 0.14mg, respectively). The values were within safe levels for cadmium and lead allowed by World Health Orginisation (WHO) standard for food substances (SAFS). The phytochemicals of the vegetables were in small quantities relative to the nutrients. The phytochemical levels were higher in the extracts than in the fresh leaves. The leaves contained 0.06 - 0.12mg saponins; 0.01mg - 0.04mg flavonoids, 0.03mg ï 0.21mg alkaloids, 0.01 ï 0.02mg glycosides; 0.09mg - 0.21mg terpenes and 0.09mg - 0.16mg phytosterols. The extracts had 5.40% ï 9.84% moisture, 14.56% - 26.42% protein, 0.68% ï 1.23 % fat; 4.34% ï

8.51% ash, 0.62% ï 0.83% crude fibre and 54.64% - 74.44% carbohydrate. Mineral values for the extracts were 873.64 ï 1423.44mg sodium, 1122.61 ï 1425.30 mg potassium, 1571.94 ï 1924.34 calcium, 138.37 ï 224.19mg phosphorus, 0.18 ï 0.27mg/100g zinc, 18.74 ï 34.19mg iron, 0.28 ï 0.83mg copper, and 229.37 ï 341.55mg/ magnesium. The extracts contained 7.60 ï 13.70µg ß carotene, 1.22mg - 2.40mg thiamin, 0.54 ï 2.32mg riboflavin, 14.86 ï 26.34mg vitamin C, 0.84 ï 9.52mg niacin and 21.30mg - 25.72 mg vitamin E. The antinutrients contents of the extracts were 0.66mg - 1.78mg phytate, 4.57 - 7.07mg tannins and 0.22mg - 0.48mg hydrocyanides. 0.01mg - 0.03mg cadmium and 0.02mg ï 0.21mg lead. Phytochemicals value for the extracts were 2.40 - 3.73mg saponins, 0.09 - 0.29mg flavonoids, 4.91mg - 6.77mg alkaloids, 2.40 - 3.84mg glycosides, 1.09mg - 2.30mg terpenes and 1.26mg - 2.50mg phytosterols. Feeding the rats with rat chow supplemented with graded doses of *Hibiscus cannabinus, Adansonia digitata, Sesamum indicum and Cassia tora* leaves extracts reduced blood glucose concentrations and improved lipid profiles. The *Adansonia digitata and Cassia tora* leaf extracts fed at higher doses (1000mg) decreased blood glucose concentrations of rats (33.63% and 23.92%, respectively) more than those fed standard antidiabetic drug glibenclamide (17.23%). They improved lipid profile of the rats by (26.92% and 25.46%). They decreased the total cholesterol (TC) and triglyceride (TG)) 54.72 and 67.70% respectively more than those fed standard drug (21.15% TC and 45.83% TG). The vegetables extracts could be used for management of diabetes and some other related non ï communicable diseases due to their rich nutrients, antioxidants and phytochemical constituents.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Vegetables include those leafy outgrowths of plants or parts of plants that are used in making soup or eaten with the principal part of the meal (Onimawo & Egbekun, 1998). Green leafy vegetables and fruits occupy an important place among the food crops as these provide adequate amounts of many vitamins and minerals for humans. They are rich source of carotene, ascorbic acid, riboflavin, folic acid and minerals like calcium, iron and phosphorous (Nnam, Onyechi & Madukwe, 2012). They are important protective foods and highly beneficial for the maintenance of good health and prevention of diseases (Kubmarawa, Andenyang & Magomya, 2009). Studies have shown that phytochemicals found in large quantities in fruits and vegetables are responsible for this protective effect (Sundarrayanan, Kumia & Sekar, 2011).

Over the past 25 years epidemiological studies have shown a diminished risk of chronic diseases in populations consuming diets high in fruits and vegetables (Kearo, Popkin & Frison, 2010). Countries like South Korea, a high income country that have undergone rapid social change and economic development since the 1970s, still have lower rate of obesity and other non-communicable diseases than the countries with comparable average income. This is because South Korea has protected its traditional food systems. These foods are relatively high in vegetables and fruits (Lee, Popkin & Kim 2002). Equally numerous empirical and investigative reports have indicated that current non-communicable diseases (NCDS) trend in Africa can be attributed to rapid shift from traditional foods which contain mostly vegetables to western food products resulting in elevated intake of saturated fats and food preservatives with reduced intake of dietary fibre, vital nutrients and phytochemicals when compared to basic dietary guidelines (Nahurung, 1997; Gupta, 2011). The shift

from traditional foods to western food products has been dubbed the nutrition transition and is directly implicated in the rise of type 2 diabetes, cardiovascular and other NCDs (Uguru, 2005).

Past generations whose diets consisted mainly of herbs, fruits, vegetables, nuts and starchy tubers lived longer than the present generation (Sathanaraynan, Thomas, Fashik & Sekher, 2009). They were not victims of the many health problems faced by the present population (Uguru, 2005). Life expectancy was better in the past because vegetables were a major component of the diet (Sathanaraynan *at a*l., 2009). Vegetables should be adequately included in the diet to help to fight against the deadly scourge diseases. According to Socrates, a Greek philosopher, Fruits and vegetables are the earliest source of food to mankind (Largen, 1984). Equally Tutare (2000) reported that there are over 200 varieties of vegetables to which majority of Nigerians are not accustomed to. The major reason for less exploitation and utilization of fruits and vegetables in Nigeria is due to ignorance of their contribution to adequate nutrition (Kubmarawa *et al*., 2009; Nnam, 2011).

Leafy vegetables are known to add taste and flavour as well as substantial amounts of protein, fibre, minerals and vitamins to the diet, (Nahurung, 1997; Willel, 2002; Sundarrayanan *et al*., 2011). The amounts of the nutrients and constituents in the more commonly used leafy vegetable species in Nigeria have been studied to some extent (Oguntona, 1998; Kubmarawa, 2009; Ene-obong, 2001). However the lesser known regional and local vegetables remain virtually neglected. Lack of information on the specific nutrients in a large number of locally consumed vegetable species with which Nigeria is richly endowed is partly responsible for their under exploitation especially in areas beyond the traditional localities where they are found and consumed.

During the last decade the concept of health promotion using fruits and vegetables has become legitimate part of health care (Nielsen, 2010). There is an increasing preference expressed by many patients in recent time towards the popular use of alternative therapies that include food supplements

and herbal/folklore preparation with anti diabetic activity. This is because of the much scientific evidence available to support their efficacies in the control of diabetes related metabolic disorders and long term complications (WHO, 1980; Shittu, Bankole & Ashiro, 2007). The diabetes blog is all over research linking increased consumption of vegetables with protective health benefits (http://news.ufl.edu/2009/10121/phytochemicals.multimedia/). Treatment with diets has fewer side effects. Moreover foods are cheap and readily available even in the rural community.

There is dearth of information on the efficacy of *Hibiscus cannabinus* (rama), *Adansonia digitata* (baobab), *Sesamum inducum* (karkashi) , *Cassia tora* (tabsa) leaves as remedy to manage diabetes. The thrust of this study was to explore the detailed nutrients and phytochemicals composition of these foods and their use in animal models to treat diabetes mellitus.

1.2 Statement of the Problem

Increasing incidence of chronic diet related non communicable diseases (NCD_S) is one of the health challenges world over (Nnam, Onyechi & Madukwe, 2012). The diseases which include cardiovascular diseases (CVD_S), diabetes mellitus, obesity, hypertension and cancers are increasingly becoming public health problems in Nigeria (Nnam *et al.,* 2012). NCDs account for 60% of global deaths. It is predicted that by 2020 NCDS would account for 73% deaths and 60% disease burden (Ene-Obong, 2010). The causes are linked to poverty, globalization and adoption of western dietary patterns.These are facilitated by advertisement for consumption of unhealthy foods and lack of physical exercise (Onyechi and Ibeanu, 2010; Nnam *et al.*, 2012).

Diabetes mellitus is one of the chronic non communicable diseases.Diabetes is a serious complex chronic condition and a major cause of ill health worldwide (Willel, 2012). This metabolic disorder is characterized by hyperglycemia and disturbances of carbohydrate, protein and fat metabolism. These could be as a result of an absolute or relative lack of the hormone insulin (Sathanaraynan, Thomas &

Sekher, 2009; Rajjkiran, Nusrath & Sujatta, 2011). Currently, there are over 150 million diabetic patients worldwide. The number is likely to increase to 300 million or more by the year 2025 (Rajikeran *et al*., 2011). No modern medicine has reached the satisfactory level in the treatment of diabetes. The International Diabetes Federation (IDF) (2013) reported that diabetes is no longer a disease of the poor as four out of five people (80%) have diabetes in the world live in low and middle income countries. A country by country summary table by IDF 2012 showed that 3,165.31 million Nigerians between the ages of 20 and 79 years have diabetes, while 2,532.25million Nigerians living with the conditions are unaware and undiagnosed. Nigeria lost 88.681million persons in 2012 due to diabetes related illnesses and has a 4.83% comparative prevalence according to World Health Organization (WHO) standard. As the global burden of diabetes accelerates the call to address the world wide care of diabetics intensifies daily. It has been estimated that by 2025 the incidence of diabetes mellitus would double. There are several drugs for the treatment of diabetes. They however have prominent side effects (Gupta, Medratia, Singh & Sharm, 2006) and most often out of reach for most diabetics. The next option is dietary treatment using foods that are locally available with hypoglycemic effect (Onyechi and Ibeanu, 2010). There has been increasing demand for the use of natural plant products with anti diabetic activity (Fuentes, Sagua, Morale & Bongue, 2005). This is because of their wide biological activities, high safety margins and low costs (Fuentes *et al*., 2005).

Use of plant products to treat diabetes mellitus is of growing interest as most plant foods contain many bioactive substances with therapeutic potentials. Many leafy vegetables and their extracts are effective in the treatment of many non-communicable diseases (NCDs) (Fuentes *et al*., 2005; Nnam *et* al., 2012). WHO also recommends the evaluation of traditional plant extracts, for the treatment of diabetes as such extracts have fewer side effects and possess better glycemic control over the synthetic medicines (WHO, 2007). Nnam *et* al. (2012) reported that some leafy vegetables have

medicinal properties and can be used for the sick and convalescence. Anecdotal evidence suggests that there is antidiabetic activity in *Hibiscus cannabinus* (rama*), Adansonia digitata (baobab), Sesamum indicum (karkashi)* and *Cassia tora* (*tabsa)* leaves. The leaves are major soup vegetables in different parts of northern Nigeria where they are grown and utilized. The present study focused on the scientific investigation of the nutrient, phytochemical composition and anti diabetic activity of *Hibiscus cannabinus* (*rama*), *Adansonia digitata* [*baobab*], *Sesamum indicum* (*karkashi*) and *Cassia tora* (*tabsa*) leaves. The study would provide evidence based information for further research work on the hypoglycemic potentials of the vegetables.

1.3 Objectives of the Study

General objective

The general objective of the study was to determine the nutrients and phytochemical composition of some leafy vegetables (*Hibiscus cannabinus*, *Adansonia digitata*, Sesamum *indicum*, *Cassia tora leaves*) used for preparing soups in Adamawa State of Nigeria and their anti diabetic activity in alloxan-induced diabetic rats.

Specific Objectives

The specific objectives of this study were to determine:

1. some nutrients, antinutrients and food toxicant content of *Hibiscus cannabinus*, *Adansonia digitata*, *Sesamum inducum*, *Cassia tora* leaves and their extracts;
2. some phytochemical constituents of the leaves (alkaloids, carotenoids, phytosterol, glycosides, terpenoids , flavonoids, phenols, saponins) and their extracts; and
3. the effect of the vegetable extracts on blood glucose , serum cholesterol, high density lipoprotein, low density lipoprotein and triglycerides in alloxan - induced diabetic rats .

1.4 Significance of the Study

The result of this work will be of great significance to the scientific community because it would provide evidence based information on the vegetables for Nutritionists and Dietitians preparing diets for diabetic patients in various communities. The vegetables are cheap and abundant and information on their contribution to dietary management of diabetes would be very meaningful. The result could be useful information to Pharmacists to elucidate the medicinal potentials of the vegetables.

The result if positive would create awareness to the general public on the use of the vegetables as remedy to manage diabetes. It would also increase their consumption in many parts of the country where the vegetables are less known and consumed even when they are available in large quantities in the places. The result would also provide valuable information for use in compiling the food composition table on Nigerian foods.

Equally, the result if positive would attract Scientists for further biochemical investigations as well as determine mechanism of action of their active constituents responsible for antidiabetic activities and properties.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Vegetables

The term vegetable usually refer to the fresh edible portion of a herbaceous plant ï root, stem, leaves, flower or fruits (Encarta, 2009). Onimawo & Egbekun (1998) described vegetables as leafy outgrowths of plants used as food and included those plants and parts of plants used in making soups or served as integral part of the main meal. The use of leafy vegetables is part of Africaôs cultural heritage and vegetables play important roles in the food culture of African households (Ene-Obong 2008). Tutari (2000) reported that Nigeria is endowed with a variety of vegetables. Different ethinic groups consume various types of vegetables for different reasons. Vegetables are the cheapest and most available sources of important proteins, amino acids, vitamins and minerals (Okaka, 2000). Vegetables in the diet have many positive effects upon health because of their constituents. Some vegetables have medicinal prosperities and can be used for the sick and convalescences (Kubmarawa, 2009; Nnam *et al*., 2012).

Vegetables are naturally low in fat and calories. None have cholesterol; many are good sources of fibre, minerals and vitamins (lloveindia, 2004). Vegetables equally contain carbohydrates and protein. Until most recently a group of chemicals known as phytochemicals were discovered. They are found only in plant based food in very small amount (Nnam, 2011). They perform numerous preventive and healing functions within the body (Pamplona-roger, 2005).

Vegetables may be raw, cooked, fresh, frozen, canned or dried/dehydrated. They may be used whole, cut up or mashed (Enwere 1998). Vegetable consumption is affected by seasons of the year, however improved farming systems (e.g. use of irrigation) has helped to keep most types of vegetables available throughout the year.

Diets based on roots, tubers and legumes can be supplemented with vegetables to enhance their health and nutritional values. Vegetables are nutrient dense, this means that for the small amount of calories they contain; the level of nutrients is high.

2.1.1 Classification of vegetables

Vegetables can be categorized according to their type and taste

Table 2.1 Classification of Vegetables

TYPE	EXAMPLE
Bulb Vegetables	onions, garlic and shallots
Fruit Vegetables	avocadoes, cucumbers, okra, tomatoes, pepper and egg plant
Inflorescent Vegetables	broccolis and artichokes
Leafy Vegetables	bitter leaf, scent leaf, lettuce, spinach and cabbage.
Root Vegetables	carrots, beets, radishes and turnips
Stalk Vegetables	asparagus, bamboo and celery.
Tuber Vegetables	cassava, yam, sweet potato and taro

Source: Iloveindia, 2004.

Leafy vegetables

Leafy vegetables are many, ranging from leaves of annuals and shrubs to leaves of trees. Leaf vegetables are also called potherbs, greens, vegetable greens, leafy greens or salad greens, they are plant leaves eaten as vegetables sometimes accompanied by the tender petioles and shoot. Although they come from a wide variety of plants most share a great deal with other vegetables in nutrition and cooking methods. Nearly one thousand species of plants with edible leaves are known. Leafy vegetables most often come from short lived herbaceous plants such as lettuce and spinach. Woody

plants whose leaves can be eaten as leaf vegetables include *Adansonia*, *Aralia*, and *Moringa*. The leaves of many foodder crops are also edible by humans, but usually only eaten under famine conditions example alfalfa, clover and most grasses. They are generally good sources of nutrients (Sundarrayanan *et al*., 2011). They contain lots of carbohydrates, and are rich in carotenoids and vitamin C. They are also good sources of fibre, folate and supply varying amounts of iron and calcium.

Leafy vegetables contain many typical plant nutrients but since they are photosyntehetic tissues their vitamin k levels in relation to those of other fruits and vegetables as well as other foods is particularly notable (Pamplona-roger, 2005).The reason is that phylloquinone the most common form of vitamin K is directly involved in photosynthesis.This causes leafy vegetables to be the primary food class that interacts significantly with the anticoagulant pharmaceutical werferin (www.healthy-eating.and. nutrition. co).

2.1.2 Uses of vegetables in foods

Vegetables are used in foods depending on the purposes to be achieved. They may be used as major or minor ingredients in soups, sauces, stews, pottage, porridge and salads to enhance the flavour of foods, garnish prepared dishes so as to enhance eye appeal, serve as fillings for sandwiches, pies and Indian egg rolls. They can serve as a critical part of the ingredients in the preparation of certain dishes such as vegetable soup, vegetable pottage, vegetable parcels and salads (Enwere, 1998).

2.1.3 Importance of vegetables.

Vegetables provide essential vitamins, minerals, fiber and other substances that are important to good health. Eating plenty of vegetables everyday can help reduce risk of heart disease, high blood pressure, type II diabetes and certain cancers. Vegetables have many important phytochemicals that

help to protect health. Phytochemicals are usually related to colour. Vegetables of different colours ð green, yellow-orange, red, blue-purple, and white ð contain their own combination of phytochemicals and nutrients that work together to promote good health. Vegetables are low in calories and fat. They are high in fibre and are filling thus they can help to control weight. Vegetables are natural source of energy; they give the body many nutrients needed to keep going. Busy lives require food that is nutritious, energizing, and easy to eat on-the-go, like fresh fruits and vegetables (National cancer institute, 2009).

2.1.4 Health benefits of vegetables

Recent researches show that most people will benefit from increasing their fruit and vegetable intake (University of Warwick, 2012). Life time habit of eating adequate amount of fruits and vegetables every day can help prevent coronary heart disease, constipation, some forms of cancer, overweight and obesity. It can also reduce blood pressure and blood cholesterol levels and improve control of diabetes (www.nutrition.org.uk/healthyliving/.).

2.1.5 Vegetables in diabetes management

Numerous epidemiological studies have correlated human consumption of diets rich in fruits and vegetables containing high levels of phytochemicals to lower risk for specific chronic diseases such as diabetes, cancer, cardiovascular diseases (Bokange 1994; Chakaraburthy 2008; Shittu 2009). Vegetables are usually high in fibre and researches have shown that eating foods more in fibre lower blood sugar levels. In general, high fibre foods take longer to digest and therefore produce a slower rise in blood glucose levels (Balch & Balch, 1998). The high fibre foods include vegetables (Willell, 2012). Nahurung (1997) reported that green leafy vegetables have shown beneficial hypoglycemic influence in both experimental animals and humans. The hypoglycaemic influence is claimed to be

mediated through an insulin secretagogue effect or through an influence on enzymes involved in glucose metabolism.

Grover *et al*. (2002) identified that herbs play important role in diabetic therapy, particularly in developing countries where most people have limited resources and do not have access to modern medical treatment. WHO has also authenticated the use of herbal remedies for treatment of diabetes (WHO, 1980). In the same vain the WHO expert committee on diabetes has recommended that traditional methods of treatment of diabetes should be further investigated (WHO, 2007). The increase in demand of the use of plant based medicine to treat diabetes may be due to the side effects associated with the use of orthodox drugs such as insulin and oral hypoglycemic agents (Ashraluzzaman, 2011). Another factor that strengthens the use of plant materials as antidiabetic could be attributed to the belief that herbs do provide some benefits over and above allopathic medicine and allow the users to feel that they have some control on their choice of medication (ADA, 1997; Gupta, 2011).

Consumption of vegetables is a major source of micronutrients. Sathanarayan *et al*. (2009) affirmed that turning to green vegetables will be a great advantage in the diabetes management as it provides safety, quality and efficacy thus ensuring complete freedom from the hazardous effects of other systems. There are not much published research studies to confirm the glycemic index of vegetables. Most authors place the glycemic index of vegetables between 15 and 50. This range is considered to be based on their low carbohydrate and high fibre content. A food is generally considered to have a high glyceamic index if it is rated above 60 so the best is 40 or less (Willell, 2012). Eboh (2006) reported that some African indigenous vegetables showed hypoglycemia activity as antidiabetic agents. They are good sources of phytochemicals, especially flavonoids. Flavonoid may be responsible for the observed antidiabetic activity of the vegetables (Eboh, 2006).

Vegetables have high concentration of micronutrients. They provide little dietary energy making them valuable in energy limited diets (Solanke & Awonorin, 2002). Their content of phytochemicals and antioxidants in correct combination keep the blood sugar in balance, create better energy in the body and build up the immune system (Jane, 2005). The WHO expert committee on diabetes recommends that plants possessing hypoglycemic activity may provide a useful source of oral compound for the development of hypoglycemic treatments. Jane (2005) confirmed that many of the side effects of diabetes can be prevented if glucose level at normal range is controlled using natural plants and herbal medicines.

2.1.6 Effect of processing on green leafy vegetables

Processing of vegetables involves cleaning, sorting, grinding pounding, trimming, blanching, canning, storage, freezing and drying (Enwere, 1998). Squeeze washing and cutting are popular procedures among Nigerians in preparation of certain green leafy vegetables. The choice of processing method depends on the product desired and storage facilities available. It may have beneficial or harmful effects on different properties of the vegetable. According to Mepba, Eboh and Banigo (2007) leafy vegetables are highly perishable food items and require special processing treatments to prevent post harvest losses. Mepba *et al*. (2007) further reported that in Nigeria, leafy vegetables are preserved by sun-drying and used like freshly harvested vegetables in soups. They can also be cooked or dried, depending on the mode of utilization (Shittu & Ogunmoyela, 2001). Moshe, Pace, Adeyeye, Laswai and Mtebe (1997) reported that-traditional sun drying of cowpea leaves resulted in severe losses of provitamin A. Shade drying and storing in airtight containers produced better results (Mosha *et al*., 1997). Boiling and then discarding the water used for boiling vegetables provides a good means of reducing the oxalate content of some leafy vegetables and consequently the associated food safety problems (Ogbadoyi *et al*., 2006).

2. 1.7 Recommended daily intake of vegetables

Table 2.2 the recommended daily intake of fruits and vegetables for children and adolescents

Age (yrs)	fruit serves	vegetable serves
4-7	1-2	2-4
8-11	1-2	3-5
12-18	3-4	4-9

Source the Australian guide to healthy eating, (2012).

Lower serve is recommended for those with diet high in cereal foods (e.g. pasta, rice, bread). Higher serve for those who eat more evenly spread across five food groups. Pregnant and lactating women should aim to eat 4 to 5 serves of fruits and 5 to 7 serves of vegetables to meet extra demands of the body (Time in life style GROUP, 2013).

The right amount of fruits and vegetables depends on several factors including age, gender and physical activity (Healthy living www.cookinglight.com.). Based on this, physical activity has been categorized into three levels. Lightly active. Less than 30 minutes exercise a day; Moderately Active average of 30 to 60 minutes of exercise a day; Very active average of 60 minutes exercise or more a day.

Table 2.3 recommended daily intake of fruits and vegetables for adults.

Physical activities	Gender	Age	cups of fruits Per day	cups of vegetables per day	total
Lightly active					
	Women	19-30	2	2 ½	4½
		31-50	1 ½	2 ½	4
		51+	1 ½	2	3½
	Men	19-50	2	3	5
		50+	2	2½	4½
Moderately active exercise.					
	Women	19-50	2	2 ½	4 ½
		50+	1½	2½	4
	Men	19- 30	2	3½	5½
		31+	2	3	5
Very active exercise or more					
	Women	19-30	2	3	5
		31-50	2½	3½	6
		51+	2	2 ½	4 ½
	Men	19-30	2½	4	6½
		31-50	2½	3½	6
		51+	2	3	5

Source www.com/healthy living.cooking light, (2012).

The goal is to match the number of servings of fruits and vegetables consumed in a day with the total recommendation for a day.

2. 2. 0 Nutrient Composition of vegetables

Nutrients are the essential substances obtained from food (www.nutrition.org.uk/healthy_living/.) They are what the body needs to perform its functions properly. The classes of nutrients are carbohydrates, proteins, fats, vitamins, minerals and water. Water which helps to assimilate nutrients and fibre which helps with regular elimination of toxins and wastes are very important nutrient

facilitators in foods (www.healthy-eating.and.nutrition.co.). Fruits and vegetables are naturally good. They contain vitamins, minerals and other compounds like antioxidants and phytochemicals. These substances help to protect the body against diseases. Some vegetables contain some antinutrients and toxicants (Bokanga, 1994).

2.2.1 Moisture Content of vegetables

Vegetables naturally have high levels of water. This is the reason why they are generally fat free and low in calories (www.organicfacts.net/health). Both weight and health are controlled with diets rich in vegetables. Fresh green leafy vegetables are high in moisture. The level in individual sample depends on several factors such as age, agronomic practices prevailing during cultivation and freshness (Oguntona, 1998). The moisture in green leafy vegetables ranges from 72% in cassava leaves to 93% in water leaf (Onimawo & Egbekun, 1998). Proper moisture content is essential for maintaining fresh healthy foods (www.ehow,> eHow> Healthy living). The moisture content of the dried vegetables also varies. Generally, vegetables are traditionally sun- dried. Eka and Osagie (1998) reported that vegetables continue to lose moisture while in storage or display depending on the local environmental condition. Vegetables with high moisture content are called high water content foods with 80-95% of their total composition being water. The more vegetables consumed the more water intake that flushes out waste products from the body (www.healthy-eating.and. nutrition. co).

2.2.2 Carbohydrates.

Carbohydrates are present in foods in form of sugars, starches and fibre. Vegetables are important sources of both digestible and indigestible carbohydrates. The digestible carbohydrates are present largely in the form of sugars and starches (Eka & Osagie, 1998). The indigestible carbohydrates are in the form of fibre. Carbohydrates are eventually metabolized by the body into blood glucose. All cells of the body utilize glucose as the primary energy source, particularly in the brain, for which

glucose provides the only source of fuel. Excess carbohydrates are converted into triglycerides for storage in adipose or fat cells, leading to weight gain. Incomplete carbohydrate metabolism leads to accumulations of sugar in the blood a condition known as hyperglycemia, a manifestation of diabetes.

Starch also known as complex carbohydrate or polysaccharide, is present in foods such as cereals, whole grains, rice, pasta, potatoes, peas, corn and legumes. Sugar is found naturally in many foods. They have simpler chemical structure than starch. Food sources of natural sugar include fruit, vegetables, milk and yoghurt. Low sugar vegetables include tender green leafy vegetables, spinach, lettuce etc. Foods containing natural sugars are generally very nutritious, providing many vitamins, minerals, phytochemicals (natural plant chemicals) and antioxidants. These foods also tend to be good sources of fiber, such as that found in fruits, vegetables and whole grains. However, foods high in added sugars are often referred to as sources of "empty calories," meaning they add calories to the diet but provide little benefit in terms of vitamins, minerals or fiber. Most plant foods are good sources of fibre.

Dietary fibre

Dietary fibre sometimes referred to as roughage or bulk encompasses all substances and compounds that pass through the intestine undigested. Fibre is found only in foods of plant origin. Fibre is divided into two general categories _soluble fibre which are compounds that dissolve in water and insoluble fibre which are those that bind to water. Vegetables including green leaves are significant sources of both soluble and insoluble dietary fibre (Egbuna 2000). Available evidence suggests that the soluble components represents 25% or less of the fibre present in most natural food stuffs while the insoluble fraction accounts for 75% of the fibre content (Onimawo & Egbekun, 1998). Soluble fibre promotes healthy cholesterol and blood sugar levels. Dietary fiber increases the weight and size of stool and softens it. In the presence of adequate amount of fluid insoluble fibre makes stool

larger, softer and easier to pass thereby decreasing the chance of constipation (Cumming, 1981). A high-fiber diet may lower the risk of specific disorders, such as hemorrhoids, irritable bowel syndrome and the development of small pouches in the colon (diverticular disease). While most food sources contain varying amount of both soluble and insoluble fibre, some foods are especially rich in one type. Soluble fibre found in beans, oats, flaxseed and oat bran may help lower total blood cholesterol levels by lowering low-density lipoprotein, or "bad," cholesterol levels (Onimawo & Egbekun, 1998). Soluble fibre can slow the absorption of sugar, which for people with diabetes can help improve blood sugar levels. A high-fiber diet may also reduce the risk of developing type 11 diabetes (www.healthy eating. Sfgate.com>). High-fiber foods generally require more chewing time, which gives the body time to register when the body is no longer hungry, cutting down overeating (Cumming, 1980). Also, a high-fiber diet tends to make a meal feel larger and linger longer, so the body stays full for a greater amount of time. High fiber diets also tend to be less "energy dense," which means they have fewer calories for the same volume of food.

2.2.3 Protein content of vegetables

Every cell and tissue in the body contains protein. Different proteins work as enzymes, hormones, neurotransmitters, antibodies and specialized proteins such as heamoglobins and others (Bean, 2000). Proteins are constantly repairing body tissues to keep it healthy. They are made up of amino acids .There are two types of amino acids- essential and non- essential. The eight essential amino acids cannot be made in sufficient amounts in the body and most therefore be supplied in the food. The twelve non essential amino acids can be made from other amino acids in the diet. Foods containing animal protein such as meat, milk and eggs, contain ample amounts of all essential amino acids. Vegetable protein sources have one or more of the essential amino acids missing or have less than the adequate amounts (http://www.eufic, org). These foods however can be combined in a diet that

supplies the required amounts (WHO, 1985). Crude protein content of green leafy vegetables ranges from 1.5% to 1.7 %.(WHO, 1985; Aletor & Adeogun 1995). Some vegetables such as legumes are great sources of plant proteins. Proteins combine well with green leafy vegetables and non starchy vegetables (www.marilu.com/../ Food combining...)

2.2.4 Fats in vegetables

Green leafy vegetables are known to be poor sources of fat (en.wikipedia.org/wiki/leaf. Veg.). Fat represents the lowest among the proximate components. Oguntana (1998) reported that the ether extract scarcely exceeds 1.0% in fresh leafy vegetables. The values from dry samples range from 1.0-3.0%. However, dark green leafy vegetables contain omega 3 fatty acids. Omaga 3 fatty acids are called essential fatty acids because the body cannot manufacture them from other nutrients. It must be obtained from the diet. Omaga 3 fatty acids come in three varieties namely Alpha Linoleic Acid (ALA), Decosol Hexanoic Acid (DHA) and Eicosoi Pentonoic Acid (EPA).They give important health benefits to the body. They may help to prevent breast and colon cancer, high blood pressure and can reduce the risk of suffering a stroke among other benefits. ALA is found primarily in dark green vegetables, flax seeds, hemp seeds, walnuts and a variety of vegetable oils. EPA is found primarily in cold water fish like salmon,cold mackerel and tuna as well as fresh seaweed. DHA are found in the same foods that EPA is found. Dark green vegetables are among the highest sources of ALA (www.young women health org>). Most humans can convert ALA found in plant foods to DHA and EPA in the body to provide all its health benefits. Theoritically eating foods containing ALA or dark geen vegetables can produce enough DHA and EPA but the controversy is that some people cannot efficiently convert ALA to DHA and EPA. It is wise and safe to eat a variety of foods that are naturally rich in ALA, EPA and DHA rather than to rely on a supplement that contains just one or more of this omega 3 fatty acids as isolated nutrients. Researches confirmed that the body

needs a little dietary fat to absorb some of the vitamins found in dark green leafy vegetables (www.young women health org>).

2.2.5 Ash

The ash content is a measure of the total amount of mineral present within a food whereas the mineral content is a measure of the specific inorganic components present within a food (www.soil and health.org/06clipfile/RC). The percentage of ash and each of the constituents of ash of any given species of plants are known to vary widely. They vary with the variety and with the age of the plant and the environmental condition under which it was grown (Onimawo & Egbekun, 1998). Such variations are of considerable significance to animals and man since these creatures depends on plants for most of their mineral matter.

2.2.6 Minerals

Minerals are elements that originate in the soil and cannot be created by living things such as plants and animals (www.health.alternatives.com/minerals). They are also known as micronutrients. They do not provide the body with energy but they help the body to carry out the metabolic processes. Plants, animals and humans need minerals in order to be healthy. There are two kinds of minerals: macrominerals and trace minerals. The macrominerals include calcium phosphorius, magnesium, chlorine, sodium and potassium. The trace minerals are required in trace amounts and they play catalytic functions in the body. They include copper, iron, cobalt, iodine, molybdenum, selenium and manganese.

Plants absorb minerals from the soil; animals get their minerals from the plants or other animals they eat. Most of the minerals in the human diet come directly from plants such as fruits and vegetables or indirectly from animal sources. Minerals may also be present in drinking water depending on the type

of water and sources (www.heaalth.alternatives.com/mineralé). Minerals from plant sources may also vary from place to place because the mineral content of the soil varies according to the location in which the plant was grown. Vegetables are important sources of mineral elements calcium, phosphorus and iron (Onimawo & Egbekun, 1998). Green leafy vegetables are, particularly rich in iron and calcium but low in sodium (Egbuna, 2000). Some vegetables like swisschord and spinach that are high in oxalic acids are often low in iron and calcium because the oxalic acid tends to chelate the calcium and iron in these vegetables.

Green leafy vegetables are high in magnesium content and have low glycemic index thus they prove to be helpful for patients with type 2 diabetes (www.heaalth.alternatives.com/mineralé). Dietitians advice that one serving of green leafy vegetables each day will considerably lower the risk of diabetes (www.organicfacts.net/health-benefits). Some minerals are essential to health while others can be toxic example lead, mercury, cadmium and aluminum. While all minerals play key roles in the body processes manganese, copper and zinc are particularly important in energy metabolism. Calcium, phosphorus, iron, magnesium, iodine and selenium are also important in carbohydrate metabolism.

Copper

The recommended daily intake of copper is 1.5 - 3.0 mg/day. Copper is involved in the absorption, storage and metabolism of iron and formation of red blood cells. It helps to supply oxygen to the body. Copper helps in energy metabolism. It works closely with the enzyme cytochrome c oxidase to produce energy. Cytochrome c oxidase starts the process that converts oxygen to water, creating an electrical element that the mitochondria (the part of the cell that makes energy) uses in its processes. Most vegetables contains some amount of copper but it is more significant in lima beans, amaranthus leaves, artichokes, kale, pumpkin and taro.

Manganese

The recommended daily intake of manganese is 2.0 - 5.0mg/day for adults and 2.0 - 3.0mg / day for children. The functions of manganese are not specific since other minerals can perform in its place; however it functions in enzyme reactions concerning blood sugar metabolism and thyroid hormone function. The body uses manganese to activate several of the enzymes involved in energy metabolism for example the body uses pyruvate carboxylase an enzyme that contains manganese in the process of converting glucose from proteins and fats. In the process of converting sugar to glucose which is the fuel for the body, manganese activates the enzyme phosphoenolpyruvate (Pauline, 2006). Sources of manganese include most green vegetables, okro, potatoes, amaranthus leaves. Most legumes are also itsô good sources. The deficiency of manganese is rare in humans (Pauline, 2006).

Zinc

Zinc is important in a number of key functions in the body ranging from protein and carbohydrates metabolism to immune system, wound healing and vision. The recommended daily intake of zinc varies; men 15mg/day, women 12mg/day and children 10 to 15mg/day. Vegetarians need about 50% more zinc in their diet than meat eaters ((www.organicfacts.net/health-benefits). In energy metabolism, zinc is primarily used to make the enzymes active in the chemical reactions involved in the metabolic process. It is also used in the release of insulin; insulin enables the cells to absorb glucose which produces energy. Zinc is also important in the management of diarrhoea. WHO and UNICEF recommend daily supplement of 20mg for 10 -14 days for children with acute diarrhea, 10mg / day for infants under 6 months (WHO, 2007). Severe deficiency of zinc can, contribute to stunted growth. Most fruits and vegetables contain small amounts of zinc but the following has

significant amounts corn, amaranthus, whole grain cereals, meat and eggs (Onimawo & Egbekun 1998).

Magnesium

Magnesium is required in energy production. It catalyses conversion of ATP to ADP in carbohydrate metabolism. Intake of magnesium supplement helps the diabetic patient to improve insulin and glucose level (www.bodybuildingtipsguide.com. Studies have shown that 80% of the people suffering from diabetes have magnesium deficiency. Thus, intake of magnesium reduces the duration, intensity and frequency of urination (www.bodybuildingtipsguide.com). Its other functions include making new cells, activating B vitamins, relaxing nerves and muscles and blood clotting. Insulin secretion and function also requires magnesium. It also assists in the absorption of calcium, vitamin C and potassium. Deficiency of magnesium results in fatigue, nervousness, heart problem, weakness and cramps. Rich vegetable sources of magnesium include spirulina, okra, green vegetables, amaranthus leaves and legumes. Its recommended daily intake for adult is 310 to 420mg/day; children 130 to 240mg/day (www.magnesium. org).

Other minerals

Calcium is the most abundant mineral in the human body .The recommended daily intake of calcium for adults is 1000mg/day; children 800 - 1300mg/day. Good sources of calcium include dark green vegetables, okra, beans, Brussels and milk. Potassium acts as catalyst in energy metabolism. Phosphorus aid in metabolic reactions (as component of DNA and RNA, ADP, ATP and TPP). It is widely distributed in both plant and animal foods. Iodine aids in regulating basal metabolism (as a component of thyroxin and tri-iodothyroninine. Its sources include iodized salt, salt water fish. Sodium aids in the absorption of glucose.

2.2.7 Vitamins

Vitamins are organic compounds that are necessary for normal growth and maintenance of life. The body cannot synthesize vitamins; they must be taken in food and food supplements. Adequate intake is necessary for normal functioning of the body. Thirteen essential vitamins have been isolated and these are divided into 2 categories; Water soluble vitamin and fat soluble vitamins. The water soluble vitamins include vitamin C and the B group of vitamins. These water soluble vitamins are not stored by the body and can be readily depleted. The fat soluble vitamins include vitamin A, D, E and K. They can be stored in the body (www.livestrong.com). Vitamin D sometimes is not regarded as essential because it can be synthesized in the body by the action of ultra violet rays of sun on 7 dehydrocholcalciferol in the skin of humans. Carnitine a vitamin like compound very indispensable for survival and health are not strictly "essential" because the human body has some capacity to produce them from other compounds (www.nutrition. Org. uk/healthyliving).

Specific conditions are known to arise as a consequence of a dietary deficiency of one or more of the vitamins. These conditions can be avoided if meals are well planned and carefully prepared (Ene-obong 2001; Nnam 2010). Dietitians advice that the practice of warming or heating vegetable soups every morning should be discouraged as many vitamins are lost in the process. Excess of some vitamins especially the fat soluble vitamins could be dangerous to health.

In general vegetables are good sources of vitamins. The factors that influence the amount of vitamins in green leafy vegetables are cultivars, maturity and light (Egbuna, 2000). Green leafy vegetables are the richest source of thiamin and riboflavin, ascorbic acid and beta carotene (Pro-vitamin A). Oguntona, (1998) and Egbuna (2000) reported that Niacin and folate are found in reasonable amount in green leafy vegetables. Other sources of vitamins include meat, eggs poultry, fish, and cereals.

Vitamins involved in energy metabolism

All vitamins play key roles in the body processes, vitamin B_{12} and pantothenic acid are particularly important in energy metabolism. Niacin, thiamin, riboflavin, biotin, are also important in carbohydrate metabolism. When vitamin B_{12} is taken in the body, it is broken down into several compounds including 5 Deoxy adenosylcobalamin. This compound is used by an enzyme to catalyze important biochemical process that the body uses in the metabolism of energy from protein and fat. Pantothenic acid also called vitamin B_5 is used by the body to form an enzyme called coenzyme A. Coenzyme A is used in biochemical reactions that result in the bodyôs production of energy from carbohydrates, fats and proteins.

2. 3.0 Antioxidants

Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species such as singlet oxygen, superoxides, peroxyl radicals, hydroxyl radicals and peroxylnitrite (Buhler & Miranda, 2004). They are essentially the chemical substances that mop up harmful free radicals from body cells and tissues (Ene-obong, 2001). They assist to prevent extremely broad spectrum of diseases like cancer, heart disease, stroke, Alzheimers disease, rheumatoid arthritis and cataracts (Lakshmi, Tiak, & Janard, 2004)). Free radicals are those harmful and unstable types of oxygen that damage cells and attack the fats that provide structure to cell membrane surrounding the cells. The reactions provide progressive adverse damage that accumulates and manifest as diseases with age (Nutrihealth, 2005). Antioxidants neutralize the free radicals (Kendall, 2000).

Antioxidants are classified into two broad groups, depending on whether they are soluble in water (hydrophilic) or in lipids (hydrophobic). The water soluble antioxidants react with oxidants in the cell cytoplasm and the blood plasma, while lipid ï soluble antioxidants protects cell membranes from

lipid peroxidation. These compounds may be synthesized in the body or may be obtained from the diet (Docampo, 1995).

The enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), and the glutathione peroxidase (GP_X). The non enzymatic antioxidants are the vitamins such as vitamin C, E and beta carotene and the minerals such as selenium and zinc (Hayek, 2000). Selenium and zinc are commonly referred to as antioxidant nutrients but they have no antioxidant action themselves. They are instead required for the activity of some antioxidant enzymes. Other nutrients and compounds that have antioxidant properties include the co-enzyme Q_{10} or ubiquinone which is essential to energy production and can also protect the body from destructive free radicals (Hayek, 2000). Uric acid a product of DNA metabolism has become increasingly recognized as important antioxidant (Johnson & Giuilivi, 2005). Plants produce antioxidant phytochemicals that include carotenoids, flavonoids, cinnamic acids, folic acids ascorbic acid, tocopherols and tocotrienols to prevent oxidation of the susceptible substrate (Hollman, 2001).

2. 3.1 Antioxidants in vegetables

Antioxidants are naturerally found in fruits and vegetables. Examples of dietary antioxidants are vitamins, A, C, E, phenolic acids, selenium, flavonoids, glutathione, lycopene, chlorophyll and melatonin. Plants rich in antioxidants include several fruits, vegetables, nuts, seeds, ginger, soybeans, onions, garlic, cabbage, spinach, cauliflowers and other food sources. Antioxidants obtained from food sources, including fruits and vegetables are potentially active in disease risk reduction and beneficial to human health (Warmer, 2002).

Lutein, an antioxidant found in spinach is the main pigment in the macular-the region of maximum visual sensitivity. Studies have shown that people who consume spinach are less likely to develop cataracts and macular degeneration, the two most common causes of vision loss. Lutein shields the

retina from sun damage and fights free radicals that can harm the eyes. Some preliminary studies suggested that lutein prevents heart disease (Tuner, 2002). Ene-Obong (2001) documented that spinach is a source of alpha ï lipoic acid a powerful antioxidant that counteracts the effect of ageing and heart disease. Broccoli and other cruciferous vegetables cabbage, cauliflowers and Brussels sprout prevent cancer and ward off heart disease.

Tomatoes contain lycopene a relatively rare member of the carotenoid family. Lycopene has been shown to prevent prostate cancer (Ene-Obong, 2001). The benefits of tomatoes were found in both raw and cooked forms. Men who eat 4-7 serving per week have a 22% of reduced risk compared with those eating less than 2 serving weekly. Tomatoes also contain the antioxidant glutathione which boost immune functions (Warmer, 2002).

Carrots are highly concentrated with a potent antioxidant beta carotene, a member of the healing family of carotenoids. Ginger contains phenol compounds that have antioxidant activity greater than that of vitamin E. Garlic and onions are good sources of flavonoids which are powerful antioxidants. Studies have shown that garlic and onions keep the heart healthy by lowering cholesterol levels, reducing blood pressure, fighting free radical and keeping blood from clotting. Other studies suggested that eating garlic and onion regularly assists to prevent cancer.

The most well known components of food with antioxidant activity are vitamins C and E, beta carotene and the mineral selenium (Buhler & Miranda, 2004).

Vitamin C

Vitamin C also known as ascorbic acid, is a water soluble antioxidant that scavenges free radicals and reactive oxygen molecules produced during metabolic pathways of detoxification (Proteggente, Pannak, Wagner & Evans 2002). Vitamin C as an antioxidant protects the DNA of the cells from

damages caused by free radicals and mutagens .Gaby and Singh (1991) reported that vitamin C prevents harmful genetic alterations within cells and protects lymphocytes from mutation to the chromosomes. Another way in which vitamin C protects the body is by preventing the development of nitrosamines, the cancer causing chemical that stem from the nitrates contained in foods (Gaby & Singh,1991). Vitamin C is an excellent source of electrons to free radicals such as hydroxyl and superoxide radicals; being water soluble it works both in and outside the cells to stop their reactivity by donating electrons to them (Bendich, 1990). Vitamin C works with glutathione peroxidase (a major free radical fighting enzyme) to revitalize vitamin E, a fat soluble antioxidant. Vitamin C being water soluble cannot be stored in the body It is important to obtain it regularly from its major sources- fruits and vegetables. The important sources include citrus fruits, green pepper, broccoli, green leafy vegetables, kiwi, strawberries, raw cabbage and potatoes (Kendall, 2000).

Vitamin E

Vitamin E, also known as alpha tocopherol is a fat soluble antioxidant vitamin. It is the collective name for eight compounds, four tocotreinols and four tocopherols. It is present in all cellular membrane and mainly stored with fat in adipose tissue, the liver and muscles (Prior, 2007). Vitamin E is promoted for a range of health purposes_ from delaying ageing to healing sun burn (Frei, 1994). It is a powerful antioxidant. Vitamin E is the most effective non enzymatic antioxidant for terminating the chain reactions of lipid peroxidation in cell membranes. It is especially effective in protecting low density lipoproteins (LDL) from oxidation. It corroborates with vitamin C to slow progression of cardiovascular disease and protects the double bonds of beta carotene from oxidation and thus exhibits a sparing effect. Salomen, Nyssomen, Touma and Nem (1995) established that vitamin E status has a strong independent inverse association with the risk of diabetes. Important

sources of vitamin E include wheat germ, nuts, seeds, wholegrains, green leafy vegetables, vegetable oils and fish liver oil (Proteggente, *et al*., 2002).

Carotenoids

Carotenoids are organic pigment occurring in plant and some types of algae and fungi. More than 600 types of carotenoids have been identified. The molecular structures of caroteinoids identify them as very efficient free radical scavengers. They posses powerful antioxidant effects. Carotenoids are the naturally occurring pigments that give fruits and vegetables their bright colours. Some important carotenoids include beta carotene, alpha carotene, lycopene and xanthaxanthin.

Beta carotene

Pauline (2006) emphasized that beta carotene is the most widely used carotenoid. It is in the category of provitamin A, from where animal body synthesizes vitamin A (retinol) a potent antioxidant itself (Frei, 1994). Frei (1994) reported that beta carotene is the one most easily converted to vitamin A in the body. Kendall (2000) observed that beta carotene protects dark green vegetable, yellow and orange vegetables and fruits from solar radiation damage. It has been found to play a simillar role in the body (Nnam, 2012). Beta carotene as antioxidant stops singlet oxygen scavenges free radical and protects cell membrane liquids from the destructive effects of oxidative degradation (Brio, Wu & Schaich (2005). Beta carotene is beneficial in cataract prevention. Rich sources of beta carotene are dark green, yellow, orange vegetables such as carrots, tomatoes, spinach, peppers, watercress, kale and others. There are over 500 carotenoids found in dark green vegetables (Brio *et al*., 2005).

Lycopene

Lycopene is another important carotenoid. It plays important role in cataract prevention (Pauline, 2006). Red coloured fruits and vegetables are high in lycopene. Tomato, red pepper, red onions and papaya are high in lycopne. They also protect the skin against harmful rays of the sun.

Selenium

Selenium is a mineral. It is included as an antioxidant. However it is not itslf an antioxidant. It is an essential component of the endogenous antioxidant enzyme glutathione peroxidase **(**Salomen, Nyssomen, Touma & Nem ,1995). Kanner and Granit (1994) reported that selenium is a mineral thought to assist fight cell damage by oxygen derived compounds and thus protects against cancer. Selenium fed to cells prevents their conversion to cancer cells by radiation. Selenium protects tissues from damage by peroxides. It increases levels of catalase and glutathione peroxidase and destroys peroxides. Selenium deficiency in humans correlates with high rates of cancer of the colon, breast, and ovary, prostate, lung, bladder and skin (Blocks & Menkes, 1989).

Selenium and vitamin E are interchangeable for some antioxidant functions. Vitamin E and selenium protect the cells against breakdown (Blocks & Menkes, 1989).

It is best to get selenium through foods as large dose from the supplement form is toxic. Plants foods like rice and wheat are the major dietary sources of selenium in most countries. The amount of selenium in soil which varies by region, determines the amount of selenium in foods grown in the soil. Animals that consume plants grown in selenium rich soils have higher levels of selenium in their muscles. In the United States, meats and bread are common sources of dietary selenium. Braziilnut nuts also contain large quantities of selenium (Blocks & Menkes, 1989).

2.3.2 Antioxidants in prevention and management of diabetes

Endothellum-dependent vasodilation is impaired in humans with diabetes mellitus. Experiments and clinical data suggest that the supplementation of insulin resistance diabetic state with antioxidants such as vitamin E normalizes oxidant stress and improves both endothelium dependent vasodilation and insulin sensitivity (Wiley, 2003). Inactivation of endothelium-derived nitric oxide by oxygen derived free radicals contributes to abnormal vascular reactivity in experimental model of diabetes. Hypothesis was tested to determine whether same observation is relevant to humans. It was concluded that endothelium dysfunction in forearm resistance vessels of patients with non-insulin dependent diabetes mellitus is improved by administration of the antioxidant vitamin C. The findings support the hypothesis that nitric oxide inactivation by oxygen-derived free radicals contribute to abnormal vascular reactivity in diabetes (Laight, Carrier & Anggard, 2000). Laight *et al*., (2000) explained that alpha lipoic acid is a potent antioxidant that prevents lipid peroxidation in vitro and in vivo. Alpha lipoic acid dose and time dependently prevented the deficits in nerve conduction and nerve blood flow and biochemical abnormalities (reduction in reduced glutathione and lipid per oxidation) (Laight *et al*., 2000).

2. 4 .0 Phytochemicals

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties (Oguntona, 1986, Nnam, 2011). Phytochemicals are of plant origin as the name implies. The word ñphytoò is derived from the Greek word for a plant. They are natural bio-active non nutrient compounds found in plant foods (Stanner, 2004). They work with nutrients and dietary fiber to protect against diseases (Tantilio, 2009). A wide spectrum of phytochemicals has been discovered in plant foods mostly fruits and vegetables (Pauline, 2006). Green leafy vegetables are rich sources of bioactive phytochemicals including carotenoids and flavonoids (Pamplona-roger, 2005; Nnam 2011).

There are more than a thousand known phytochemicals (Bokanga, 1994). It is known that plants produce these chemicals to protect itself (Bokange, 1994). Phytochemicals give plants natural defense against diseases and they perform similar function for humans (Nnam, Onyechi & Madukwe, 2012). They occur in small quantities in a food (Nnam 2011). It is most common to find mixtures of phytochemicals within a plant food and rarely is one class found in a food (Nnam, *et al*., 2012).

Many of the phytochemicals are reasonably heat stable and most are not water soluble. They are not appreciably lost during conventional cooking methods (Bokenga, 1994). This means that one does not have to eat raw food to obtain the health benefits of the foods that are rich in phytochemicals. Many phytochemicals have antioxidant properties (Tantilio 2009). Wardlaw & Kessel (2002), Nnam *et a*l. (2012) reported that the merits of the preventive and curative potentials of these phytochemicals would be attained by diversification of diets. .This is because various fruits and vegetables contain varied levels of phytochemicals. It is advisable to consume a wide variety of plant foods that contain different phytochemicals at varied levels to prevent diet related non-communicable diseases (Tantillo, 2009).

2.4.1 Classes of phytochemicals.

More than 1,000 different phytochemicals have been identified (Be Healthy Enterprises, 2007). Phytochemicals are classified for easy identification. Some of the classes include

1. Carotenoids - alpha-carotene, beta-carotene, beta-cryptoxanthin, lutein, lycopene, zeaxanthin and others.

2. Flavonovoids / bioflavonoids / polyphenols ï anthocyanins organosulfides, allicin, catechins, querecetin, tangeritin, resveratrol, hesperidin, coumarins, isoflavones (phytoestrogenes), flavanones, flavanols and others.

3. Glutathione, indonle-3-curbinol, isothiocyannates, sulforaphane etc alkaloids -caffeine, theobromine, theophylline and others
4. Lignans - pinoresinol, caricresinol secoisolarciresnol, matairesinol and others
5. Saponins- forosterol saponins,spirosterol saponins,triterpenoid saponins etc
6. Phenolic acids-capsaicin,ellagic acid, Gallic acid,rosmaric acid, tannic etc
7. Terpenes/Mono-terpenes-limonene,linolyl acetate,menthol,thymol etc
8. Inositol phosphates(phytates)
9. Phenols and cyclic compounds - a ginerols b diarylhaptanoids etc

Source (Pamplona-roger, 2005).

2.4.2. Mechanisms of actions of phytochemicals

Most phytochemicals have antioxidant activity (Ene-Obong 2001). They protect the cells against oxidative damage and reduce the risk of developing certain types of cancer. Phytochemicals with antioxidant activity include allyl sulfides (onions, leeks, garlic), carotenoids (fruits, carrots), flavonoids (fruits, vegetables), polyphenols (tea, grapes) (http//www.phytochemicals.info). Vassort (2010) reported that the antioxidant phytochemicals act through one of the three mechanism to prevent oxidant induced cell damages; they can reduce the generation of reactive oxygen species (ROS); Scavenge ROS or interfere with ROS- induced alterations. Phytochemicals from natural plant based foods prevent oxidative stress.

2.4.3 Specific phytochemicals in foods and their actions

Anthocyanins found in red and blue fruits and vegetables e.g. raspberries and blue berries help to slow the aging process, protect against heart disease and tumors, prevent blood cloths and fight inflammations and allergies

Isoflavones, found in soybean and soybean products imitate human estrogens and help to reduce menopausal symptoms and osteoporosis (http//www.phytochemicals.info). Indoles, which are found in cabbages, stimulate enzymes that make the estrogen less effective and could reduce the risk for breast cancer. Other phytochemicals, which interfere with enzymes, are protease inhibitors (soy and beans), terpenes (citrus fruits and cherries) (http//www.phytochemicals.info).

Saponins found in beans interfere with the replication of cell DNA, thereby preventing the multiplication of cancer cells. Capsaicin, found in hot peppers, protects DNA from carcinogens (http//www.phytochemicals.info).

The phytochemical allicin from garlic and onions has anti-bacterial properties. Allicin blocks or eliminates certain toxins from bacteria and viruses. Some phytochemicals bind physically to cell walls thereby preventing the adhesion of pathogens to human cell walls. Proanthocyanidins are responsible for the anti-adhesion properties of cranberry. Consumption of cranberries will reduce the risk of urinary tract infections and will improve dental health (http//www.phytochemicals.info).

Flavonoids are polyphenolic compounds that are ubiquitous in nature. They are found in fruits, vegetables and certain beverages. They have diverse beneficial biochemical and antioxidant effects. The antioxidant activities of flavonoids depend on their molecular structure (Buhler & Miranda, 2004).

Lutein is found in leafy green vegetables. It may prevent macular degeneration and cataracts as well as reduce the risk of heart disease and breast cancer.

Phenolics are found in citrus fruits, fruit juices cereals legumes and oilseeds. Phenols are thought to be extremely powerful with variety of health benefits including slowing the aging process, protecting against heart disease and tumors, fighting inflammation and allergies.

Lycopene found primarily in tomatoes, when cooked appear to reduce the risk of cancer and heart attacks.

Beta carotene

Beta carotene also known as provitamin A may help to decrease the risk of developing cancer. .According to American Cancer Society this substance may prevent certain cancers by enhancing the white blood cells in the immune system (white blood cells help to work to block cell damaging free radicals). Good sources of beta carotene are dark green vegetables and yellow orange fruits. Large doses of beta carotene can cause the skin to turn yellow orange colour, a condition called carotenosis. There is no RDA for beta carotene but there is RDA for vitamin A. Foods high in beta carotene includes carrots, swiss collad spinach, sweet potatoes.

Alkaloids

The term alkaloid means natural nitrogen-containing bases found in plants. An alkaloid is a nitrogenous organic molecule that has a pharmacological effect on humans and animals (http/www.buzzle.com). Alkaloids are colourless and bitter-tasting. Many alkaloids have toxic properties. If taken in larger doses, alkaloids can be extremely poisonous. Caffeine is an alkaloid. Many are found in plant foods, including potatoes and tomatoes (the *Solanum* alkaloids), or as the

products of fungal action (e.g. ergot), although they also occur in animal foods (e.g. tetrodotoxin in puffer fish, tetramine in shellfish) (http/www.buzzle.com).

Glycosides

Glycosides are compounds containing a carbohydrate and non carbohydrate residues in the same molecule. The carbohydrate residue is attached by an acetyl linkage to a non-carbohydrate residue or aglycone. The non sugar component is known as aglycone while the sugar component is known as glycone. If the carbohydrate component is glucose, the resulting compound is glycosides. Glycosides are therefore compounds or substances found in combination with sugars. They are described according to their classes: cardiac glycosides, cyanogenic and steroidal glycosides are known to pose some toxicological effects (Nwaogu,Ujwundu,&Mgbemena, 2006). A high level of cyanogenic glycosides can pose toxicity problem to consumers. Consuming foods rich in glycosides helps in fighting cancer, reducing pain associated with arthritis and also, in lowering high blood pressure (http://www.brighthub.com/health/alternative). Food sources of glycosides are apple seeds, blackberries, raspberries, millet and millet seeds, barley, brown rice, cashew nuts, cassava, plum kernels, and sorghum cane syrup (http://www.brighthub.com/health/alternative).

2.4.4 Recommended dietary allowance of phytochemicals

There is no recommended dietary allowance for phytochemicals. Experts advice consumption of a variety of foods including plenty of fruits and vegetables to ensure adequate amounts in diet. In order to get enough protective phytochemicals daily, researchers advice that people should consume plant based foods such as leafy greens, fruits, vegetables, nuts and legume at the start of a day (htt://news.ufl.edu/ 2009/10/21/phytochemical. Multimedia/).

2. 4. 5 Phytochemical Index

To encourage people to get enough phytochemicals from meals and snacks, the use of phytochemical index is used. Phytochemical Index compares the number of calories consumed from plant based nutrients rich foods with the overall number of calories taken in each day. The best diet for good health and diabetes is a diet with a high nutrient per calorie ratio (htt://news.ufl.edu/ 2009/10/21/phytochemical.Multimedia/).

2.4.6 Health benefits of phytochemicals

Recent researches demonstrate that phytochemicals can protect humans against diseases (Adisa, Oke, & Olorunsogo, 2004; Yang & kuo 2008; Nnam *et al*., 2012) and can assist the body to defend itself against damage (Auta & Goje, 2011). They may prevent formation of carcinogens, and diseases such as diabetes and heart disease and boost immune functions (Adisa *et al*., (2004). Compounds such as phytates, saponins, alkaloids and flavonoids are phytochemicals. Most of these compounds are detoxified by several processing methods (Soetan, 2008). They have potent preventive, curative, anti-oxidant and anti- inflammatory properties. Carotenoids have significant antioxidant activities (Seddon, Ajani & Grannit, 1994). Flavonoids reduce oxidative damage that can lead to thrombosis and blockage of coronary arteries (Kitts, 1994). Saponins found predominantly in legumes have demonstrated anti carcinogenic and hypocholesterolemic activity (Rao & Sung 1995,). Phytates are associated with reduced risk of cardiovascular disease and cancer (Thomson, 1992).

2.4.7 Phytochemicals and diabetes

Diabetes mellitus has assumed epidemic proportions in most part of the world. It is a major source of morbidity and mortality in developed world (Fardoun, 2007). Diabetes is often associated with a variety of metabolic abnormalities including abdominal obesity, insulin resistance, hypertension, dyslipidemia and hyperglycemia. There is considerable evidence that hyperglycemia causes the generation of reactive oxygen species (ROS), ultimately leading to increased oxidative stress in a

variety of tissues (Ruhe, 2001; David, 2007; Fardoun, 2007). In the absence of an appropriate compensatory response by the endogenous antioxidants such as vitamin C and E, catalase, glutathione and superoxide dismutase, oxidative stress dominates resulting in the activation of stress sensitive intracellular signaling pathways. One of the major consequences is the generation of gene products that cause cellular damage and ultimately responsible for the late complications of diabetes (Davi, 2005). Fardoun (2007) also reported that in the absence of an appropriate response from endogenous antioxidant mechanisms the redox imbalance causes stress signaling pathways that leads to insulin resistance (i.e. resistance to insulin mediated glucose uptake by some cells) and impaired insulin secretion. Intake of antioxidant phytochemicals helps to improve the endogenous antioxidants mechanisms and therefore reduce the vascular complications in diabetes and other effects of hyperglycemia (Fardoun, 2007).

Cardiac dysfunction occurs during type 1 and type 2 diabetes. This results from multiple parameters including glucotoxicity, lipotoxicity, fibrosis and mitochondrial coupling (Vassort, 2010). Oxidative stress arises from an imbalance between the production of ROS and the biological systemôs ability to readily detoxify the reactive intermediates. This happens in diabetes induced cardiac dysfunctions. Antioxidant phytochemicals help to rectify the redox imbalance. Several studies have reported beneficial effects of a therapy with antioxidant phytochemicals against the cardiovascular system consequence of diabetes (Ruhe, 2001; Davi, 2005; Vassort, 2010). Studies in humans and laboratory animals indicate that vitamin E and lipoic acid supplements lessen the impact of oxidative damage caused by deregulation of glucose metabolism (Ruhe, 2001).

Ruhe (2001) reported that dietary phytochemicals of which polyphenols form a considerable part may affect the risk of chronic diseases like type 2 diabetes. Phytochemicals especially polyphenols in fruits and vegetables may modify imbalanced lipid and glucose homeostasis thereby reducing the risk

of the metabolic syndrome and type 2 diabetes complications. The two most important factors contributing to the development of non insulin dependent diabetes mellitus NIDDM are obesity and physical inactivity. Current therapies for NIDDM focus primarily on weight reduction. An alternate approach to the control of type 2 diabetes is to arrest the progress of the pathology. To this end investigators have attested that dietary antioxidant phytochemicals are of value (Ruhe, 2001). Researchers reported that nutritional rehabilitation by way of efficient antioxidants like vitamin E reactivates the enzymatic antioxidants system and guards against the insult caused by ROS during diabetes. Hence well balanced antioxidant defense system through the use of dietary phytochemicals is vital for proper prevention against diabetic damages (Fardou, 2007).

2.5.0 Antiphysiological factors in green leafy vegetables

Plant species often naturally contain chemicals which when consumed in large quantities, over long periods may prove toxic. Such naturally occurring toxicants are called anti-physiological factors. Examples of these include protease inhibitors mainly present in legumes, they inhibit digestion of proteins; heamagglutinins that agglutinate blood cells; goitrogens that may cause hypothyroidism found in mustard, cabbage and such vegetables; cyanogenic glycosides that produce hydrocyanic acid, lathyrogens, saponins, solanins as in potatoes and such others. Muller (2008) identified that where such substances occur in foods, it is necessary to inactivate these by heating, leaching or by special treatments, or by developing plant species that contain low concentrations of these.

2.5.1 Antinutrients

Antinutrients are inevitably present in vegetables. This limits the importance of vegetables in nutrition, as the nutritional importance of any given food is a function of its nutrient and antinutrient composition (Ogbadoyi, Hussaini, & Makin, 2006). The major anti nutrients commonly found in

green leafy vegetables is phytic and oxalic acids (Aletor & Adeogun, 1995). Green leafy vegetables when processed and cooked are often free of food toxicant (Bokanga, 1994). Willy (2003) observed that tannins are anti nutrients with antioxidant effects. They were traditionally considered antinutritional but it is now known that their beneficial or antinutritional properties depend upon their chemical structure and dosage. They act as cation agents, preventing availability of certain nutrients and as well act as beneficial antioxidants.

Boiling and then discarding the water used for boiling vegetables provides a good means of reducing the oxalate content of some leafy vegetables and consequently the associated food safety problems (Ogbadoyi *et al*., 2006). Green leafy vegetables when processed and cooked are often free of food toxicant (Bokanga, 1994).

2.6 Toxicants in vegetables

Toxicants or toxic factors are those substances found in foods that may produce deleterious effect on health when ingested by man or animals (Joshi, 2002). The contaminants include dust,dirt, plant material from the same plant or other sources, residues of pesticides .and chemicals used in the farm or storage. Some heavy metals often found in fruits and vegetables include lead and cadmium. Lead and cadmium are naturally present in the environment. Both of them in the body cause varying degrees of toxicity. Thus they are not needed at any reasonable amount in the diets. Although such contaminations are difficult to avoid completely, a limit on the proportion of these is placed in food standard specifications (Nkefamiya & Haggai, 2010). The WHO/FAO has the safe value of some heavy metals in fruits and vegetables for a guide. The value of such metals like lead and cadmium are 0.3 and 0.20mg/kg respectively. Nkefamiya and Haggai (2010) reported that fruits and vegetables collected from production and market sites in Nigeria contained measured heavy metals content within the safe limits prescribed by WHO/FAO. Many of these contaminants can be removed by

manually removing the physical contaminants and washing before the raw food is processed for food preparation and consumption. Bokanga (1994) reported that leafy vegetables when processed and cooked are often free of food toxicants.

2.7 *Hibiscus cannabinus* plant and leaves

Hibiscus cannabinus is an annual tough herbaceous plant belonging to the malvaceae family. It is a woody tropical plant commonly known as *Kenaf* in Cameroon and other parts of the world and as *Rama* in Nigeria (Kubamarawa, 2009, Auta & Goje, 2011). It has varying colours of flowers. Its leaves have an acid flavour and are used for soups (sundarrayanan *at al*., 2011). The leaves are eaten as vegetables (Agbor *et al*., 2001). It is a non-conventional leafy vegetable consumed largely by the rural dwellers in Adamawa state of Nigeria (Kubmarawa *et al*., 2001). Ahmed (1979) also reported that the tender leaves are used for soup in Cameroones. Matured *Kenaf* plants are distinguished by the presence of small prickly hairs on the stem (Schippers, 2000). In Nigeria, they are sometimes used to mark farm boundaries or planted as kitchen garden crops (Seck, 1997).

In recent times, the interest in growing kenaf throughout the world for its high biomas yield and elevated fibre content has been increased (Hossan, 2011). It can grow up to 3m and is usually cultivated for vegetables or fibre. *Hibiscus cannabinus* is commonly known as *rama* in northern Nigeria where it is used as vegetables in the preparation of local dish called ópateô (maize portage) and the preparation of a local salad (Audu, Auta & Goje,2011).

2.7.1 Nutrient composition of *Hibiscus cannabinus* leaves

Glew, Amoako, Ankar, and Presley (2010) reported that *Hibiscus cannabinus* contained a large amount of protein (15.5 ï 22.8%) in dry weight bases. Kubamarawa *et al*. (2009) reported that *Hibiscus cannabinus* has high percentage of essential amino acid except methionine and cystine which are commonly deficient in green leafy vegetables (Okaka *et al*., 2000). Kobaisy, Tellez, and Webber (2001) analysed the essential oil of kenaf (*Hibiscus cannabinus*) by GC-NS, fifty eight components were characterized with five major ones as ephytol (28.19%), zyphtol (l8.02 %,), n-nonanal (5.70%), benzene acetaldehyde 4.39%, E-2 hexane (3.10%) . The oil has no algicidal activity.

Pascoal, Seca, and Fradinho (1996) reported that there is relative abundance of cellulose and ashes in the foliage of Hibiscus cannabinus at different stages of maturity. The plant also has rich fibre content (Enwere, 1998). In a wide toxicity study, Sundarayanan *et al*. (2011) reported that methonolic extract of *Hibiscus cannabinus* did not produce lethality up to the dose level of 2000mg/kg body weight of rats The major antinutrient factor commonly found in *Hibiscus cannabinus* leaves are phytic acid and oxalic acid (Osagie & Offiong, 1995).

2.7.2 Phytochemical content of *Hibiscus cannabinus* leaves

Sundarayanan *et al*. (2011) reported that methanoic leaf extract of *Hibiscus cannabinus* showed presence of phytosterol, flavonoids and glycosides. Agbor (2001), Neera and Sherma (2008) isolated from Hibiscus cannabinus compounds such as flavonoids, phenolic acid and polysaccharides.

2.7.3 Hypoglycemic activity of *Hibiscus cannabinus* leaves

The scientific basis for the statement that plants and their active constitute play an important role in the prevention of chronic and degenerative disease is continuously advancing (Falusi, 2009). In line with this Sundarrayanan *et al*. (2011) reported that the methanoic extract of *Hibiscus cannabinus* leaf exhibited hypoglycemic activity in streptozotocin induced diabetic rats. Natural plant products have shown great potential in the management of various ailement and *Hibiscus cannabinus* is one of such plant and has long been used as folk medicine in India and Africa for the treatment of various disease conditions (Duke, 1983).

Audu (1989) indicated that *Hibiscus cannabinus* in combination with *Reptadenia hastate* is used as an anti-diabetic remedy in Bauchi State Nigeria. Auta and Goja, (2011) also reported that administration of *Hibiscus cannabinus* methanolic leaf extract inhibits glycosylation of hemoglobin and as such the formation of advanced glycated end-pt (AGEs).

2.8 *Adansonia digitata* plant and leaves

Adansonia digitata belongs to the family Bambacaceae, genus *Adansonia* and specie *Adansonia digitata* Linl. It is a deciduous majestic tree up to 25m high, Audu (1989) reported that it may live for hundreds of years. The form of the trunk varies. In young trees it is conical, in mature trees it may be cylindrical, bottle shaped or tapering with branching near the base (Yusha, Hamza, & Abdullahi, 2010). The leaves, the stem bark and soult pulp are all very useful foodstuffs. *Adonsonia digitata* is the most widespread of the Adonsonia species on the African continent found in hot dry savannah of sub-saharan African (Vertueni, Broccoli & Buzzona, 2002). The English common names include baobab, deadrat-tree, monkey bread tree (Vertueni *et al*., 2002). It is also known as the small ñpharmacyò or ñchemist treeò because of its numerous uses for medicinal purposes.

The specific epithat digitata refers to the fingers of a hand which the five leaflets (typically) in each cluster bring to mind. All parts of the plant have medicinal properties (Vertuani *et al*., 2002). The leaves can be eaten as relish and be used for soups in some African countries. The native African populations commonly use the baobab fruit as famine food to prepare decoctions and sauces. Adonsonia digitata is locally called ñKukaò and ñOsheò in Hausa, Kanuri and Yoruba languages in Nigeria. Typically the plant is found in Northern parts of Nigeria, especially Adamawa, Borno, and Yobe states where the leaves are eaten as soup condiments (Venter & Ventes 1996, Pet, 2011). Assagbadjo, Chidere, Kakau and Farson (2012) reported that leaves of baobab are sources of nutrients in Africa where the species occur. Assagbadjo *et al*. (2012) also reported that the leaves (fresh and dried) are used in cooking as a type of spinach and can also be used as foragie. Equally, Sena, Jagt, Rivera, Millson, and Glew, (1998) reported that *Adansonia digitata* leaves were nutritionally superior to the fruit of the tree.

In Nigeria, the leaves are locally known as ókukaô and are used to make ñkuka soupò (http://en.wikipedia.org/wiki/baobab). The baobab leaves are tender in rainy season and are harvested fresh in the last month of the rainy season, sun dried and either stored as whole leaves or pounded and sieved into a fine powder. In the market the powder is the most common form (Sidebe *et al*., 1998). Dried green leaves are used throughout the year, mostly in soups served with the staple dish of millet (Delisle *et al*., 1997). Baobab shades its leaves at beginning of the dry season and new leaves appear after flowering. Baobab has economic potential locally and internationally (Vertueni *et al*., 2002). Nnam & Nwofor (2001) reported that baobab leaves, fruits and seeds are used as articles of food in the northern states of Nigeria where it grows extensively but are not consumed in the southern states.

2.8.1 Nutrient composition of *Adansonia digitata* leaves.

The leaves of the baobab tree have rich nutrient potentials. FAO (1990) reported its protein value as 12.3%, 3.1% fibre, 9.6% ash, 11.8% moisture, 221mg calcium, 24mg iron, 275mg phosphorus, and traces of ascorbate. Nnam and Nwofor (2001) identified that baobab leaf would be useful in providing macro and micronutrients to the diets of people who consume it. They ascertained that pulverized baobab leaf soup is a potential good source of calcium (147mg), phosphorus (0.02mg) and provitamin A (89.61mcg) per 100g dry weight basis (Nnam & Nwofor 2001). Kamali and Khalifa (1999) detected a content of provitamin A of 27mg Retinol equivalents per gramme of the dried leaves and identified that shade drying increases the value. Equally Stefano (2002) reported content of rhamanose and other sugers in dry leaves of *Adansonia digitata.* The leaves of *Adansonia digitata* are important protein sources in complementing the amino acid profile and improving the protein quality of diets (Nordeide, Hatloy, Folling & Oshaug, 1996). Glew, Vanderjafit , Lockett, and Millsan (1997) reported a total lipid of 55mg/g in dry weight bases in *Adansonia digitata* leaves.

Ethnobotanical studies have confirmed the high content of antioxidant vitamins in *Adansonia digitata* fruit constituents and leaves. Baobab fruit pulp can be considered a much valuable source containing levels of vitamin C ranging 2.8-3 g/kg (Vertuani *et al*., 2002).The *Adansonia digitata* leaves are also rich in vitamin C (55mg/100g), iron (23mg/100g) and calcium (400mg/100g) (www,actahor ./book/806/506.40htmtorg) . Baobab leaves contain high amounts of tannins as tannic acid. Stefano (2002) reported that high tannins content of the leaves of *Adansonia* has a marked negative effect on their digestibility in livestock.

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2.8.2 Phytochemical content of *Adansonia digitata* leaf

Adansonia digitata leaves have higher content and concentration of saponin than those of the fruits (Dike 2010). The leaves contain substantially high crude fibre. Stafano (2002) reported that *Adansonia digitata* dry leaves and fruit pulp have higher values of antioxidant phytochemicals when compared with other vegetables. Scheuring, Sidibe and Frigg (1999) identified baobab leaf as rich source of beta-carotene, the precausor of vitamin A (156.5mcg/g).

2.8.3 *Adansonia digitata* leaves in the management of diabetes mellitus

The leaves bark and fruits of *Adansonia digtata* are traditionally employed in several African regions as food stuffs and medicinal purposes (Tanko, Yerima, mahde & Mohammed 2008). Offia *et al*., (2011) reported that *Adansonia digitaita* leaf is important in the management of metabolic diseases in plateau State of Nigeria when they compiled a survey of medicinal plants in plateau state in Nigeria. Kamli and Khalya (1999) in their compilation of Sudanese natural medicines identified that *Adansonia digitata* young leaves eaten as vegetables is important in treatment of diabetes.

2.9 *Sesamum indicum* plant and leaves

Sesamum indicum also known as sesame is a flowering plant in the genus sesamum and family *pedaliaceae* (Raphav *et al*., 1990). It is widely naturalized in tropical regions around the world and cultivated for its edible seeds which grow in pods and leaves. Bedigian (2003) reported that sesame is very drought tolerant. It has been called a survivor crop with an ability to grow where most crops fail (Bedigian, 2003). *Sesamum indicum* is an annual plant growing to 50 or 100cm (1.6m) tall with opposite leaves 4 to 14cm long and an entire margin. The leaves are broad lenceolate (Wikipedia, 2012). Sesame seed has one of the highest oil content of any seed. It is a common ingredient in cuisines across the world. The leaves and seeds are astringent. The leaves of sesamum indicum are

popularly consumed in Adamawa state of Nigeria largely by the rural communities where it is known as ñ*karkashi*ò (Kubamarawa, 2009). Shittu, (2007) also reported that all parts of sesame plant(leaves and seed) are useful and is locally consumed as foods for many years by rural subsistence farmers in south-west, middle belt, northern areas of Nigeria where they are largely cultivated. Shittu, Ajai and Benson (2008) reported that *sesamum* leaf is very important in Nigeria because of its nutritive nature and its reported health benefits. The decoction of the leaves serve various traditional and medicinal purposes (Bankole *et al*., 2007).

2.9.1 Nutrient composition of *Sesamum indicum* leaves

Ijomah, Igwe and Audu (2000) reported a high moisture content of *Sesamum indicum* leaves. Kubmarawa *et al*. (2009) reported a crude protein content of 18.59% for *Sesamum indicum* dry leaves. Seventeen amino acids were found in varying proportions in the protein. Sesame is the only oil seed (legume) that is high in methionine. The leaves of *sesamum indicum* contain low soluble carbohydrates (Konan, 2004). Reports on the vitamin potentials of this leaf have not been reported in Nigerian literature.

2.9.2 Phytochemical content of *Sesamum indicum* leaves

Phytochemically, the sesamum plant is rich in phenolic compounds (phenols, sterol, lignans and flavonoids) (Ijomah *et al*., 2000). *Sesamum indicum* leaves have high fibre content. Kubmarawa (2000) reported a value of 27.58% of fibre content in *Sesamum indicum* leaves. Extraction processes show that *Sesamum indicum* plant leaves have Lignans mainly sesaminnol (Thompson *et al*., 1991).

2. 9.3 *Sesamum* leaves in diabetes management

Sesamum leaves are popularly used in folk medicine of the southern Nigeria to treat diabetes (Shittu *et al*., 2009).

2.10 *Cassia tora* plant and leaves

Cassia tora is a legume belonging to the caesalpiniaceae family. It grows wild mostly in the tropics and is considered a weed in many places (Kubmarawa, Magomija, Yebjoella & Adebayos, 2011). The plant is an annual herbaceous herb, almost an under shrub up to 30-90cm high with pinnate leaves which is about 10cm long. Each leaf has three pairs of leaflets that are opposite and oblique at the base. The yellow coloured flowers are bearded in the axel of the leaves .The plant bears flowers in rainy seasons. Common names of *Cassia tora* are sickle pod (English), sickle senna (English), foetid cassia (English), chueh-mintsu (China), ɑ́tapsaô (Nigeria). The leaves of *Cassia tora* is used for soup preparations in some parts of northern Nigeria (Kubmarawa *et al*., 2011) and popularly consumed in Adamawa state of Nigeria.

2.10.1 Uses of *Cassia tora* leaves

Culinary and Folkloric

Cassia tora is edible wild vegetable. The leaves are used as pot herb. The roasted seeds are used as coffee substitute. In Philippines, the entire plant in decoction is used as purgative and vermifuge. The leaves and seeds are used as a remedy for ringworm and scabies. Infusion of the leaves is used for intestinal disorders. Malayas use decoction of the leaves as a mild purgative or as a cure for coughs and in children suffering from fever while teething. In Ayurveda (Indian medical science), the seeds and leaves are used for cough, leprosy, ringworm,colic flactulence, dyspepsia and bronchitis (http://www.iloveinda.com.> Indian herbs).

2.10.2 Constituents and chemical properties of *Cassia tora* plant and leaf.

Study of the seed extract of *Cassia tora* isolated nine anthroguinones with two exhibiting inhibitory activities on protein glycation and aldose reductase (Hyun, Sook, & Lee, 2009) . The seeds yielded

tannins and dyes (yellow, blue and red). Its volatile oil showed a high content of aliphatic acids (>75%) and anthroquinones. The plant yields emodin to which the medicinal properties are attributed to. The leaves are mucilaginous,foetid smelling and yield a principle similar to cathartin (http://www.iloveinda.com.> Indian herbs).

2.10.3 Medicinal potentials of *Cassia tora* leaves.

The leaves of *Cassia tora* have been widely claimed against different diseases by rural and traditional practitioners of Septura region of Madhya Pradesh (Mishara, 2010). It is the most popular ingredient in Ayurvedic formulations (www.academia.edu/239988/Medicinal). The leaves are rich in glucosides and anthraguinone (Smith & Patil 2010). The Consumption of cooked leaves or use of leaf extract of this plant helps the body in maintaining the normal level of cholesterol (Barmines, Charles & Emmanuel 1998**).** It is useful as digestion and metabolism corrective substance, as a tonic and posses hyperglycemic actions (Barmines *et al*., 1998; Mishra 2010).

Cassia tora leaves have antioxidant and antiproliferative potentials. Rejiya, Iben and Annie (2009) reported that the plant leaf extract induced a marked concentration dependent inhibition of proliferation, reduced DNA content and Apoptosis in rats. *Casia tora* leaf is effective against free radical mediated diseases. A study with albino rats showed the protective effect of *Cassia tora* leaves against carbon tetrachloride induced hepatoxity attributed to its effective free radical scavenging that accounts for its antioxidant property (Rejiya *et al*., 2009). Tape,Subhesk and Mande (1998) reported that methanol extract of cassia tora leaves exhibited siginificant anti-inflammatory activities against carrageenin, histamin, serotonin and dextran induced rat hindpaw edema.

In antidiabetic studies, Hyun, et al. (2009) reported that *Cassia tora* seeds have beneficial effect on postprandial blood glucose control which may be partly due to mediation by stimulated insulin secretion from the pancrease of diabetic rats. Cho, Chun and Lee (2009), in their study on the effect

of *Cassia tora* fibre supplement on serum lipids in Korean diabetic patients concluded that *Cassia tora* leaf fibre supplement can help improve serum lipid status in type 2 diabetic patients without any serious adverse side effects. Ethanolic extract of seeds of *Cassia tora* have been reported to decrease total and low density lipoprotein cholesterol, triglycerides and increased high density lipoprotein (Hyun *et a*l., 2009).

2.11 Blood Glucose

Glucose, a simple monosaccharide sugar, is one of the most important carbohydrates and is used as a source of energy in animals and plants. Glucose is one of the main products of photosynthesis and respiration. The natural form, (D-glucose) is also referred to as dextrose, especially in the food industry. Blood sugar concentration or glucose level refers to the amount of glucose present in a mammalôs blood. Normally, in mammals the blood glucose level is maintained at a reference range between about 3.6 and 5.8 mM (mmol / l) (Rother, 2007). It is tightly regulated in the human body as a part of metabolic homeostasis. Other sugars (e.g. fructose) do not participate in the control mechanisms and are, thus, largely irrelevant to metabolic control. Normal blood glucose levels are about 90mg/100ml, equivalent to 5mM (mmol) (John & Henry, 2001). Glucose levels rise after meals for an hour or two by a few grams and are usually lowest in the morning, before the first meal of the day. Glucose is the primary source of energy for bodyôs cells. Fats and oils (i.e. lipids) being primarily a compact energy store (Inzucchi & Sherwin, 2007).

2.11.1 Blood glucose regulation

The homeostatic mechanism which keeps the blood value of glucose in a remarkably narrow range is composed of several interacting system of which hormone regulation is the most important. There are two types of mutually antagonistic metabolic hormones affecting blood glucose levels: catabolic hormones such as glucagon, growth hormone e.g. pituitary hormone, glucocorticoiid e.g. cortisol and

catecholamines e.g. epinephrine, norepinephrine, dopamine which increase blood glucose; anabolic hormone (insulin), which decreases blood glucose.

The human body wants blood glucose (blood sugar) maintained in a very narrow range. Insulin and glucagon are the hormones which make this possible. Both insulin and glucagon are secreted from the pancreas, and thus are referred to as pancreatic endocrine hormones. It is the production of insulin and glucagon by the pancreas which ultimately determines if a patient has diabetes, hypoglycemia, or some other sugar problem (John & Henry, 2001).

Glucagon is secreted by the alpha cells of the pancreatic islets in much the same manner as insulin except in the opposite direction. If blood sugar is high, then no glucagon is secreted. When blood glucose goes low, however, (such as between meals, and during exercise), more glucagon is secreted like insulin, glucagon has an effect on many cells of the body, but most notably the liver. The effect of glucagon is to make the liver release the glucose it has stored in its cells into the blood stream, with the net effect of increasing blood glucose. Glucagon also induces the liver (and some other cells such as muscle) to make glucose out of building blocks obtained from other nutrients found in the body (e.g. protein)

2.12 Diabetes mellitus

The term diabetes, without qualification, usually refers to diabetes mellitus, which is associated with excessive sweet urine (known asñglycosuriaò). There are several other conditions also named diabetes. The most common of these is diabetes insipidus in which the urine is not sweet (insipidus meaning ñwithout tasteò in Latin); it can be caused by either kidney or pituitary gland damage. Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia and defective metabolism of glucose and lipids (WHO, 1999). Read, Barritt & Hewer (1991) defined diabetes as a condition of impaired carbohydrates utilization caused by an absolute or relative deficiency of

insulin. Diabetes is a chronic disorder that affects the metabolism of carbohydrate as well as fat and protein metabolism. This usually results to glucosuria, which is associated with polyuria and weight loss. Diabetes is a primary disorder in many patients. It may also arise as secondary disorder to other diseases that impair the function of the pancreas. The pancreas release insulin that regulates blood glucose levels as well as converts glucose to energy (Kumar and clark, 1999). Glucose is simple sugar in the blood stream, produced when starchy foods are eaten and digested in the body. Non-diabetics release insulin from the pancreas to keep this blood glucose at constant level irrespective of the amount of carbohydrate foods eaten. Diabetic with impaired pancreas function could not keep the blood glucose at constant level. The long standing metabolic derangement caused by this lack of insulin is freely associated with permanent and irreversible functional and structural changes in the body cells. The changes lead to the development of the complications of diabetes (www.ncbi.nim.nih.gov/pubmed/18503731).

2.12.1 Classification of diabetes

Diabetes can be divided into two main groups based on their requirement of insulin namely Insulin dependent diabetes (Type 1) and non-insulin dependent diabetes (Type 2). However, other types of diabetes have also been identified. Maturity onset Diabetes of the young (MODY) is now classified Type 3 and gestational diabetes classified as Type 4 (Tanko, Yerima, Mahde, & Mohammed, 2008).

Type 1 diabetes also called juvenile onset diabetes is a chronic disease in which insulin production from the pancreas is diminished or absent. It usually starts in childhood. The patients are managed with insulin and diet therapy.

Type 2 diabetes is much more common. It is seen mainly in adults. The patientôs pancreas may produce some quantity of insulin that would be unable to keep the glucose at constant level. Reavin

(2003) reported that the basic pathogenic mechanism of Type 2 diabetes patients is insulin resistance. Management can be diet alone or diet and oral hypoglycemic drugs.

Maturity onset Diabetes of the young (MODY) is characterized by a history of childhood protein energy malnutrition, presentation of malnutrition during diagnosis, pancreatic calcification and fibrosis (Akinji, 1990). It could be managed with diet alone.

Gestational diabetes is common in women during pregnancy. It may abate after delivery to reoccur with subsequent pregnancies. The development of this diabetes is attributed to the hormone secreted by the placenta (human placenta lactogen). This hormone is insulin antagonist (Tanko, Yerima, Mahde, & Mohammed, 2008)

2.12.2 Prevalence of diabetes mellitus

Diabetes is a health condition that affects an estimated 23.6 million people in the US. In Nigeria, Onyemelukwe (1993) estimated that there are more than 6 million cases of diabetes mellitus. Most studies on diabetes suggested that the prevalence is less common amongst the Africans (Rajikeran *et al.,* 2011). The ósloow-releaseô carbohydrate in Africanôs traditional diets helped protect susceptible populations from developing this disease (Onyemelukwe 1993). Currently, there are over 150 million diabetic patients worldwide. This is likely to increase to 300 million or more by the year 2025 (WHO, 1980; Rajikeran *et al*., 2011), and no modern medicine has reached the satisfactory level in the treatment of diabetes.

2 .12.3 Aetiology

Diabetes is caused when insulin is deficient or ineffective. It is mainly a primary disorder, though, it could manifest as secondary disorder to some diseases that impair the function of pancrease. Factors

such as genetic, dietary, infections, stress, drugs, age, hormones may increase the risk of developing diabetes (Passmore and Eastwood, 1986).

2.12.4 Common symptoms

Some common symptoms of diabetes include frequent urination (polyuria), unusal thirst or weight loss and fatigue or irritability**,** excessive hunger, reduced visual activity, hyperglycemia.

2.12.5 Diabetes treatment and management

Diabetes mellitus is currently a chronic disease without a cure. Medical emphasis must necessarily be on managing /avoiding possible short- term as well as long-term diabetes related problems. The goal of diabetic treatment is to keep blood glucose levels stable and stay healthy. Depending on the type of diabetes, treatment can include insulin medication, changing diets and exercise habits. Due to the associated higher risk of cardiovascular disease, lifestyle modifications should be undertaken to control blood pressure and cholesterol by exercising more, smoking cessation, consuming an appropriate diet and taking drugs to reduce pressure (Vinik, Fishwick and Pitttenger, 2004).

2.12.6 Diabetes complications

If blood glucose levels remain chronically elevated because of poorly controlled diabetes, tissue damage will eventually occur. This damage can happen anywhere in the body but the kidneys, eyes, nerves and heart are at greatest risk. The diagnosis and management of early cases or less acute cases of diabetes may prevent or delay later complications. The complications are the major causes of morbidity and mortality in diabetic patients. Chumello, Bognetti & Mesch (1991) reported that diabetics are at the risk of multiple complications both metabolic and vascular. Type 2 DM are mainly at risk with macrovascular diseases leading to coronary heart diseases (CHD), stroke, peripheral vascular disease. Several herbal preparations and dietary supplements such as

antioxidants, essential fatty acids, vitamins and trace elements are prescribed as useful adjuvant to a diabetic diet. This will improve glycemic control and reduce the impact of chronic diabetic complications (www.ncbi.nim.nih.gov/pubmed/18503731).

2.13. Alloxan

Alloxan is an oxygenated pyrimidine derivative. It is present as alloxan hydrate in aqueous solution (Lenzen, 2008). Alloxan is a toxic glucose analogue, which selectively destroys insulin ï producing cells in the pancreas when administered to rodents and many other animal species. This causes an insulin- dependent diabetes mellitus (called ñAlloxan Diabetesò) in these animals, with characteristics similar to type 1 diabetes in humans. Alloxan is selectively toxic to insulin-producing pancreatic beta cells because it preferentially accumulates in beta cells through uptake via the glucose transporter 2 (GLUT2). Alloxan generates reactive oxygen species (ROS) in a cyclic reaction with its reduction product dialuric acid. The beta cell toxic action of alloxan is initiated by free radicals formed in this redox reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of beta cells. One study suggests that alloxan does not cause diabetes in human (Lenzen, 2008). Other studies show some correlation between alloxan plasma levels and diabetes Type1 in children (Mrozikiewicz, Lstrokowicki & Chmara, 1994).

2.14 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 (http://.wikipedia.org/wiki/lipid profile). Lipid profile is often ordered together to determine the risk of coronary heart diseases. Lipid profile is used together with other risk factors to asses a personôs risk of cardiovascular

diseases. The tests typically include total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL) (NCEP, 2012).

2.14.1 Cholesterol

Cholesterol is a fat (lipid) which is produced by the liver and is crucial for normal body functioning. It is a waxy steroid and is transported in the blood plasma of all animals. It is the main sterol synthesized by animals. Small amounts are also synthesized in plants and fungi.The word ñcholesterolò comes from the greek word chole , meaning bile and the greek word stereos, meaning solid, stiff (www.webmed.com). Cholesterol is found in certain foods such as food from animals like dairy products eggs and meat. The body needs only a limited amount of cholesterol to meet its needs .When too much of cholesterol is present health problems such as heart disease may develop.

Functions of cholesterol

Cholesterol builds and maintains cell membtanes (outer layer) and prevents crystallization of hydrocarbons in the membrane. It is essential for determining which molecules can pass into the cell and which cannot (cell permeability) (Nordqvist, 2009). Cholesterol is involved in the production of sex hormones (androgens and estrogens) and is essential for the production of hormones released by the adrenal glands (cortsol, corticosterone, and aldosterone). It aids in the production of bile. Cholesterol converts sunshine to vitamin D and is important for the metabolism of fat soluble vitamins, including vitamins A, D. E and K. It insulates nerve fibers.

2.14.1.1 Types of cholesterol

Cholesterol is carried in the blood attached to a protein. This Cholesterol protein package is called a lipoprotein. A lipoprotein is any complex or compound containing both lipid (fat) and protein. Lipoproteins are classified as High density lipoprotein (HDL), Low density lipoprotein (LDL) and Very low density lipoprotein (VLDL) depending on how much protein is in relation to fat. The three main types are:

Low density lipoprotein (LDL), often refered to as bad cholesterol. LDL carries cholesterol from the liver to cells. If too much is carried, too much for the cells to use, there can be a harmful buildup of LDL. This lipoprotein can increase the risk of arterial disease if levels rise too high. Most human blood contains approximately 70% LDL - this may vary, depending on the person (Nordqvist, 2009).The more the LDL there is in the blood the greater the risk of heart disease.

LDL = TC - (triglyceride/5) +HDL

High density lipoprotein (HDL), often refered to as good cholesterol. HDL prevents arterial disease. HDL does the opposite of LDL. HDL takes the cholesterol away from the cells and back to the liver. In the liver it is either broken down or expelled from the body as waste (Nordqvist, 2009).The higher the levels of HDL cholesterols the better. If levels of HDL are low, the risk of heart disease is increased (www.webmed.com).

Triglycerides are another type of fat that is carried in the blood by VLDL. Triglycerides are the chemical forms in which most fat exists in the body, as well as in food. They are present in blood plasma. Triglycerides, in association with cholesterol, form the plasma lipids (blood fat) (Nordqvist, 2009). Triglycerides in plasma originate either from fats in our food, or are made in the body from other energy sources, such as carbohydrates. Calories consumed but are not used immediately by the tissues are converted into triglycerides and stored in fat cells through out the body. When the body needs energy and there is no food as an energy source, triglycerides will be released from fat cells and used as energy - hormones control this process (Nordqvist, 2009; www.webmid.com/,, /cholesterol-basics). Many people who have heart disease or diabetes have high triglyceride levels.

Very low density lipoproteins (VLDLs), are lipoproteins that carry cholesterol from the liver to organs and tissues in the body. They are formed by a combination of cholesterol and triglycerides. VLDLs are heavier than low density lipoproteins and are associated with atherosclerosis and heart disease. The VLDL is obtained by dividing the triiglyceride levels by 5.

Cholesterol levels

The amount of cholesterol in human blood can vary from 3.6 mmol/liter to 7.8 mmol/liter. The National Health Service (NHS), UK, reported that any reading over 6 mmol/liter is high, and will significantly raise the risk of arterial disease (Nordqvist,2009). The UK Department of Health recommends a target cholesterol level of under 5 mmo/liter. Unfortunately, two-thirds of all UK adults have a total cholesterol level of at least five (average men 5.5, average women 5.6) (Nordqvist, 2009).

Desirable - Less than 200 mg/dL

Borderline high - 200 to 239 mg/dL

High - 240 mg/dL and above

Optimum level: less than 5mmol/liter

Mildly high cholesterol level: between 5 to 6.4mmol/liter

Moderately high cholesterol level: between 6.5 to 7.8mmol/liter

Very high cholesterol level: above 7.8mmol/liter Source: (Nordqvist,2009).

High cholesterol levels can cause:

Atherosclerosis - narrowing of the arteries. Higher coronary heart disease risk - an abnormality of the arteries that supply blood and oxygen to the heart (Nordqvist, 2009). Heart attack - occurs when the supply of blood and oxygen to an area of heart muscle is blocked, usually by a clot in a coronary artery. This causes the heart muscle to die (Nordqvist, 2009). Angina - chest pain or discomfort that occurs when the heart muscle does not get enough blood (Nordqvist, 2009).Other cardiovascular conditions - diseases of the heart and blood vessels. Stroke and mini-stroke - occurs when a blood clot blocks an artery or vein, interrupting the flow to an area of the brain. It can also occur when a blood vessel breaks. Brain cells begin to die (Nordqvist, 2009). When both blood cholesterol and triglyceride levels are high, the risk of developing coronary heart disease rises significantly (Nordqvist, 2009).

Total cholesterol levels

Total cholesterol score is calculated by the following HDL +LDL+20% of the triglyceride levels.

< 200mg/dl desirable

200 ï 239mg/dl borderline high

240mg/dl and above high

HDL cholesterol level

<40mg/dl (for men) é é é é low HDL cholesterol. A major risk factor for heart disease

<50mg/dl (for women)é é .. low HDL cholesterol. A major risk factor for heart disease

60mg/dl and aboveé é é é High HDL cholesterol. Considered protective against heart disease.

LDL cholesterol(Bad) cholesterol level

< 100mg/dl optimal

100 to 129mg/dl near or above optimal

130 to 159mg/dl borderline high

160 to 189mg/dl high

190mg/dl and above very high

SOURCES Third report of the national cholesterol Education program (NCEP), Expert panel on detection, evaluation and treatment of high blood cholesterol in adults(pdf).

CHAPTER THREE

MATERIALS AND METHODS

3.1.0 Materials

3.1.1 Procurement of materials

The vegetables used in the study were *Hibiscus cannabinus* (*rama*), *Adansonia digitata* (*baobab*), *Sesamum indicum* (*karkarshi*), and *Cassia tora* (*tabsa*) leaves. The vegetables (Two kilogrammes each) were bought fresh in June 2013 from Mubi daily market in Adamawa State Nigeria. The vegetables were identified at the Herbarium in the Department of Botany, University of Nigeria Nsukka, Nigeria. Rat chow was bought from rodent diet retailers at Nsukka town Enugu State, Nigeria.

3.1.2 Preparation of vegetables

All the four vegetables (2kg each) were separately sorted by removing extraneous materials, washed with deionized water and separately pulverized using Gallenkamp mixer Kenwood ï MPR 201. A half of the vegetables were used for chemical analysis and a half for their extracts production.

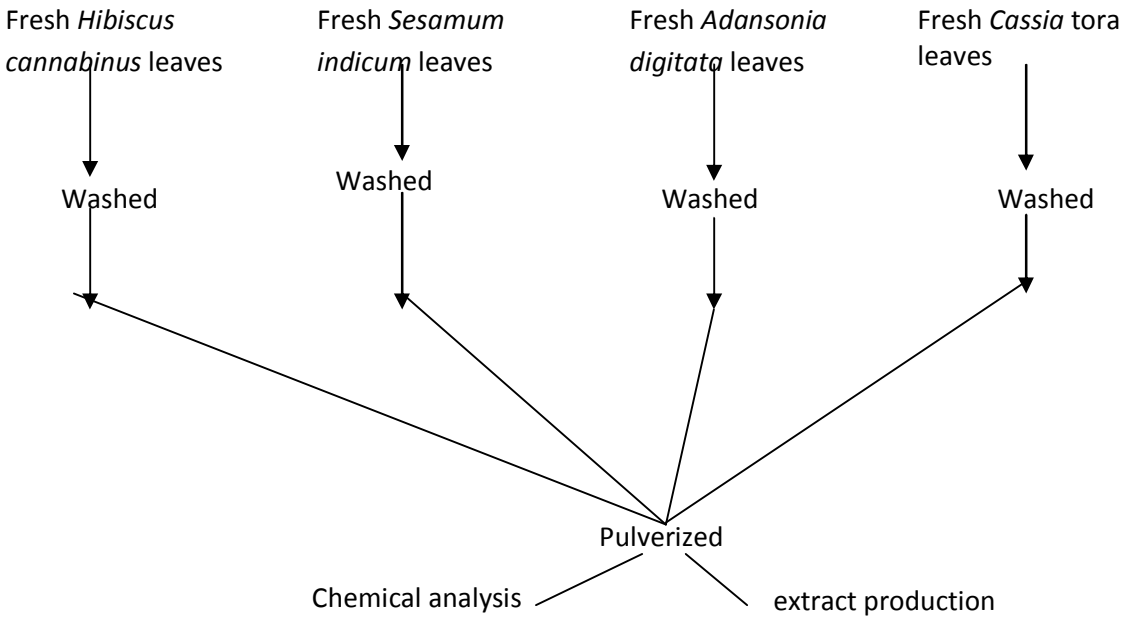

Fig 1 shows flow chart for processing the four leaves for use in the study.

3.1.3 Preparation of vegetable extracts for the rat study.

Five hundred grammes each of the pulverized vegetables were soaked in the extracting solvent (methanol) in the ratio of 200ml solvent to 10 g of vegetable and stirred (with the aid of a magnetic stirrer) for one hour, then stored in the dark for 48 hours after which the mixtures were first filtered with muslin cloth and again with cotton wool in a funnel. The filtrates were concentrated with the aid of rotary evaporator, dried under vacuum, cocked in a glass tube and kept as the crude methanol extract (Harborne, 1984) of each vegetable. The desired consistency for feeding of the rats, water was added to the crude extract in a ratio of 1:10 (weight/volume).This provided 100mg / ml of extract of each vegetable. The daily ration of each rat is calculated as

weight of rat X dose / conc. X 1000 = daily ration

3.2 Chemical analysis.

Chemical analysis was carried out in triplicate for the pulverized vegetables and their extracts. Proximate, some minerals, vitamins, antinutrients, food toxicants, and phytochemicals were determined in triplicate using standard methods of AOAC (2005).

Proximate composition

The proximate compositions of fresh *Hibiscus cannabinus*, *Adansonia digitata*, *Sesamum inducum* and *Cassia tora* leaves and their methanol extracts were determined using standard methods of AOAC (2005). All analyses were done in triplicate.

3.2.1 Moisture determination

The moisture contents of the samples were determined using AOAC (2005) procedure. Washed porcelain dishes were dried in a gallenkemp oven at 100^0C for about 2 hours, cooked in desiccators and reweighed. Two grammes (2kg) of each of the samples were weighed into the weighted dishes

and placed in the oven at 100^0C for 24 hours.The dishes containing the samples were cooled in desiccators, weighed and dried repeatedly until a constant weight was obtained. The percentage moisture was calculated using formula below:

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

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

3.2.2 Crude protein determination

Crude protein was determined by automatic micro-Kjeldahl method (AOAC, 2005). Crude protein was estimated by multiplying nitrogen value with N conversion factor 6.25 (Nx6.25) proteins. The method involves digestion, and titration. About 0.2g of each sample was weighed into a 100ml Kjeldahl digestion flask. Two and a half grammes (2.5g) of anhydrous sodium sulphate, 0.5 copper sulphate (catalyst) and 5ml of concentrated sulphuric acid was added. The flask was placed on a heater and heated gently initially, until the solution turns black. After this, heat was increased to obtain a clear solution, cooled, washed transferred into 25ml volumetric flask and rinsed with distilled water to mark.

Distillation

A combination of boric acid and methyl indicator was poured in a conical flask, placed under a condenser tip under the liquid. Five milliliters (5ml) of the digest plus 10ml of 60% concentrated sodium hydroxide was poured into Markham distillation apparatus. Steam was let down through the distillation apparatus for 5 minutes when ammonia droped into the indicator and changed the colour of the indicator from purple to green which is characteristic of alkaline gas.

Titration:

The distillate was titrated with 0.01 normal hydrochloric acid (Hcl) until a neutral point was reached (light purple or pink).

Titre value (T) = final burette reading minus initial burette reading.

$$\% \text{ crude protein} = \frac{14.01 \times 0.01 \times 6.25 \times T \times 100}{200mg}$$

Where,

14.01 = atomic wt of nitrogen

0.01 = molarity of acid

100 = percentage

6.25= protein conversion factor

T = Titre value

3.2.3 Fat determination (Soxhlet method)

The fat contents of the samples were determined using the soxhlet extraction method AOAC (2005). The extraction flask was washed, dried, cooled and weighed prior to adding 2kg of each sample. The samples were weighed into filter paper and introduced into thimble. Petroleum ether was added to the flask for extraction in the soxhlet apparatus. After this, the extract was dried in an oven for 15 minutes at 100^0C to remove any remaining solvent, cooled in the desiccators and reweighed.

Calculation:

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

3.2.4 Ash determination

The ash contents of the samples were determined using the method of AOAC (2005). Two grammes (2kg) of each sample was weighed into reweighed crucibles and put into muffle furnace at 600°c for three hours until light gray ash is obtained. The crucibles was removed from the furnace, put in desiccators to cool and reweighed to obtain the weight of ash. The percentage ash was calculated using the formular below.

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

3.2.5 Crude fibre

The crude fibre content of the samples was determination using the procedure of AOAC (2005). Two grammes (2g) of each sample was put in a 250m1 beaker, boiled for 30minutes with l00ml of 0.12M l-H_2S04 and filtered through a funnel. The filtrate was washed with boiling water until the washing water was no longer acidic. The solution was boiled for another 30 minutes with l00mI of 0.02M sodium hydroxide solution, filtered with hot water and methylated spirit three times. The residue was transferred into a crucible and dried in an air oven for l hour. The crucible with its content was cooled in a desiccator and weighed (W2). This was taken to furnace for ashing at 600°C for one hour. The ashed sample was removed from the furnace after the temperature has cooled and put into desiccator and later weighed (W2). The crude fibre content was obtained between the weight before and after the temperature had cooled and put into the desiccator and later weighed (W3). The crude fibre content was obtained between the weight before and after incineration. The percentage crude fibre was calculated thus

$$\% \text{ crude fibre} = \frac{W_2 - W_3}{W_1} \times \frac{X}{1} \times 100$$

3.2.6 Carbohydrate determination

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

Carbohydrate = 100 ï (% protein + % fat + % ash + % crude fibre + % moisture).

3.2.7 Mineral determination

Mineral contents of the samples were determined by atomic absorption spectrophotometer (AOAC, 2005) method. The samples were wet digested with concentrated nitrate and perchlorate. Iron (Fe), copper (cu), zinc (Zn), magnesium (Mg) were determined by polarized Zeeman atomic absorption spectrophotometer. The sodium, potassium, calcium, lead and cadmium contents were determined using the EDTA titration method. Stock solution was used.25ml sample was diluted with 50ml of water. 2ml buffer solution was dissolved (16.9g NH4CL in 143 ml NH4OH), and 1.25 EDTA was added and diluted to 250ml with water. 250 NaCN (PH 10.0) was added and 200mg indicator erichrome black T (mix 0.5 erichrome black T and 100g Nacl). One liter of water was used to dilute 0.01M EDTA by titration. End point was observed when the solution turned reddish.

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

A = ml EDTA

TCA = Titration factor of EDTA

V_1 = Total volume

V_2 = Aliquot for determination

W = weight of sample

Vanadomolybate method was used for phosphorus determination.

3.2.8 Vitamins determination

Vitamin E and beta carotene were determined by High performance liquid chromatography (HPLC, model C030) of AOAC (2005). The samples were digested and injected into the HPLC equipment. A pressure pump was used in moving the mobile phase and the components of the mixture through the stationary column. The pressure pump of different sizes was used and the detector mounted was capable of providing characteristic retention times for the sample components. The detector also takes care of the area counts which reflected the amount of each analyte passing through the detector.

Vitamin C, B_1, B_2, and niacin were determined using AOAC (2005) method. Two grammes of each sample were dissolved with distilled water (2ml). Trichloroacetic acid (TCA) was added and colour was developed with 2, 6 ï dichloroindophenol. The colour (pink) developed was read with spectrophotometer.

Calculation:

$$\frac{A \times (v_2 - v_1) \times 0.0052}{\text{Weight of sample}}$$

Where

A = volume of filtrate

v_1 = initial burette reading

v_2 = final burette reading

3.2.9 **Anti-nutrients determination**

3.2.9.1 Oxalate

Oxalate was determined by AOAC (2005). About four (4g) grams of the sample was extracted with 6N, HCL. The oxalates in this extract were precipitated with $CuCl_2$ and calcium salts. The precipitated oxalates were washed with 25% H_2SO_4 and dissolved in hot water before titrating against 0.05N $KMNO_4$ (1ml of 0.05N $KMNO_4$) 2.2mg.

3.2.9.2 Phytate

Phytate was determined by photometric method adapted from the method of Latta and Eskin (1980). Five grammes (5kg) of each sample were extracted with 2.46 HCL. One tenth sodium chloride (0.1m NaCl) was added to elude inorganic phosphorus and seven tenths mol of sodium chloride was added to elude phytate. One millimeter (1ml) of Wade reagent was added and read at 500nm in a spectrophotometer.

3.2.9.3 Tannins

Tannins were determined by Van ï Burden and Robinson (1981) method. About 500mg of each sample were weighed into a 50ml plastic bottle. 50ml of distilled water was added and shaken for one hour in a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to the mark. Then 5ml of the filterate was pipette out into a test tube and mixed with 2ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide.The absorbence was measured at 720nm within 10 minutes.

3.2.9.4 Hydrocyninde

3.2.10 Toxicants determination

3.2.10.1 Cadmium and lead

Lead and cadmium were determined by the method of determination of lead and cadmium in vegetables by stripping chronopotentiometry as described by Lococo (2004). 20g of the samples were digested with concentrated sulphuric acid and dry ashed at high temperature with sulphuric acid as ashing aid. Metal ions were concentrated as their amalgams on a glass by carbon working electrode previously coated with a thin mercury film and then stripped by a suitable oxidant. Potential and time dials were digitally derived. Method of standard addition coefficient was determined as n-4, 0.998 for cadmium and n-4, and 0.993 for lead. Accuracy was matched with a reference sample.

3.2.11 phytochemical determination

3.2.11.1 Saponins

Saponins were determined by the method of Obadoni and Ochuko (2001). The samples were ground and 20g of each were put into a conical flask and 100cm^3 of 20% aqueous ethanol were added.The samples were heated over a hot water bath at about 90^{0}C. The concentrate was transferred into a 250ml separatory funnel and 20 ml of diethyl ether was added and shaken vigourously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60ml of n ï butanol was added. The combined n ï butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a waterbath. After evaporation, the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

3.2.11.2 Flavoniod determination

The flavoniod contents were determined by the method described by Bohn and Kocipai- Abyazan (1994). Ten grammes (10g) of each sample were extracted repeatedly with 100ml of 80% aqueous

methanol at room temperature; the whole solution was filtered through Whatman filter paper N0 42 (125mm). The filtrate was later transferred into a crucible and evaporated into dryness over a bath and weighed to a constant weight.

Calculation

Weight of empty beaker = WI

Weight of empty beaker + sample after drying = w2

Weight of empty residue = W_2-W_1

Flavoniod = W_2-W_1 X 100

3.2.11.3 Alkaloids

This was determined using Harborne (1973) method. About 5g of the sample was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one ï quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole situation was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

3.2.11.4 Glycosides

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

3.3.0 Animal experiment

3.3.1 Sourcing of animals and housing.

Forty five male adult albino rats (150-200g) were purchased from the Department of Veterinary Pathology, University of Nigeria, and Nsukka. They were divided into nine groups of five rats each based on body weight. The average body weight of each group did not differ by more than 5 grammes (AOAC, 1995). The rats were put in metabolism cages equipped to separate faeces and urine of the animals during a 12 - day study period in the Department of Home Science, Nutrition and Dietetics, University of Nigeria, Nsukka metabolic house.

3.3.2 Diet Composition

Altogether one control and eight experimental diets were formulated as follows.

Diet 1-rat chow and 0.5mg glibenclamide drug (standard control diet)
Diet 2 - rat chow and 500mg *Hibiscus cannabinus* leaf extract
Diet 3 - rat chow and 500mg *Adansonia digitata*leaf extract
Diet 4 ï rat chow and 500mg *Sesemum indicum* leaf extract
Diet 5 - rat chow and 500mg *Cassia tora* leaf extract
Diet 6 ï rat chow and 1000mg *Hibiscus cannabinus* leaf extract

Diet 7 ï rat chow and 1000mg *Adansonia digitata*leaf extract

Diet 8 ï rat chow and 1000mg *Sesemum indicum* leaf extract

Diet 9 ï rat chow and 1000g *Cassia tora* leaf extract

Table 3.1 Composition of the diet

GD1*	GD2**	GD3**	GD4**	GD5**	GD6**	GD7**	GD8**	Diet9**
RC + 0.5mg Glb drug	RC + 500mg Hc leaf extract	RC + 500mg Ad leaf extract	RC + 500mg Si leaf extract	RC+ 500mg Ct leaf extract	RC+ 1000mg Hc leaf exrract	RC+ 1000mg Ad leaf extract	RC + 1000mgSi leaf extract	RC+ 1000mg Ct leaf extract

GD1 to GD9 ï Group1 Diet1 to Group9 Diet9

RC - Rat chow

Glb - Glibanclamide drug

Hc - *Hibiscus cannabius* leaf extract

Ad ï *Adansonia digitata*leaf extract

Si ï *Sesamum indicum* leaf extract

Ct - *Cassia tora* leaf extract

* - standard control diet

** - Experimental diet

GD1 - rat chow and 0.5mg glibenclamide drug- (standard control diet)

GD2 - rat chow and 500mg *Hibiscus cannabinus* leaf extract

GD3 - rat chow and 500mg *Adansonia digitata*leaf extract

GD 4 ï rat chow and 500mg *Sesemum indicum* leaf extract

GD 5 - rat chow and 500mg *Cassia tora* leaf extract

GD 6 ï rat chow and 1000mg *Hibiscus cannabinus* leaf extract

GD 7 ï rat chow and 1000mg *Adansonia digitata*leaf extract

GD 8 ï rat chow and 1000mg *S esemum indicum* leaf extract

GD 9 ï rat chow and 1000g *Cassia tora* leaf extract

3.3.3 Induction of diabetes

The rats in each group were induced diabetes using alloxan on the 4th day. The rats were fasted overnight. Freshly prepared 5% aqueous solution of alloxan monohydrate were injected intraperitonealy to the rats at a single dose of 150mg/kg body weight of animal. The rats were allowed free access to 5% glucose solution to avoid possible effect of hypoglycemia for 48hrs. After 48h of induction of diabetes (6th day), blood was taken from each rat to estimate blood glucose

levels to confirm establishment of diabetes**. .** Blood samples were collected by making an incision on the four veins in the tail of the rats using a 15 scalpel blade for blood glucose determination. Determination was done by Trinders glucose-oxidase method (Lott & Tuner, 1975) using the one Touch Basic (lifescan Milpitas, CA) instrument. The results were reported as mg/dl. Rats with blood glucose concentration of 130 mg/dl and above were considered diabetic (Etuk, 2010) and used in the study.

3.3.4 Feeding trial

The rats were fed normal rat chow and water for 3 days to acclimatize them to the environment and diet. The rats were tested to confirm their blood glucose status. The rats with normal blood glucose (<100mg/dl) were used in the study (Etuk, 2010). The rats in each group were induced diabetes using alloxan on the 4^{th} day. The rats were fasted overnight. Freshly prepared 5% aqueous solution of alloxan monohydrate were injected intraperitonealy to the rats at a single dose of 150mg/kg body weight of animal. The rats were allowed free access to 5% glucose solution to avoid possible effect of hypoglycemia for 48hrs. After 48h of induction of diabetes (6^{th} day), blood was withdrawn from each rat to estimate blood glucose levels to confirm establishment of diabetes**.** Blood samples were collected by making an incision on the four veins in the tail of the rats using a 15 scalpel blade for blood glucose determination. Determination was done by Trinders glucose-oxidase method (Lott & Tuner, 1975) using the one Touch Basic (lifescan Milpitas, CA) instrument. The results were reported as mg/dl. Rats with blood glucose concentration of 130 mg/dl and above were considered diabetic (Etuk, 2010) and used in the study.

After the inducement of diabetes, the rats were fed rat chow in addition to vegetable extracts in graded levels. One group was given 500mg/kg body weight of vegetable extract. Another group was given 1000mg/kg BW of the same vegetable extract.

The vegetable extracts were given orally with an intubation tube daily. Rats in group 1 were fed rat chow and 0.5mg of standard drug glibenclamide (standard control diet). Rats in groups 2-5 were fed rat chow and 500mg/kg BW of each of the vegetable extract, respectively. The rats in groups 6-9 were fed rat chow and 1000mg/kg BW of each of the vegetable extract, respectively (Table3.1).

The study was carried out for 12days consisting of 3 days acclimatization, 1 day induction of diabetes, 2 days for establishment of diabetes and 6 days on experimental diet. The rats were allowed rat chow and water *ad libitum.* Blood samples were collected for biochemical indices determination on days 6, 10 and 12. The blood samples were analyzed to determine the blood glucose concentrations, serum total cholesterol concentration, serum triglyceride; serum high density lipoprotein (HDL) and serum low density lipoprotein (LDL) levels of the rats. The result of the biochemical indices on day 6 served as base line information. Blood collected on days 10 and 12 were tested for the same biochemical indices to establish the efficacy of each vegetable to manage diabetes. The study was carried out for 12days consisting of 3 days acclimatization, 1 day induction of diabetes, 2 days for establishment of diabetes and 6 days on experimental diet. The rats were allowed rat chow and water *ad libitum.* Blood samples were collected for biochemical indices determination on days 6 and 12.

3, 3.5 Blood sample collection

Blood samples were collected from the retro-bulba plexus of the medial canthus of the eye of the rats for biochemical analysis. A nucrocapillary tube was carefully inserted into the canthus of the eye to puncture the retro-bulbar plexus and thus enabled outflow of about 2ml of blood into a clean glass test tube. The blood samples were kept at room temperature for 30 minutes to clot. Afterwards, the test tubes containing the clotted blood samples were centrifuged at 300 revolutions per minute for ten minutes using a table centrifuge, to enable a complete separation of the serum from the clotted blood.

The clear serum supernant were carefully aspirated with syringe and needle and stored in a clean sample bottle for the biochemical indices determination. Blood samples were collected on days 6, 10 and 12 for the biochemical indices determinations. The bloods were analyzed to determine the blood glucose, cholesterol, LDL, HDL and triglyceride levels of the rats.

3.3.6 Biochemical indices determination

3.3.6.1 Determination of blood glucose

Determination of blood glucose was by the Trinders glucose-oxidase principle (Lott & Tuner, 1975) using the one Touch Basic (lifescan Milpitas, CA) instrument.

Method

The test strip was inserted in the meter. The meter turns on automatically .A small drop of blood was put on the top white edge of the test strip. The test strip automatically draws the blood into the reaction cell where the reaction takes place. The blood glucose level was read on the meter.

3.3.6.2 Determination of serum total cholesterol.

Enzymatic colorimetric test (CHOD- PAP method) for the in-vitro determination of cholesterol in serum, using Quimica Clinica Applicada (QCA) cholesterol test kit was used to determine serum cholesterol (Allain, Poon, Chan, Richmond and Fu, 1974).

Reagents

i. Working reagent composed of cholesterol esterase, cholesterol oxidase, peroxidase, pipes buffer Ph 6.8, phenol, 3,5-Dichlorophenol, 4- Aminoantipyrine.

ii Standard, equivalent to 200mg/dl cholesterol

Method

Accurately 1 ml of the working reagent was added to a set of labeled test tube. Two of the test tubes was labeled Standard ó1ô and ó2ô while another was labeled óBlankô apart from the sample to be tested that was labeled according to the group names and numbers (e.g. sample G1, G2, G3 to G10).Accurately 0.01ml of the sample was added to a labeled test tube, the sample was mixed well with the working reagent and allowed to stand for 10minutes at room temperature. Also 0.01ml of standard was added to each of the test tubes labeled standard1 and 2. It was mixed well and allowed to stand for 10 minutes at room temperature. Reading was taken at absorbance of the samples and standard against the reagent blank at 505nm.Cholesterol content of each sample was calculated using the following formular:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{200}{1}$$

The result was expressed in mg cholesterol/dl

To convert to SI units: (mg/100dl) x 0.0259=mm0L/L

3.3.6.3 Determination of serum Triglyceride

The glycerol-phosphate oxidase method (enzymatic test) for the in-vitro determination of triglycerides in serum, using Quimica Clinica Applicada (QCA) Triglyceride test kit was used to determine triglyceride (Assman *et al*., 1984).

Reagents

i. Working reagent composed of 4-chlorophenol, 4-aminoantipyrine, ATP.MgCl2, Glycerol 3 phosphate oxidase, peroxides and lipases..

ii. Standard, equivalent to 200mg/dl triglyceride.

Method

Accurately 1ml of working reagent was added to a set of clean labeled test tubes. Two of the test tube was labeled Standard 1 and 2 while another was labeled blank apart from the test samples which were labeled according to group names and numbers (e.g. Sample G1-9 etc). Approximately 0.01ml of the sample mixture was added to the labeled test tube. It was mixed well and allowed to stand for 10 minutes at room temperature. Also 0.01ml of the standard was added to each of the test tubes labeled standard 1 and 2. This was mixed well and allowed to stand for 10 minutes of room temperature. Reading was taken at absorbance of the samples and standard against the reagent blank at 590nm.

Triglyceride content of the sample was calculated using the following formular:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{200}{1}$$

The result was expressed in mg triglyceride/dl

3.3.6.4. Determination of serum high density lipoprotein

Dextran sulphate-mg (II) method for the in-vitro determination of HDL-cholesterol in serum, using Quimica Clinica Applicada (QCA) HDL test kit was used to determine HDL (Alberts, Warnick and Cheung, 1978).

Reagents

i. Precipitant solution containing dextran sulphate and magnesium acetate. peroxidase, pipes buffer Ph 6.8, phenol, 3,5-Dichlorophenol, 4- Aminoantipyrine.

ii. Working reagent composed of cholesterol esterase, cholesterol oxidase, peroxidase, pipes buffer Ph 6.8, phenol, 3, 5-Dichlorophenol, 4- Aminoantipyrine.

iii. Standard, equivalent to 200mg/dl HDL-cholesterol.

Method

Accurately 0.03ml of the serum sample was added to a clean labeled 1ml test tube. A drop of the precipitant solution was added to 1ml test tube. It was mixed and allowed to stand for 15 minutes at room temperature. It was centrifuged at 300 revolutions per minutes for 10 minutes. Approximately 1ml of cholesterol working reagent was added to a set of cleaned test tubes. Two of the test tube was labeled Standard 1 and 2 while the other was label blank apart from the test samples that were labeled according to group names and numbers (e.g. Sample G1-10). Approximately 0.01ml of the supernant derived from centrifugation of the precipitant-serum sample mixture was added to the test tube. It was mixed well and allowed to stand for 10 minutes at room temperature. Also 0.01ml of the standard was also added to each of the test tubes labeled standard 1 and 2. It was mixed well and allowed to stand for 10 minutes at room temperature. Reading was taken at absorbance of the samples and standard against the reagent blank at 505nm.

Cholesterol content of each sample was calculated using the following formular:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{200}{1}$$

The result was expressed in mg HDL-cholesterol/dl.

3..3.6.5 Determination of serum low density lipoprotein

Polyvinyl sulphate method for the in-vitro determination of LDL-cholesterol in serum using Quimica Clinica Applicada (QCA) LDL test kit was used to determine LDL (Assman, Jab and Hohnert, 1984).

Reagents

i. Precipitant solution containing polyvinyl sulphate, sodium EDTA and polyethleneglycol monomethyl ether.

ii. Working reagent composed of cholesterol esterase, cholesterol oxidase, peroxidase, pipes buffer Ph 6.8, phenol, 3, 5-Dichlorophenol, 4- Aminoantipyrine.

iii. Standard, equivalent to 200mg/dl LDL-cholesterol.

Method

Accurately 0.03ml of the serum sample was added to a set of clean labeled 1ml test tubes. A drop of the precipitant solution was added to 1ml test tube. It was mixed and allowed to stand for 15 minutes at room temperature. It was centrifuged at 300 revolutions per minutes for 10 minutes. About 1ml of cholesterol working reagent wasl also added to a set of cleaned test tubes. Two of the test tube labeled Standard 1 and 2 while another test tube was label blank apart from the samples to be tested that was labeled according to the group names and numbers (e.g. Sample G1-10). Approximately 0.01ml of the supernant derived from centrifugation of the precipitant-serum sample mixture was added to the test tube. It was mixed well and allowed to stand for 10 minutes at room temperature. Also 0.01ml of the standard was added to each of the test tubes labeled standard 1 and 2. It was mixed well and allowed to stand for 10 minutes of room temperature. Reading was taken at absorbance of the samples and standard against the reagent blank at 505nm.

Cholesterol content of each supernant was calculated using the following formular:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{200}{1}$$

The LDL cholesterol was obtained using the formular:

LDL (mg/dl) = Total cholesterol ï 1.5 x supernant cholesterol

The result was expressed in mg LDL-cholesterol/dl.

3. 4 Statistical analysis

Data collected were entered into the computer and the analysis was ran with statistical package for Social Sciences (SPSS) version 20. The result of the triplicate was pooled and analyzed using descriptive statistics (mean, standard deviation) and Post Hoc.

CHAPTER FOUR

RESULTS

Table 4. 1 presents the proximate composition of *Hibiscus cannabinus, Adansonia, digitata, Sesamum indicum and Cassia tora* leaves on wet weight basis. *Hibiscus cannabinus* had 90.04% moisture, 2.01% protein, 0.05% fat, 1.35% ash, 1.56% crude fibre and 4.99% carbohydrate *Adansonia, digitata* had 80.20% moisture, 3.89% protein, 0.05% fat, 0.19% ash, 4.16% crude fibre and 11.52% carbohydrate. *Sesamum indicum* had 95.05% moisture, 1.62% crude protein, 0.06% fat, 0.33% ash, 1.90% crude fibre and 1.04% carbohydrate. *Cassia tora* had 82.38% moisture, 1.64% crude protein, 0.05% fat, 0.06% ash, 2.16% crude fibre and 13.71% carbohydrate.

Table 4. 1: Proximate composition of fresh *Hibiscus cannabinus, Adansonia digitata, Sesamum indicum* and *Cassia tora* leaves (wet weight basis).

Nutrient %	*Hibiscus cannabinus*	*Adansonia, digitata,*	*Sesamum indicum*	*Cassia tora*
Moisture	90.04±0.16	80.20±0.79	95.09±0.05	82.38 ± 0.69
Crude protein	2.01±0.03	3.89±0.19	1.62 ± 0.39	1.64 ± 0.37
Fat	0.05±0.03	0.05±0.10	0.06 ± 0.03	0.05 ± 0.03
Ash	1.35±0.33	0.19±0.03	0.33± 0.03	0.06 ± 0.04
Crude fibre	1.56±0.30	4.16±0.10	1.90 ± 0.19	2.16 ± 0.19
Carbohydrate	4.99±0.51	11.52±0.83	1.04 ± 0.33	13.71 ± 0.50

Mean ±SD, n=3

Table 4.2 presents the mineral contents of fresh *Hibiscus cannabinus, Adansonia, digitata, Sesamum indicum and Cassia tora* leaves on wet weight basis. *Hibisucs cannabinus* contained 367.09mg sodium, 2.67mg potassium, 2.28mg calcium, 201s.77mg phosphorus, 0.56mg zinc, 0.53mg iron, 3.44mg copper and 0.28mg magnesium. *Adansonia digitata* contained 437.11mg sodium, 0.94mg potassium, 1.37mg calcium, 235.70mg phosphorus, 0.57mg zinc, 0.49 iron, 3.44mg copper and 0.37mg magnesium. *Sesamun indicum* had 236.68mg sodium, 0.87mg potassium, 0.63mg calcium, 172.50mg phosphorus, 0.51mg zinc, 0.26mg iron, 3.42mg copper and 0.24mg magnesium. *Cassia tora* had 317.90mg sodium, 2.67mg potassium 4.97mg calcium, 197.63mg phosphorus 0.59mg zinc, 0.59mg iron, 3.37mg copper and 0.26mg magnesium.

Table 4. 2: Mineral contents of fresh *Hibiscus cannabinus, Adansonia, digitata, Sesamum indicum and Cassia tora* leaves (wet weight basis).

Nutrients (mg/100g)	*Hibiscus cannabinus*	*Adansonia, digitata,*	*Sesamum indicum*	*Cassia tora*
Sodium	367.09±-0.88	437.11±1.07	236.68±0.33	317.90±0.10
Potassium	2.67±0.06	0.94±0.07	0.87± 0.15	2.67±0.12
Calcium	2.28±0.02	1.37±0.09	0.63± 0.32	4.97±0.92
Phosphorus	201.77±0.94	235.70±0.66	172.50±0.58	197.63±0.34
Zinc	0.56 ± 0.04	0.57±0.03	0.51 ± 0.02	0.59±0.03
Iron	0.53 ± 0.04	0.49±0.00	0.26 ± 0.32	0.59±0.01
Copper	3.44 ± 0.01	3.44±0.06	3.42 ± 0.19	3.37±0.03
Magnesium	0.28 ± 0.02	0.27±0.03	0.24±0.02	0.26±0.02

Mean ±SD, n=3

Table 4. 3 presents the vitamin content of fresh *Hibiscus cannabinus, Adansonia, digitata, Sesamun indicum and Cassia tora* leaves on wet weight basis. *Hibiscus cannabinus* had 13.71µg β carotene, 1.25mg vitamin B_1, 0.87mg vitamin B_2, 23.45mg vitamin C, 1.11mg niacin and 29.25mg vitamin E. *Adansonia digitata* had 22.28µg β carotene, 2.88mg vitamin B_1, 2.82mg vitamin B2, 29.37mg vitamin C, 1.61mg niacin and 31.43mg vitamin E. *Sesamum indicum* had 11.57µg β carotene, 2.12mg vitamin B_1 1.87mg vitamin B_2, 15.60mg vitamin C, 0.74mg niacin, and 25.89mg vitamin E. *Cassia tora* had 19.65mg β carotene, 1.53mg vitamin B_1, 2.13mg vitamin B_2, 18.96mg vitamin C, 1.45mg niacin and 28.79mg vitamin E.

Table 4. 3: Vitamin contents of fresh *Hibiscus cannabinus, Adansonia digitata, Sesamum indicum and Cassia tora* leaves (wet weight basis).

Nutrients (mg/100g)	*Hibiscus cannabinus*	*Adansonia, digitata,*	*Sesamum indicum*	*Cassia tora*
β carotene (µg)	13.71 ± 0.27	22.28±0.29	11.57±0.41	19.65±0.07
Vitamin B_1	1.25±0.28	2.88±0.17	2.12±0.05	1.53±0.45
Vitamin B_2	0.87±0.07	2.82±0.03	1.87 ± 0.18	2.13±0.05
Vitamin C	23.45±0.53	29.37±0.64	15.60±0.21	18.96±0.09
Niacin	1.11±0.02	1.61±0.03	0.74± 0.16	1.45 ± 0.10
Vitamin E	29.25±0.45	31.43±0.06	25.89 ± 0.09	28.79±0.27

Mean ±SD, n=3

Table 4. 4 presents the anti nutrients and food toxicants contents of fresh *Hibiscus cannabinus, Adansonia, digitata, Sesamum indicum and Cassia tora* leaves on wet weight basis. *Hibiscus cannabinus* contained traces of oxalate, 0.35mg tannins, 0.02mg phytate, 0.01mg hydrocyanins, 0.02mg cadmium and 0.02mg lead. *Adansonia digitata* contained traces of oxalate, 0.43mg tannins, 0.01mg phytate, 0.02mg *Hydrocyannin*s, 0.03mg *cadmium* and 0.21mg lead. *Sesamum indicum* had traces of oxalate, 0.41mg tannins, 0.05mg phytate, 0.01mg hydrocynanins, 0.21mg cadmium and 0.14mg lead. *Cassia tora* had traces of oxalate, 0.41mg tannins, 0.03mg phytate, 0.02mg hydrocyannins, 0.03mg cadmium, and 0.21mg lead.

Table 4. 4: Antinutrient and food toxicant content of fresh *Hibiscus cannabinus, Adansonia, digitata, Sesamum indicum and Cassia tora* leaves on wet weight basis.

Antiutrients & toxicants (mg/100g)	***Hibiscus cannabinus***	***Adansonia, digitata,***	***Sesamum indicum***	***Cassia tora***
Oxalate	Traces	Traces	Traces	Traces
Tannins	0.37±0.03	0.43±0.02	0.40±0.02	0.41±0.02
Phytate	0.02±0.01	0.01±0.00	0.05±0.01	0.03±0.01
Hydrocyannins	0.01±0.01	0.02±0.01	0.01± 0.01	0.02±0.01
Cadimum	0.02±0.02	0.03±0.01	0.01± 0.00	0.03±0.00
Lead	0.02±0.01	0.21±0.01	0.14±0.01	0.21±0.01

Mean ±SD, n=3

Table 4. 5 presents the phytochemcial compositions of fresh *Hibiscus cannabinus, Adansonia, digitata, Sesamum indicum and Cassia tora* leaves on wet weight basis. *Hibiscus cannabinus* contained traces of oxalate, 0.08mg of saponins, 0.01mg flavonoid, 0.12mg alkaloids, 0.02mg glycosides, 0.09mg terpenes, and 0.09mg phytosterols. *Adansonia digitata* had traces of oxalate, 0.12mg Saponins, 0.04mg flavonoids, 0.21mg alkaloids, 0.01mg glycosides, 0.09mg terpenes and 0.12mg phytosterols. *Sesamum indicum* had traces of oxalate, 0.06mg saponins, 0.01mg flavonoids, 0.03mg alkaloids, 0.01mg glycosides, 0.16mg terpenes and 0.08mg phytosterols. *Cassia tora* had traces of oxalate 0.12mg saponins, 0.02mg flavonoids, 0.15mg alkaloids, 0.01mg glycosides, 0.21mg terpenes and 0.16mg phytosterols.

Table 4. 5: Phytochemcial composition of fresh *Hibiscus cannabinus, Adansonia digitata, Sesamum indicum and Cassia tora* leaves on wet weight basis.

Phytochemcial mg/100g)	***Hibiscus cannabinus***	***Adansonia, digitata,***	***Sesamun indicum***	***Cassia tora***
Oxalate	Traces	Traces	Traces	Traces
Saponins	0.08 ± 0.06	0.12 ± 0.03	0.06±0.02	0.12±0.02
Flavonoids	0.01±0.01	0.04±0.02	0.01±0.01	0.02±0.01
Alkaloids	0.12±0.02	0.21±0.02	0.03±0.01	0.15±0.01
Glycosides	0.02±0.01	0.01±0.01	0.01±0.01	0.01±0.01
Terpenes	0.09±0.02	0.09±0.04	0.16±0.30	0.21±0.02
Phytosterols	0.09±0.02	0.12±0.02	0.12±0.02	0.16±0.01

Mean ±SD, n=3

Table 4. 6 presents the proximate composition of the methanol extracts of *Hibiscus cannabinus, Adansonia, digitata, Sesamum indicum and Cassia tora* leaves on wet weight basis. *Hibiscus cannabinus* extract had 8.65% moisture, 25.93% protein, 0.95% fat, 8.45% ash, 0.78% fibre and 55.19% carbohydrate. *Adansonia digitata* had 5.40% moisture, 19.56% protein, 0.87% fat, 8.51% ash, 0.62% crude fibre and 67.93% carbohydrate. *Sesamum indicum* had 5.44% moisture, 14.56% protein, 0.68% fats, 4.34% ash, 0.58% crude fibre and 74.44% carbohydrate. *Cassia tora* had 9.84% moisture 26.42% protein, 1.23% fat, 7.11% ash, 0.83% crude fibre and 54.64% carbohydrate.

Table 4. 6: Proximate composition of the methanol extract of *Hibiscus cannabinus, Adansonia, digitata, Sesamum indicum and Cassia tora* leaves on wet weight basis.

Nutrient %	***Hibiscus cannabinus***	***Adansonia, digitata,***	***Sesamum indicum***	***Cassia tora***
Moisture	8.65±0.30	5.40±0.07	5.44±0.60	9.84 ± 0.81
Protein	25.93±0.11	19.56 ± 0.59	14.56±0.27	26.42 ±0.86
Fats	0.95±0.05	0.87 ± 0.11	0.68±0.08	1.23 ± 0.03
Ash	8.45±0.58	8.51 ± 0.55	4.34±0.47	7.11 ± 0.16
Crude fibre	0.78±0.03	0.62±0.04	0.58±0.03	0.83 ± 0.15
Carbohydrate	55.19±0.76	67.93±0.29	74.44±0.59	54.64 ± 0.71

Mean ±SD, n=3

Table 4. 7 presents the mineral content of the methanol extract of *Hibiscus cannabinus, Adansonia, digitata, Sesamun indicum and Cassia tora* leaves. *Hibiscus cannabinus* contained 873.64mg sodium, 1122.61mg potassium, 1571.94mg calcium, 138.37mg phosphorus, 0.18mg zinc, 18.74mg iron, 0.28mg copper and 229.37mg magnesium, *Adansonia digitata* had 1234.22mg sodium, 1425.30mg potassium, 1724.12mg calcium, 0.18 mg zinc, 34.19mg iron, 0.83mg copper and 341.55mg magnesium; *Sesamum indicum* had 1132.10mg sodium, 1341.63mg potassium 1634.06mg calcium, 142.67mg phosphorus, 0.27mg zinc, 21.45mg iron, 0.35mg copper and 267.47mg magnesium. *Cassia tora* had 1423.44mg sodium, 1147.50mg potassium 1924.34mg calcium, 178.30mg phosphorus, 0.22mg zinc, 21.31mg iron, 0.40mg copper and 285.40mg magnesium,

Table 4. 7: Mineral contents of methanol extracts of *Hibiscus cannabinus, Adansonia digitata, Sesamum indicum and Cassia tora* leaves.

Mineral mg/100g	*Hibiscus cannabinus*	*Adansonia, digitata,*	*Sesamum indicum*	*Cassia tora*
Sodium	873.64±0.32	1234.22±0.18	1132.10±0.23	1423.44±0.64
Potassium	1122.61±0.60	1425.30 ±0.43	1341.63±0.45	1147.50±0.12
Calcium	1571.94±0.09	1724.12±0.49	1634.06±0.52	1924..34±0.04
Phosphorus	138.37±0.64	224.19.±0.24	142. 67±0.28	178.30±0.42
Zinc	0.18 ± 0.03	0.18±0.03	0.27±0.03	0.22±0.01
Iron	18.74 ± 0.23	34.19 ± 0.18	21.45±0.50	21.31±0.36
Copper	0.28±0.02	0.83 ± 0.20	0.35 ± 0.02	0.40 ± 0.02
Magnesium	229.37±0.02	341.55±0.72	267.47±0.59	285.40 ± 0.39

Mean ±SD, n=3

Table 4. 8 presents the vitamin contents of methanol extracts of *Hibiscus cannabinus, Adansonia, digitata, Sesamum indicum and Cassia tora* leaves. *Hibiscus cannabinus* contained 7.60 µg β carotene, 1.22mg vitamin B_1, 0.54mg vitamin B_2, 26.34mg vitamin C, 0.84mg niacin and 22.72mg vitamin E. *Adansonia digitata* had 13.70µg β carotene, 2.40mg vitamin B_1, 2.32mg vitamin B_2, 18.30mg vitamin C, 1.43mg niacin and 25.22mg vitamin E. *Sesamum indicum* had 8.59µg β carotene, 1.74mg vitamin B_1,1.25mg B_2, 14.86mg vitamin C, 0.52mg niacin and 21.30mg vitamin E. *Cassia tora* had 10.42µg β carotene, 1.96mg vitamin B_1, 1.68mg vitamin B_2, 16.40mg vitamin C, 1.21mg niacin and 23.50mg vitamin E.

Table 4. 8: Vitamin contents of the methanol extracts of *Hibiscus cannabinus, Adansonia digitata, Sesamum indicum and Cassia tora* leaves.

Vitamin (mg/100g)	***Hibiscus cannabinus***	***Adansonia, digitata,***	***Sesamum Indicum***	***Cassia tora***
β-Carotene (µg)	7.60±0.33	13.70±0.65	8.59±0.61	10.42±0.62
Vitamin B_1	1.22±0.40	2.40±0.15	1.74±0.08	1.96±0.09
Vitamin B_2	0.54±0.05	2.32±0.07	1.25±0.05	1.68 ± 0.41
Vitamin C	26.34±0.49	18.30±0.39	14.86±0.17	16.40 ±0.52
Niacin	0.84±0.24	1.43±0.17	0.52 ± 0.03	1.21± 0.15
Vitamin E	22.72±0.75	25.22±0.04	21.30±0.39	23.50±0.83

Mean ±SD, n=3

Table 4. 9 presents the antinutrient and toxicant contents of the methanol extracts of *Hibiscus cannabinus, Adonsonia, digitata, Sesamun indicum and Cassia tora* leaves. *Hibiscus cannabinus* extracts contained

5.10mg tannins, 0.66mg phytate, 0.34mg hydrocyanides, 0.02mg *cadimium* and 0.02mg lead. *Adansonia digitata* had 4.57mg tannins, 1.75mg phytate, 0.22mg hydrocyanides, 0.03mg cadmium and 0.21mg lead. *Sesamum indicum* had 6.96mg tannins, 1.21mg phytate, 0.45mg hydrocyanides, 0.01mg cadmium and 0.14m lead. *Cassia tora* had 7.07mg tannins, 1.78mg phytate, 0.48mg hydrocyanides, 0.03 cadmium and 0.21mg lead.

Table 4. 9: Anti nutrient and food toxicant contents of methanol extract of *Hibiscus cannabinus, Adansonia digitata, Sesamum indicum and Cassia tora* leaves.

Antinutrient & Toxicant (mg/100g)	***Hibiscus cannabinus***	***Adansonia, digitata,***	***Sesamum indicum***	***Cassia tora***
Tannin	5.10±0.02	4.57±0.46	6.96±0.14	7.07±0.87
Phytate	0.66±0.06	1.75±0.05	1.21±0.05	1.78±0.10
Hydrocyanides	0.34±0.03	0.22±0.03	0.45±0.07	0.48±0.03
Cadmium	0.02±0.00	0.03±0.00	0.01±0.00	0.03±0.01
Lead	0.02±0.01	0.21±0.01	0.14±0.00	0.21±0.00

Mean ±SD, n=3

Table 4.10 presents the phytochemical composition of methanol extract of *Hibiscus cannabinus, Adansonia, digitata, Sesamum indicum and Cassia tora* leaves. *Hibiscus cannabinus* contained 3.26mg saponins, 0.09mg flavonoid, 4.91mg alkaloids, 2.70mg glycosides, 1.09mg terpenes and 1.36mg phytosterols. *Adansonia digitata* had 2.05mg saponins, 0.14mg flavonoids, 5.22mg alkaloid, 2.40mg glycosides, 1.23mg terpenes and 2.39mg phytosterols. *Sesamum indicum* had 3.73mg saponins, 0.23mg flavonoids, 6.45mg alkaloids, 3.76mg glycosides, 1.43mg terpenes and 1.26mg phytosterols. *Cassia tora* contained 2.40mg saponins, 0.29mg flavonoids, 6.77mg alkaloids, 3.84mg glycosides, 2.30mg terpenes and 2.50mg phytosterols.

Table 4. 10: Phytochemcial contents of methanol extracts of *Hibiscus cannabinus, Adansonia, digitata, Sesamum indicum and Cassia tora* leaves.

Phytochemicals (mg/100g)	***Hibiscus cannabinus***	***Adansonia, digitata,***	***Sesamum indicum***	***Cassia tora***
Saponins	3.26 ± 0.12	2.05± 0.17	3.73±0.39	2.40±0.08
Flavonoids	0.09 ± 0.01	0.14 ± 0.02	0.23±0.03	0.29±0.02
Alkaloids	4.91 ± 0.11	5.22 ± 0.27	6.45±0.05	6.77±0.28
Glycosides	2.70 ± 0.20	2.40 ± 0.53	3.76±0.09	3.84±0.16
Terpenes	1.09 ± 0.01	1.23 ± 0.02	1.43±0.04	2.30±0.06
Phytosterols	1.36 ± 0.04	2.39 ± 0.01	1.26±0.04	2.50±0.46

Mean ±SD, n=3

Table 4. 11 presents the mean blood glucose levels of groups of rats fed rat chow and glibenclamide drug, rat chow and 500mg or 1000mg of *Hibiscus cannabinus, Adansonia digitata, Sesamum indicum and Cassia tora* leaves extracts from day 3 to 12. The blood glucose concentration of all groups of rats on day 3 the last day of acclimatization ranged from 61.80mg/dl to 83.60mg/dl. On day 6 the day diabetes was confirmed, the blood glucose levels were in the range of 246.00mg/dl - 573.60mg/dl (baseline). The blood glucose level of rats fed rat chow and standard drug (standard control group) decreased from 402.80 on day 6 to 333.40 on day 12 with a 17. 23% decrease.

The blood glucose levels for groups of rats fed rat chow and graded doses of the various vegetable extracts (group 2 ï 9) ranged from 327.40mg/dl ï 573.60mg/dl on day 6 and 221.40mg/dl ï 547.00mg/dl on day 12. The blood glucose concentrations of rats in groups 3, 4 and 6 had some increases. Group 3 increased from 404.80mg /dl on day 6 to 479.20mg /dl on day 12 with an increase of 18.38%. Group 4 had increase from 336.60mg/dl on day 6 to 363.80mg/dl on day 12 with an increase of 8.08%. The group 6 had increase from 389.80mg/dl on day 6 to 513.00mg/dl on day 12. There was an increase of 31.61%. The blood glucose concentration of other groups (2, 5, 7, 8, and 9) had decreases of 32.38%, 7.83%, 33.63%, 9.06%, and 23.96%, respectively on day 12.

Table 4.12 presents the mean serum total cholesterol (TC) levels (mg/dl) of rats fed chow and glibenclamide drug, rat chow and 500mg or 1000mg of *Hibiscus cannabinus, Adansonia digitata, Sesamum indicum and Cassia tora* leaf extracts from day 6 ï 12. On day 6 when diabetes was estab0lished (baseline), the mean TC levels of all groups of rats ranged from 216.16mg/dl ï 265.70mg/dl. On day 12 the mean TC level in all groups ranged from 171.13mg/dl ï 225.17mg/dl. There were decreases in TC in all groups of rats in the range of 9.62% ï 26.92%.

The group of rats fed rat chow and standard drug (GD1) had a decrease in total cholesterol from 234.17mg/dl on day 6 to 184.64mg/dl on day 12. This group had a decrease of 21.15% on day 12. The mean TC levels of the group of rats fed rat chow and graded doses of the various vegetable extracts (Gp2 ï 9) had values that ranged from 216.16mg/dl ï 252.19mg/dl on day 6 and 171.13mg/dl ï 211.16mg/dl on day 12. The total decrease in TC levels had a range of 9.62% - 26.92% on day 12. The TC of the group of rats fed rat chow and 500mg of *H.cannabinus* (diet2) had decrease from 234 .17mg/dl on day 6 to 193.63mg/dl on day 12 (17.31%). The group of rats fed 1000mg/dl *H. cannabinus* (diet 6) had a decrease from 229.67mg/dl on day 6 to 207.15mg/dl on day 12 (9.81%). The groups of rats fed rat chow combined with 500mg or 1000mg *A digitata* (diet3 and 7) each showed decreases of 18.75 and 26.92% each. The groups fed rat chow and 500mg or 1000 mg each, of *S. indicum* (diet 4 and diet 8) had decreases of 15.38% and 19.64 %, respectively. The groups fed rat chow combined with 500mg and 1000mg (diets 5 and 9), each of *C.tora* leaf extract had decreases of 9.62% and 25.46%, respectively.

Table 4. 13 presents the mean serum triglyceride (TG) levels (mg/dl) of rats fed rat chow and glibenclamide drug, rat chow and 500mg or 1000mg of *Hibiscus cannabinus, Adansonia digitata, Sesamum indicum and Cassia tora* leaves extracts from day 6 ï 12. On day 6 the mean TG levels of all the groups of rats ranged from 94.42mg/dl ï 133.42mg/dl (baseline). On day 12 the mean TG levels for all group of rats ranged from 43.10mg/dl ï 78.00mg/dl. The decrease ranged from 28.30% - 67.70%.

The group of rats fed rat chow and the standard drug (diet1) had a decrease from 98.53mg/dl on day 6 to 53.37mg/dl on day 12. There was a decrease of 45.83%. The mean TG levels for the groups of rats fed graded doses of vegetable extracts (diets 2 to 9) had decreases from 94.42mg/dl ï 133.42mg/dl on day 6 and 43.10mg/dl ï 69.79mg/dl on day 12, there was a decrease that ranged from 34.62% - 67.70%. The groups of rats fed rat chow and 500mg or 1000mg each of *H. cannabinus* leaf extract (diet 2 and 6) had decreases in TG levels 47.83% and 34. 62% each. The groups fed 500mg or 1000mg each of *A. digitata* leaf extract (diet 3 and 7) had decreases 39.21% and 54.72% each. The groups fed rat chow and 500mg or 1000mg (diet4 and 9) of *S. indicum* leaf extract had percent decreases of 40.74% and 46.29% in TG levels each. The groups of rats fed 500mg or 1000mg (diet5 and 9), *Cassia tora* leaf extract had decreases of 50.94% and 67 .70% in TG levels, respectively. The groups of rats fed 1000mg of *Cassia tora* leaf extract (diet 9) had highest decrease (67.70%) TG levels among all the groups.

Table 4. 14 presents the mean serum high density lipoprotein (HDL) levels of rats fed rat chow and glibenclamide drug, rat chow and 500mg or 1000mg of *Hibiscus cannabinus, Adansonia digitata, Sesamum indicum and Cassia tora* leaf extracts. On day 6, the base line mean HDL cholesterol for all the groups of rats ranged from 71.70mg/dl - 87.29mg/dl. On day 12, the mean HDL cholesterol for all groups ranged from 74.82mg/dl ï 106.00mg/dl. The mean HDL cholesterol of the group of rats fed rat chow and standard drug (diet1) had increase from 84.18mg/dl on day 6 to 102.88mg/dl on day 12 with an increase of 22.21%. The mean HDL cholesterol of group of rats fed rat chow and various doses of vegetable extracts (Diet2 to 9) ranged from 71.70mg/dl ï 87.29mg/dl on day 6 and 74.82mg/dl ï 102.88mg/dl on day 12. There was an increase in HDL (4.35% - 30.77%) at the end of the study. The HDL of groups of rats fed rat chow and 500mg (diet 2) of *Hibiscus cannabinus* leaf extract increased from 87.29mg/dl on day 6 to 102.88mg/dl on day 12. The group fed rat chow and 1000mg of *Hibiscus cannabinus* (diet 6) had increase from 81.06mg/dl on day 6 to 106.00mg/dl on day 12 with an increase of 30.77%. The HDL of groups fed rat chow and 500mg or 1000mg *of A.digitata* (diet 3 and 7) increased from 77.94mg/dl and 71.70mg/dl on day 6 to 96.65mg/dl and 74.82mg/dl on day 12. The groups fed rat chow and 500mg, or 1000mg of *Sesamun indicum* leaf extract (diets 4 and 8) had increases in HDL from 81.06mg/dl and 77.94mg/dl on day 6 to 96.65mg/dl (19.23%) and 99.77mg/dl (28.01%), respectively, on day 12. The HDL of group of rats fed 500mg of *cassia tora* leaf extract (diet 5) increased from 77.94mg/dl on day 6 to 96.65mg/dl on day 12 with an increase of 24.01% and that of the groups fed 1000mg *Cassia tora* leaf extract (diet 9) increased from 74.82 mg/dl on day 6 to 90.41mg/dl on day 12. This was an increase of 20.84%.

Table 4. 15 presents the mean serum low density lipoprotein (LDL) levels of rats fed rat chow, and glibenclamide drug, rat chow and 500mg or 1000mg of *Hibiscus cannabinus, Adonsonia digitata, Sesamum indicum and Cassia tora* leaf extract from day 6 ï 12. On day 6 when diabetes was established the mean LDL cholesterol for all groups of rats ranged from 117.28mg/dl ï 152.08mg/dl. On day 12, the LDL cholesterol levels ranged from 66.25 ï 104.34mg/dl. There was decrease in all the groups of rats.

The mean LDL cholesterol level for rats fed rat chow and standard drug (diet1) had decrease from 117.82mg/dl on day 6 to 71.08 mg/dl on day 12. This was a decrease of 39.67%. The mean LDL cholesterol levels for rats fed rat chow and graded doses (diet 2 to diet 9) of the various vegetable extracts had decreases 117.28mg/dl - 152.08mg/dl on day 6 and 66.25mg/dl ï 104..34mg/dl on day 12.This had a decrease that ranged from 22.64% - 43.51%. Group 3 which consumed 500mg of *Adansonia digitata* (diet3) had the highest decrease (43.51%) of LDL cholesterol among all the group of rats.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

5.1.1 Proximate composition

The high moisture (P> 0.05) (90.04%, 80.20%, 95.09% %, and 82.38%) contents of the vegetables are similar to that reported by Olaiya and Adebisi (2010) (75.0 - 91.5%) in ten leafy vegetables. Oloyede, Obuotor and Ibronke (2011) also reported moisture values of 80.50% - 90.57% in a study of five underutilized fresh green leafy vegetables in south western Nigeria. The values were also similar to the previously reported findings of Ladan *et al.* (1996) who reported values of 76.80% - 93.4% in some leafy vegetables consumed in Nigeria .The moisture values of *Adansonia, digitata,* (80.20%) and *Cassia tora (82.38%)* leaves were similar to the moisture values reported for some commonly consumed vegetables like *Solanumnigrum* leaf (87.20%) and *Teliferia occidentalis* leaf (86.0%) (Nnam *et al*., 2012). Moisture is the most abundant constituent of fresh green leafy vegetables. The high moisture content provides for greater activity of water soluble enzymes and co- enzymes needed for metabolic activities of the leafy vegetables (Iheanacho and Udebuani, 2009). The high moisture contents makes vegetables aid the digestion of food, however, shelf life is very short because the high moisture facilitates bacterial action resulting into spoilage. The high moisture content found in the fresh leaves is an indication of low total solids. Vegetables with high moisture content are called high water content foods with 80-95% of their total composition being water (Iheanacho and Udebuani 2009). This shows that the more of the vegetables consumed, the more water intake that flushes out waste products from the body (www.healthy-eating.and. nutrition. co). The low moisture values of the extracts were desirable as the levels would hinder the growth of micro-organisms and increase storage life.

The low protein values (2.01%, 3.89%, 1. 62, and 1.64%) were similar to literature reports of Sheela *et al*.(2004) who observed protein values of 0.80% ï 3.60% per 100g fresh vegetables. Similar values (2.19% - 5.78%) were reported by Nnam *et al.* (2012) on some fresh leafy vegetables, Ojimelukwe *et al.* (2012) on *Ocimum gratissmium* (3.19%) and *Lasiaanthera africana*(2.24%) on wet weight bases. The low protein levels of the vegetables were in line with literature reports which classified vegetables as poor sources of protein especially for fresh samples (Oguntana, 1998; Nnam, 2012). The protein values of the methanol extract (25.93%, 19.56%, 14.56% and 26.42%) compared favourably with the protein value of methanol extract of *Moringa olifera* (27.61%) (Bamishaiye *et al.,* 2011); *Amaranthus caudatus*(20.59%), *Manihot utilisima* (24.88%) and *Piper guineeses* (29.78%) (Akindahunsi and Salawu, 2005). The higher value of protein in the methanol extracts (14.56% ï 26.42%) than the fresh leaves (1.62% -3.89%) suggests that the process of extraction might have caused concentration of the nutrient in the extracts.

The fat contents of the vegetables (0.05% - 0.06%) (table1) were lower than the values (0.20 ï 2.60 %) reported by Sheela *et al*. (2004) on some leafy vegetables and the report of Olaiya and Adebisi (2010) (0.10 ï 1.80%) on ten leafy vegetables and Nnam *et al*. (2012) (0.08%) for some vegetables. The fat level was the least in the proximate components. This is in with the observation of Nnam *et al*.(2012) that among the proximate components, fat content represents the lowest in vegetables. Oguntona (1998) also noted that fat values for green leafy vegetables scarcely exceed 1.0%. The vegetables would be of immense importance in the preparation of low fat diets. Green leafy vegetables are known to be poor sources of fat (en.wikipedia.org/wiki/leaf. Veg.). Dark green leafy vegetables however contain omega 3 fatty acids. Omaga 3 fatty acids are called essential fatty acids because the body cannot manufacture the acids from other nutrients. The acids must be obtained from the diet. Omaga 3 fatty acids come in three varieties namely Alpha Linoleic Acid (ALA), Decosol Hexanoic Acid (DHA) and Eicosoi Pentonoic Acid (EPA).The fatty acids give important health

benefits to the body. They may help to prevent breast and colon cancer, high blood pressure and can reduce the risk of suffering a stroke among other benefits. ALA is found primarily in dark green vegetables. Dark green vegetables are among the highest sources of ALA (www.young women health org>). Most humans can convert ALA found in plant foods to DHA and EPA in the body to provide all their health benefits. Theoritically eating foods containing ALA or dark geen vegetables such as *Hibiscus cannabinus, Adansonia, digitata, Sesamum indicum and Cassia tora* leaves can produce enough DHA and EPA especially when consumed in large quantities. The presence of fat in foods however increases palatability of food by absorbing and retaining flavours (Anita *et al*., 2006).

The fat levels of the methanol extracts of the vegetables (0.68% - 1.23%) were low when compared to the levels of the methanol extracts of some leafy vegetables commonly consumed in Nigeria such as *Talinum triangulare* leaf (5.90%), *Baseila alba* (8.71%) *Amaranthus hybridus* (4.80%), *Calchorus africanum* (4.20%) (Akindahunsi and Salawu, 2005). Literature supports that a diet providing 1-2% of its caloric value of energy as fat would be sufficient to human beings as excess fat consumption is implicated in certain cardiovascular disorders such as atherosclerosis, cancer and aging (Anita *et al*., 2006).

The carbohydrate contents of the vegetables were relatively higher (4.99%, 11.52%, 1.04% and 13.17%) than the fat content (0.05% - 0.06%). The least carbohydrate content in sesamun indicum (1.04%) is not surprising considering its high moisture content of 95.09%. The carbohydrate values of 1.04% in fresh *Sesamum indicum and* 4.99% in fresh *Hibiscus cannabinus* were comparable to the values reported for some underutilized leafy vegetables *Celosia trigyna* (1.09%) and *Solanum nigrum* (4.80%) (Oloyede *et al*., 2011). However the carbohydrate contents of the fresh vegetables (1.04% - 13.71%) were lower than the values (18.23% - 90.01%) reported by Kanchan *et al*. (2011) on indigenous vegetables in India. The low levels of carbohydrates in the vegetables are in line with

literature reports that fresh leafy vegetables are not good sources of carbohydrates. The little carbohydrate contents in the vegetables could supply part of the daily requirements for carbohydrates for the individual. The end product of carbohydrate digestion (glucose) provides energy to cells in the body particularly the central nervous system (Effiong, Ibia, Udofia, 2009). The high values of carbohydrates (54.64% - 74.44%) for the extract were not surprising considering the low moisture levels (5.40% - 9.80%) of the vegetable extracts. The carbohydrate values of the extracts compares favourably with the value (53.53%) reported for methanol extract of *Moringa oliefera* (Bamishaiye *et al.,* 2011) and *Acalypha hispida* (48.48%) (Iniaghe *et al.*,2009). The low carbohydrates, fats and proteins levels of the vegetables would contribute very little to the energy value of a meal and this would be of benefit in energy controlled diets.

The crude fibre contents of the vegetables (1.56%, 4.16%, 1.90% and 2.16%) were similar with the findings of Olaiya and Adebisi (2010) who reported fibre contents of 0.8g/100g ï 9.5g/100g for ten green leafy vegetables in south-western Nigeria. The values were however lower than the fibre content of 5.62%- 15.86% reported by Nnam *et al.* (2012) on four leafy vegetables. FAO (1990) reported values of 3.1% fibre for *Adonsonia, digitata* which is within the range of values obtained in this study. Fibre is useful for providing bulk, and increasing intestinal peristalsis by surface extension of food in the intestinal tract (Mathenge, 1997). The vegetables could help in preventing constipation, bowel problems and piles when consumed in large quantities. Consumption of the vegetables in large amounts could be useful in the treatment of diseases such as obesity, diabetes, cancer and gastrointestinal disorder. Fibre has beneficial effect on blood cholesterol. In diabetics, fibre improves glucose tolerance (Ashaye, 2010). The high fibre contents of *Adonsonia digitata* (4.16%) and *Cassia tora* leaves (2.16%) are of interest. Cho, Chun and Lee (2009) in their study on the effect of *Cassia tora* fibre supplement on serum lipids in Korean diabetic patients concluded that *Cassia tora* leaf fibre

supplement can help improve serum lipid status in type 2 diabetic patients without any serious adverse side effects. The use of the vegetables could therefore be very useful in the management of diabetics.

The lower crude fibre values of the extracts (0.78%, 0.62%, 0.58% and 0.83) than the fresh leaves (1.56%, 4.16%, 1.90% and 2.16%) were not surprising. This is because the processes of extraction involve series of filtration during which most of the indigestible fibres may have been removed leading to lower crude fibre values. The values were however comparable to the values of methanol extract of *Cnidoscolus chayamansa* (0.92%), *Solaniumno diflorum* (0.78%) and *SenIecio biafrae* (0.92%) (Bamishaiyi *et a*l.,2011). Higher values than those obtained in this study (1.56% - 4.16 %,) were reported for A*calypha hispada* (10.25%), *Acalypha racemoisa* (7.02%), *Acalypha marginata* (11.50%) (Iniaghe *et al*., 2009).

The ash values obtained in this study (1.35%, 0.19%., 0.33%., 0.06%) were comparable to ash values reported for five fresh underutilized green leafy vegetables *Bidans pilosa* (1.82%), *Celosia trigyna* (0.87%), *Crassocephalum crepidioides* (1.96%), *Launaea teracifolia*(1.33%) and *solanum nigrum*(1.00%) (Oloyede *et al.,* 2011).The higher ash value obtained for *Hibiscus cannabinus(*1.35%) suggests that the vegetable may be richer in organic matter than *Adansonia, digitata* (0.19%), *Sesamum indicum* (0.33)% *and Cassia tora* (0.06%) leaves.

The ash contents of the extracts are a reflection of the mineral deposits in the food materials. The moderate ash contents of the extracts therefore suggest moderate deposit of mineral elements in the leaf extracts.

5.1.2 Mineral Composition

The result of the study showed that the fresh vegetables and their methanol extracts were good sources of some minerals as shown in Tables 4.2 and 4.7, respectively. The high sodium levels of the vegetables and their extract could be useful in the regulation of plasma volume, acid base balance, nerve and muscle contraction (Akpanyung, 2005). Sodium is a very common electrolyte not generally found in dietary supplements.

Calcium levels (0.63mg/100g ï 4.97mg/100g) of the vegetables were lower than the levels (234.30mg/100g - 279.71mg/100g) reported by Nnam *et al.* (2012) in their study of four leafy vegetables. The values were however comparable with levels (1.10mg/100g - 5.60mg/100g) reported by Olaiya and Adebisi (2010). The high calcium levels found in the fresh leaves of cassia *tora* (4.97mg/100g) relative to the other vegetables suggests that consumption of *Cassia tora* leaves could be more effective than others in bone and teeth formation and in the proper functioning of the nervous system. It has long been reported that commonly consumed leafy vegetables are superior sources of calcium to milk (Oke, 1966). Dark green vegetables are known to be good sources of calcium but the time of harvesting (June 2013) of the vegetables in this study might have affected the concentration of the minerals. This is because the month of June is the beginning of the rains in the north eastern part of Nigeria and most of the vegetables are in their early stage. At this stage some minerals are not yet fully established and may not be fully deposited in the leaf (Bamishaiye *et al*.,2011). *Adansonia, digitata* for example shades its leaves at the beginning of the dry season and new leaves appear at the beginning of rainy season (Vertueni *et al*., 2002). This might be the reason for the low level of calcium in *Adansonia digitata* (1.37mg/100g) in the study. The low calcium levels in *Hibiscus cannabinus* (2.28mg/100g), *Sasamum indicum* (0.63mg/100g) *and Cassia tora* (4.97mg/100g) leaves in the study might also be due to the time of harvesting. This might also be the

reason for the low ash values of the vegetables in the study. Nnam & Nwofor (2001) reported that pulverized baobab (*Adansonia digitata)* leaf soup is a potential good source of calcium (147mg per 100g dry weight basis) and would be useful in providing macro and micronutrients to the diets of people who consume it. The difference in the reports could be attributed to the form in which the vegetables were analyzed. The soup was prepared with dry leaves and drying is known to concentrate nutrients.

The potassium values of the fresh vegetables (0.87 ï 2.67mg/100g) were lower than the values (97.03 - 325.90mg/100g) observed by Nnam *et al*. (2012) on four leafy vegetables but comparable to values (1.34 - 3.34mg/100g) observed by Akindahusi *et al.* (2006) for fresh vegetables. Potassium is required in muscle and nerve functions. Sodium and potassium are important intracellular and extracellular cataions, respectively. Consumption of these vegetables would be of importance in maintainace of proper heart function. The values of potassium in the vegetable extracts (1122.61 ï 1425.30mg/100g) could provide more than half the required nutrient intake of 2000mg/ day of potassium for adults and adolescents.

The Magnesium values obtained in this study for the fresh leaves (0.24 ï 0.28mg/100g) were lower than the values (1.20 - 4.60mg/100g) reported by Olaiya and Adebisi (2010) on ten leafy vegetables but comparable to the value (0. 04mg - 0.32./100g) reported by Okudu (2007) on some Nigerian green leafy vegetables. The values were also similar to that (0.38mg/100g) reported by Ujowundu *et al*. (2010) for a vegetable. Magnesium is an obligate co-factor for DNA syntheses and an important mineral in energy metabolism. It is important in the reduction of blood pressure and vegetables containing it could be used to supplement low magnesium based staple foods such as cassava in Nigeria (Olaiya and Adebisi, 2010). Consumption of 100g of the extracts especially *Adansonia digitata* (341.55mg/100g) could meet the RNI for magnesium (310mg/day) for women 19 ï 39years old (FWB.1997). The result of the study suggests that the vegetable extracts could provide some health benefits as dietary supplements as they contain

significant quantities of magnesium. The extracts could help in the management of diabetes because insulin secretion and function requires magnesium. *Adansonia digitata* (341.55mg/100g) leaf extract could be more useful in the management of diabetes because of its higher magnesium content than the other leaves (229.37 ï 285.40mg/100g). Literature report shows that green leafy vegetables are high in magnesium and have low glycemic index thus they prove to be helpful for patients with type 2 diabetes (www.heaalth.alternatives.com/mineralé) Dietary advice shows that one serving of green leafy vegetables each day will considerably lower the risk of diabetes (www.organicfacts.net/health-benefits). Consumption of the vegetables studied could help to lower the risk of diabetes because of their high magnesium contents.

Consumption of 100g of the vegetables could provide sufficient copper to meet the recommended nutrient intake of copper. Copper is involved in the absorption, storage and metabolism of iron and formation of red blood cells. Consumption of the fresh vegetables could be useful in the prevention and management of anaemia. The mineral copper helps in energy metabolism as it works closely with the enzyme cytochrome c oxidase to produce energy (Ducrepene 2010).

The iron values of the fresh leaves (0.53, 0.49, 0.26 and 0.59 mg/100g) were lower than the values reported by Nnamani *et al*. (2009) (3.68 - 7.34mg/100g) and Nnam and Ngwa (2010) (4.17 - 11.04mg/100g) on some underutilized fresh vegetables they studied. Iron is an important trace element in the human body. It plays crucial role in haemopoesis, control of infection and cell mediated immunity (Bhaskeran, 2001). The iron levels of the methanol extracts of the leaves were high and could be useful as health supplements in the management of iron deficiency anaemia.

The levels of zinc (Zn) (0.56,0.57,0.51 and 0.58mg/) in the vegetables and their extract(0.18,0.18, 0.28 and 0.22mg/100g) were lower than the values reported by Nnam and Ngwa (2010) (3.00 - 23.00mg/100g) on indigenous vegetables. The values were however comparable with the levels

observed by Nnam *et al*.(2012) for *Vernonia amagdalina* (0.61mg/100g). Zn protects the skin and improves resistance to infectious diseases, inflammation and allergies (Nnam *et al*. 2009). Zn is an essential micronutrient for human growth and immune functions (Black, 2003). It plays a structural role in the storage of insulin and a catalytic role in enzymes. The zinc contents of the vegetables is in line with literature reports that most vegetables contain small amount of zinc (Akindahusi *et al.,* 2006).

The moderate values (201.77mg, 235.70 mg, 172.50mg and 197.63mg per 100g fresh vegetables) of phosphorus were not surprising because literatures report that phosphorus is widely distributed in both plant and animal foods and is very high in leafy vegetables. The values of phosphorus obtained for the vegetables are similar to those reported by Nnam *et al. (*2012) (99.36 - 409.75g/100g) for four leafy vegetables. Phosphorus is important in the energy transfer of nucleic acids. It is essential component of bone mineral. A balance proportion of calcium and phosphorus is needed in the body for healthy bones. Imbalance of phosphorus and calcium results in osteoporosis, arthritis, pyorrhea, rickets and tooth decay. Consumption of adequate quantity of the vegetables will be very helpful in the body as phosphorus aid in metabolic reactions as component of DNA and RNA, ADP, ATP and TPP.

5.1.3 Vitamins

The beta-carotene (pro-vitamin A) levels of the leaves(13.71 µg/100g, 22.28µg /100g, 11.57 µg/100g and 19.65µg/100g) and the methanol extracts (7.60 µg/100g, 13.70 µg/100g, 8.59µg/100g, and 10.24µg/100g) are significant. Beta carotene is a precursor of vitamin A, which is important in strengthening and boosting the immune system to fight infection. Carotenoid is a component of rhodopsin, the visual pigment in the mammalian eye; as such the consumption of large quantities of the vegetables would be beneficial for good vision. The lower values of beta carotene in the extracts than the fresh leaves could be because the alcohol methanol must have destroyed some of the beta-

carotene (a fat component) or formed some other compounds with beta carotene. This suggests that consumption of the fresh leaves would be more beneficial in terms of supplying ɓ- carotene.

The vitamin C levels (23.45mg/100g, 29.37 mg/100g, 15.60 mg/100g and 18.96mg/100g) of the fresh vegetables and (26.34mg/100g,18.30mg/100g,14.86mg/100g and 16.40 mg/100g) of the extracts were similar to those of O*cimum gratissmum* (18.64mg/100g), *Gongronema latifolium* (28.30mg/100g) and *Gnetum africanum* (21.07mg/100g) leaves (Nnam *et al.*,2012). The human body cannot produce vitamin C so it must be obtained entirely through the diet. The vegetables could serve as good dietary sources of the vitamin. Vitamin C is essential for the healthy formation of bones and teeth. *B-carotene* and Vitamin C are very powerful antioxidants. This suggests that consumption of large quantities of the vegetables could provide health benefits. Antioxidants are known to protect the cells by reacting with oxidizing factors and neutralizing their effects (Nnam, 2011). They help protect the body from cell damage caused by free radicals and peroxides. Consumption of the vegetables with their high ɓ-carotene and Vitamin C contents could be effective in preventing cancer and other degenerative diseases. *Adonsonia digitata* leaf with higher Vitamin C (29.37 mg/100g fresh leaf, 18.30mg/100g extract) and ɓ-carotene (22.28 µg /100g for fresh leaf, 13.70 µg/100g for extract) than the other vegetables could be better source of the antioxidant nutrients. The high levels of ɓ - carotene and ascorbate in the vegetables will synergistically facilitate the absorption of iron in the leaves. ɗɓ - carotene and ascorbate enhances iron absorption and utilization. The enhancing effect of ascorbate has been attributed to its reducing and chelating properties during the digestion of food (Hurrel and Egli, 2007). Ascorbate also enhances iron absorption by reducing the iron III ions to ferrous (fe^{2+}) state, a form in which iron is absorbed. Inclusion of the vegetables as dietary components would improve absorption of iron particularly the non haem iron from plant foods which is poorly absorbed.

The thiamin (1.25 - 2.88mg/100g for fresh leaves and 1.22mgï 2.40mg/100g for extract), riboflavin (0.87-2.82mg/100gfor fresh leaves and 0.54 ï 2.32mg/100g for extract) and niacin (0.74 - 1.62mg/100g for leaves and 0.52 -1.43mg/100g for extract) values were higher than the values reported by Olaiya and Adebisi (2010) for thiamin (0.03 - 0.16mg/100g), riboflavin (0.04 - 0.03mg/100) and niacin (0.10 - 0.90mg/100g) for ten leafy vegetables in south western Nigeria. The micronutrients thiamin, riboflavin and niacin play very important roles in nutrient metabolism. Niacin has the ability to lower blood lipids and is sometimes used in treating hyperlipidaemia (Olaiya and Adebisi, 2010). Thiamin is intricately involved with metabolism of glucose in the body while riboflavin is required to release energy from proteins, carbohydrates and fat. The significant amount of the micronutrients (B_1, B_2 and niacin) in the vegetables studied further enhances their health benefits. The micronutrients play significant role in the metabolism of proteins, carbohydrates and fat.

Consumption of 100g of the vegetables could provide about 90% of the RNI of vitamin E for adults. Vitamin E is a powerful fat soluble antioxidant vitamin. Vitamin E is the most effective non enzymatic antioxidant for terminating the chain reactions of lipid peroxidation in cell membranes. It is especially effective in protecting low density lipoproteins (LDL) from oxidation. It corroborates with vitamin C to slow progression of cardiovascular diseases and protects the double bonds of beta carotene from oxidation and thus exhibits a sparing effect. Consumption of the vegetables with the high content of vitamin C and E will help prevent cardiovascular diseases and protect the double bond of the beta carotene in the vegetables from oxidation. Salomen,Nyssomen,Touma and Nem (1995) established that vitamin E status has a strong independent inverse association with the risk of diabetes. This suggests that consumption of large quantities of the vegetables could help prevent the risk of diabetes. One of the mechanisms that form the basis of both western approaches and traditional Medicine approaches to lower blood glucose is to clear free radicals, resist lipid peroxidation and correct the lipid and protein metabolic disorder. The high Vitamin E, C, B_1, B2, niacin, beta-carotene

and Mg contents of the vegetables studied suggest that the vegetables could be very useful in the management of diabetes and other NCD_S.

5.1.4 Antinutrients, antiphysiological factors and food toxicants.

The traces of oxalate observed in the vegetables could be that the plant constituents were not yet fully established (Bamishaiye *et al.,* 2011). The phytates levels of the vegetables were below the safe limit (5.00mg/100g) (Munro and Bassir, 1969). The low levels of phytate and traces of oxalates in the vegetables are of interest because studies have shown that at lower doses the anti nutrients show photochemical beneficial effects. Phytates and oxalates at lower doses act as beneficial antioxidants. Phytic acid and oxalic acids in large amounts usually form insoluble salts with mineral elements such as zinc, calcium and iron to prevent their availability and utilization (Sariyan *et al*., 2010; Nnam and Onyeke, 2003). The low levels of phytate and oxalate would not interfer with the utilization and availability of the little concentration of calcium and iron present in the vegetables.

The tannin levels were higher than the safe level of tannins (0.15 - 0.20%) as recommended by Schiavono *et al*. (2007). However, Gibson (2007) suggested that traditional methods of food preparation could reduce certain anti nutrients and increase the nutritive value of vegetables. In line with this, proper methods of food preparation could reduce the tannin levels and boost the phytochemical properties of the vegetables. Willy (2003) observed that tannins are anti nutrients with antioxidant effects. They were traditionally considered antinutritional but it is now known that their beneficial or antinutritional properties depend upon their chemical structure and dosage. They act as cataion agents, preventing availability of certain nutrients and as well act as beneficial antioxidants. At lower levels they act as beneficial antioxidants while at higher levels they act as cataion agents, preventing availability of certain nutrients (Willy, 2003). Tannins form complexes with proteins, carbohydrates and certain metal ions (Nnam and Onyeke, 2003). The tannin- protein, tannic acid ï starch and tannin metal complexes are resistant to enzyme hydrolysis, thus inhibiting the digestibility

and absorption of nutrients (Nnam and Onyeke, 2003). The high levels of tannins in the vegetables are an indication that proper methods of preparation must be used to reduce the levels and prevent the tannin complexes that inhibit absorption of nutrients from the vegetables. Studies have also shown that tannins and saponins have the capability to lower serum cholesterol and fight cancers in low concentrations in the body (Whitney and Rolfes, 2005).

The low levels of hydrocyanides in the fresh vegetables (0,01mg ï 0.02mg/100g) are beneficial to health. This is because hydrocyanins produced from cyanogenic glycosides when consumed in large quantity over long periods may prove toxic. Simple food preparation methods would remove the little hydrocynins and leave the vegetables safe for human consumption.

Cadmium (0.03mg/g) and lead (0.1mg/g) levels of the vegetables were within the safe levels allowed by WHO standard for food substances (SAFS). Cadmium and lead are inorganic metals that are naturally present in the environment. Excess of Cadmium and lead in the body course diseases including hearth diseases. The low values obtained in the study were similar to the report of Radwan and Salami (2006) that fruits and vegetables collected from production and market sites in Nigeria contained measured heavy metals contents within the safe limits prescribed by WHO/FAO. Bokenga (1994) reported that leafy vegetables when processed and cooked are often free of food toxicants. This suggests that the small quantity of the metals in the vegetables in the study would be removed during preparation of the vegetables for food.

5.1.5 Phytochemicals

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties (Oguntona, 1986, Nnam, 2011). They are found generally in plants (Phytochemicals info). The results of the study showed that the phytochemicals (Table 4.5) were generally present in the vegetables in small quantities relative to the nutrients. The low levels of phytochemicals (Saponins 0.06-0.12,

flavonoids 0.01-0.04, alkaloids 0.03-0.21, glycosides 0.01-0.02, terpenes 0.09-0.21, phytosterols 0.09-0.16) per 100g) per 100g in the fresh vegetables were important and in line with literature reports that phytochemicals occur in small quantities in plant foods (Nnam, 2011). However the values of the phytochemicals present in the methanol extracts of the leaves (Saponins 2.05-3.73, flavonoids 0.09-0.29, alkaloids 4.91 - 6.77, glycosides 2.40-3.84, terpenes1.09-2.30, phytosterols1.26-2.50) per 100g (table 4.9) were higher than the values in the fresh leaves(Saponins 0.06-0.12, flavonoids 0.01-0.04, alkaloids 0.03-0.21, glycosides 0.01-0.02, terpenes 0.09-0.21, phytosterols 0.09-0.16) per 100g This might be due to the extracting solvent used (Tijani,Aluyu and Balogun,2009). The saponin levels of the vegetables (0.06 - 0.12mg/100g) were lower than 3% saponin value reported by Icumar (1991), which was responsible for cattle losses when they gazed on *Alfonibrilla*. Saponins contents of *Adansonia digitata* (0.12mg/100g) and *Cassia tora* (0.12mg/100g) leaves in the study were comparable to saponin value of *Vernonia amagdalina* (0.13mg/100g) reported by Nnam *et al*. (2012).

The phytosterols levels of the fresh vegetables (0.09 ï 0.16mg / 100g) were comparable to those reported by Nnam *et al*. (2012) for *0cimum gratissimum* (0.08mg/100g) and *Gnetum africanum* (0.12mg/100g). The methanol extracts of the leaves had higher levels of phytosterols (1..36mg, 2.39mg, 1.26mg, and 2.50mg/100g) than the fresh leaves (0.09mg, 0.12mg, 0.08mg, and 0.16mg/100g). The low levels of saponins and phytosterols in the fresh vegetables have the potential to lower cholesterol levels in humans due to their hypercholesteralmic effect (Nnam, 2011). Phytosterols are plant counterparts of cholesterol and thus inhibit its absorption. It lowers cholesterol level by blocking the uptake of cholesterol. The cholesterol is thus excreted from the body. This help to prevent heart diseases. Saponins form complexes with cholesterol to reduce plasma cholesterol levels. Whitney and Relfe (2005) reported that tannins and saponins have the capability to lower serum cholesterol and also fight cancer when their concentrations are low in the body. The low levels of saponins in the vegetables are of interest and will boast the phytochemical properties of the

vegetables. However saponins are bitter and could reduce the palatability of food when present in large amounts. Simple food preparation methods could reduce the bitterness in the vegetables.

The flavonoid levels of the fresh vegetables and their extracts (0.01mg, 0.04mg, 0.01mg 0.02mg/100g fresh vegetables and 0.09mg, 0.14mg, 0.23mg, 0.29mg/100g extracts) are comparable to those of *Vernonia amagdalina* (0.04mg/100g), *0cimum gratissimum*(0.08mg/100g), *Gongronoma latifolium*(0.14mg/100g) (Nnam et *al*., 2012). The presence of flavonoids in the vegetables especially the levels in *Adonsonia digitata* (0.04 mg/100g in the fresh vegetable), *Cassia tora* (0.29mg/100g in the extract) are desirable. Flavonoids have antioxidant properties to protect the body against cardiovascular diseases and some forms of cancer (Nnam, 2011). They protect the cells against the breakdown of arachidonic acid, an unsaturated fatty acid that keeps cell membrane healthy and permeable. Flavonoids lower high blood pressures as well as cholesterol in animal studies and have strong anti inflammatory properties (CSIRO, 2004).They also inhibit low density lipoprotein (LDL) oxidation by free radicals (Verena, Mario and Karl, 2006). Based on these reports consumption of the vegetables as constituents of human diet will be of health benefits.

The alkaloid levels of the extracts (4.91mg, 5.52mg, 6.45mg, and 6.77mg/100g) were much higher than the values in the fresh leaves (0.12mg, 0.21mg, 0.03mg, and 0.15mg/100g). The high values may be due to the process and solvent used for the extraction. However the alkaloid levels in the fresh leaves (0.12mg, 0.21mg, 0.03mg, and 0.15mg/100g) were lower than the values reported by Nnam et *al.* (2012) for some leafy vegetables such as *Vernonia amagdalina* (1.78mg/100g), *0cimum gratissimum* (0.95mg/100g). Bamishaiye *et al.* (2011) reported presence of alkaloids in methanol extract of leafy vegetable *Moringa oleirfera* but did not quantify the values. Alkaloids are nitrogen ï containing naturally occurring compounds. They are commonly found to have antimicrobial properties due to their ability to intercalate with DNA of the micro- organisms (Kasolo *et al*., 2010).This could be responsible for their much acclaimed medicinal values though the exact mode of

action is poorly understood. Stay (1998) reported that pure isolated alkaloids are used as basic The terpenes values in the fresh leaves (0.09mg, 0.09mg, 0.16mg, 0.21mg per 100gl) were comparable to values of 0.02 ï 0.38mg/100g of terpenoids reported by Nnam *et al*. (2012) for some leafy vegetables. Glycosides levels of the fresh vegetables were lower than those of the extract (2.07mg, 2.40mg, 3.60mg, 3.84mg /100g). Alkaloids, Terpenes and Glycosides present in the leaves are known to protect the body by decreasing the risk of heart diseases, stroke and certain types of cancers (Nnam 2011). *Cassia tora* leaves and extract contain more terpenes (0.21mg fresh, 2.30mg extract) than the other vegetables in the study. Terpenes seem to battle against cancers and heart diseases due to their antioxidant effect and their action to increase the activities of the enzyme that detoxify carcinogens (Nnam, 2011). Several studies have reported beneficial effects of a therapy with antioxidant phytochemicals against cardiovascular system consequence of diabetes (Ruhe, 2001, Dav, 2005, Vassort, 2010). Consumption of the vegetables with their concentrations of alkaloids, terpenes, and glycoside could be beneficial in the management of diabetes. Generally, the low levels of all the phytochemicals and antinutrients in all the vegetables suggest that consumption of the vegetables would help fight degenerative diseases. It is known that when anti nutrients medicinal agents because of their analgestic, antispasmodic and bacterial properties. The high concentration of alkaloids in the methanol extract (6.77mg/100g) supports the use of *Cassia tora* leaves as the most popular ingredient in Ayurvedic formulations (www.academia.edu/239988/Medicinal). This might be because of its high content of alkaloids which has been claimed as basic medicinal agent. The leaves of *Cassia tora* have been widely claimed to cure different diseases by rural and traditional practitioners of Septura region of Madhya Pradesh (Mishara, 2010). Based on these facts consumption of the vegetables especially *Cassia tora* with the high content of alkaloids would be of great importance in the management of different diseases as an analgestic, antispasmodic and antibiotic.

are present in low levels, the efficiency of phytochemiclas to fight various diseases and high levels of serum cholesterol inimical to health are higher.

5.1.6 Blood glucose

The results showed that the blood glucose levels of all the groups of rats were normal on day 3 and ranged from 62.60 to 83. 60mg/dl (Table 4.11). Blood glucose levels of 130mg/dl and above is considered diabetic for rats (Etuk, 2010). The blood glucose levels of all groups of rats were high on day 6 ranging from 327.40mg/dl to 573.60mg/dl. This shows that the alloxan drug administered to the rats induced diabetes. This is in line with the assertion that alloxan is a toxic analogue which selectively destroys insulin producing cells in the pancreas when administered to rodents and many other animal species producing an insulin dependent diabetes mellitus (called alloxan Diabetes) in the animals with characteristics similar to type I diabetes in human (Lenzen, 2008). Alloxan induces irreversible diabetes mellitus after 24hrs following its administration and the condition proves to be chronic by laboratory tests after seven days of its administration if not treated (Ahren, 1995). Thus, these animals have surviving ϐ- cells and regeneration is possible ((Prince and Menon, 2000). The use of lower dose of alloxan (150mg/kg b.w.) as in this study produced a partial destruction of pancreatic ϐ- cells even though the animals will become permanently diabetic if not treated (Prince and Menon, 2000). This must have been the reason for the increase in the blood glucose level of all groups of rats in the study on day 6.

The results showed that the blood glucose level of the group of rats fed rat chow and standard drug (group2) had a significant decrease in blood glucose (17.23%) on day 12. The findings are in line with literature report that glibenclamide is a standard drug for reducing blood sugar level in animals. Glibenclamide drug reduces blood glucose by a pancreatotrophic action. It acts by stimulating the ϐ-cells of islets of Langerhans to release more insulin.

The group of rats fed 500mg (Gp2) of *Hibiscus cannabinus* leaf extract had significant decrease (32.38%) in blood sugar level when compared with the group that was administered the standard drug (Gp1) (17.23%). The group of rats fed 1000mg (Gp 6) of *Hibiscus cannabinus* leaf extract showed decrease of blood glucose level of 31.61% which was higher than the decrease in blood glucose level of the group on standard drug (17.23%) ($p>0.05$). The rats fed the different concentrations of *Hibiscus cannabinus* leaf extracts had higher decrease in blood glucose level than those administered the standard drug. Those fed the 500mg of the extract had higher decrease (32.38%) than those fed 1000mg (31.61%). This shows that the decrease in blood sugar level with *Hibiscus cannabinus* leaf extract is not dose dependent. The significant decrease in blood glucose level in the groups that consumed *Hibiscus cannabinus* leaf extract is very important. The result is in line with several studies that reported on the hypoglycemic activities of *Hibiscus cannabinus* leaf extract (Eromosele, 1993; Ijomah *et al*., 2000. Agbo *et al*., 2005; Falusi, (2009); Sundarrayanan *et al*. (2011) reported that the methanoic extract of *Hibiscus cannabinus* leaf exhibited hypoglycemic activity in streptozotocin induced diabetic rats. The hypoglycemic activity of *Hibiscus cannabinus* could be linked to the moderate amount of some phytochemicals like alkaloids, tannins, saponins and flavonoids which have medicinal properties (Sundarrayanan *et al*., 2011). However high concentrations of the phytochemicals are toxic and may impair body metabolism. This might be the reason why the group that consumed the higher dose (1000mg/kg B.W.) had lower decrease in blood glucose level in the study showing defect in body metabolism.

The group of rats fed 500mg of *Adansonia digitata* leaf extract (Gp 3) showed increase in blood sugar level of 18.38%. The group fed 1000mg of *Adansonia digitata* leaf extract (Gp7) showed decrease of 33.67% on day 12. This suggests that the effect of *Adansonia digitata* leaf extract on blood glucose level is dose dependent. The decrease in group 7 (rats fed 1000mg of *Adansonia digitata* leaf extract) (33.63%) was higher than the decrease in the group administered the standard drug (17.23%) . The

group of rats that consumed 1000mg of *Adansonia digitata* leaf extract had the highest decrease (33.36%) ($p<0.05$) in blood sugar level among all the groups including the group of rats fed the standard drug. This is not surprising because of the high content of nutrients and antioxidant phytochemicals present in the leaf when compared with other vegetables in the study. The nutrients and antioxidant phytochemicals present in the leaf must have worked synergistically to reduce the blood glucose level. Several studies have reported that *Adansonia digitata* leaf could be useful in the management of metabolic diseases and treatment of diabetes (Kamli and Khalya 1999; Tanko, Yerima, Mahde & Mohammed, 2008; Offia *et al*. ,2011). In particular Kamli and Khalya (1999) in their compilation of Sudanese natural medicines noted that *Adansonia digitata* young leaves eaten as vegetables is important in treatment of diabetes. The leaf, bark and fruits of *Adansonia digtata* are traditionally employed in several African regions as food stuffs and for medicinal purposes (Tanko *et al.,* 2008). Offia *et al*. (2011) reported that *Adansonia digitaita* leaf is important in the management of metabolic diseases in Plateau State of Nigeria during the compilation of a survey of medicinal plants in Plateau state in Nigeria.

The group of rats fed 500mg of *Sasemum indicum* leaf extract (Gp4) had increase in blood sugar from 336.60mg/dl on day 6 to 363.80mg on day 12. This was a total of 8.08% increase in blood glucose level. However the group fed 1000mg (Gp 8) of *Sasemum indicum* leaf extract had decrease from 552.00mg on day 6 to 547.00mg on day 12. This was a total decrease of 9.06% in blood sugar level. This suggests that the 1000mg dosage was more effective in decreasing the blood sugar level indicating that the effect could be said to be dose dependent. The decrease observed for the rats fed 1000mg (Gp 8) of *Sasemum indicum* leaf extract (9.06%) was however the least among the groups fed 1000mg dosage of all the vegetables in the study (*Hibiscus cannabinus* 31.61%, *Adansonia digitata* 33.63%, *Cassia tora* 23.92%). This result is not surprising because of the low levels of most nutrients and antioxidant phytochemicals present in *Sasemum indicum* leaf extract. This might

explain why the reduction on blood sugar level was low especially at a lower dose of 500mg and could not reduce the blood sugar level in the diabetic rats. Shittu *et a*l. (2009) reported that sesame leaves are popularly used in folk medicine in the southern Nigeria to treat diabetes but the quantity was not identified.

The group of rats fed graded doses (500mg and 1000mg) of *Cassia tora* leaf extract showed decreases of 7.83 and 23.92%, respectively. There is a difference between the decreases in blood sugar level of rats fed 1000mg of *Cassia tora* leaf extract (23.92%) and the group fed standard drug (17.23%) and also the group fed 500mg *Cassia tora* leaf extract(8.08%).This shows that the decrease in blood sugar level by *Cassia tora leaf* extract is dose dependent. The positive effect on blood sugar levels might be due to high concentration of antioxidant phytochemicals such as terpenes (2.30mg), phytosterols (2.50mg), alkaloids (6.77mg) present in the *Cassia tora* leaf extract (Table 4.10). Mishara (2010) reported that *Cassia tora* leaf extract is useful as digestion and metabolic corrective substance, as a tonic and posses hyperglycemic actions.

5.1.7 Total Cholesterol Level (TC).

The result of the study showed high total cholesterol levels in all the groups of rats on day 6. Diabetic rats have been shown to have increased plasma serum lipids which are responsible for several cardiovascular disorders (Alarcon-Agular *et al.,* 2002). The increased total cholesterol levels may be due to increased mobilization of free fatty acids from peripheral depot. It may also be due to lipolysis caused by glucagon (El-soud *et al.,* 2007).

The result of the study showed decreases in total cholesterol levels in both the control and the other groups (Table 4.12) on day 12. This is not surprising because the use of alloxan usually produce partial destruction of pancreatic cells even through the animals may become permanently diabetic if not treated (Prince and Menon, 2000). Regeneration is possible because the animals have surviving

beta cells (Aybes *et al.*, 2001). The rats must have worked on this principle to have reduction in cholesterol level in all groups as shown in the study.

The higher decrease by the rats fed 500mg of *Hibiscus cannabinus* leaf extract(Gp 2) (17.31%) than for those fed 1000mg (Group 6) (9.81%) shows that the decrease in TC by *Hibiscus cannabinus* leaf extract is not dose dependent. The better effect shown by the group that consumed lower dose suggests that the phytochemicals which were abundant in *Hibiscus cannabinus* leaf extract works better in lower doses.

The group of rats that consumed 1000mg *Adansonia, digitata* leaf extract had higher reduction in TC (26.92%) than the group that consumed 500mg (18.75%) suggesting that the effect is dose dependent. The higher the dose the higher the constituents consumed and the more the effect on TC levels. Rats fed 1000mg *Adansonia digitata* leaf extract (Gp7) showed the highest reduction in TC levels among all other group of rats fed the vegetables extract. This is not surprising because *Adansonia digitata* leaf and leaf extracts contained more beta carotene, vitamin C, B_1, B_2 niacin and flavonoid. These constituents are known for their hypercholesterolemia effects which must have translated in the reduction of TC for the rats.

The observed decreases for the group of rats fed the 500mg and 1000mg *Sesamum indicum* could be due to the nutrients and photochemicals especially high flavonoid (0.23mg/100g) found in the leaf extracts. The decrease in TC of rats fed 1000mg *Cassia tora* leaf extract was higher than for all other groups except the group fed 1000mg of *Adansonia digitata* leaf extract. The result is not surprising because *Adansonia digitata* leaf extract *and Cassia* tora leaf extract had very high concentration of most nutrients and phytochemicals (Tables 4.6, 4.7, 4.8 and 4.10) than other vegetables in the study. Barmins, Charles and Emmanuel 1998 reported that consumption of cooked leaves or use of leaf extract of *cassia tora* plant helps the body in maintaining the normal level of cholesterol. Mishara

(2010) confirmed that the leaf extract of *Cassia tora* plant has hyperglycemic actions. Cho *et al*. (2009) in their study on the effect of *Cassia tora* fiber supplement on serum lipids in Korean diabetic patients concluded that *Cassia tora* leaf fiber supplement can help improve serum lipid status in type 2 diabetic patients without any serious adverse side effects. The high fiber content in the *Cassia tora* leaf extract in this study and the high decrease in total cholesterol seems to support the reports.

5.1.8 Triglyceride levels (TG)

The higher decrease (47.83%) of TG for the rats fed 500mg of *Hibiscus cannabinus* leaf extract than those fed 1000mg of the extract confirms that the effect on the lipid levels of the rats by *Hibiscus cannabinus* leaf extract is not dose dependant. The effect of *Sesamun indicuim* leaf extract on TG levels might be connected to the high content of flavonoids (0.23mg), terpenes (1.43mg) and niacin (7.00mg) in the leaf extract. Shittu, Ajai and Benson (2008) reported that *Sesamum* leaf is very important in Nigeria because of its nutritive nature and its health benefits. The results of this study seem to support the statement.

The effect of *Cassia tora* leaf extracts on the rats is dose dependant as those on 1000mg had higher ($p < 0.05$) decreases (67.70%) than those on 500mg (50.94%). The groups that consumed *Cassia tora* leaf extracts had the highest decreases in TG levels than the groups that consumed all other leaf extracts. This might be because the *Cassia tora* leaf extract contained higher quantity of the nutrients and phytochemicals (flavonoids, alkaloids, glucosides, terpenes, phytesterols and tannins) (Tables 4.8, 4.10) than all other vegetables in the study. The phytochemicals have been shown to improve serum lipid status of rats (Cho *et al.,* 2009).

5.1.9 High density lipoproteins (HDL)

The effect of *Hibiscus cannabinus* leaf extract on HDL level is dose dependant as the group of rats fed 1000mg *Hibiscus cannabinus* extract had higher increase in HDL than those fed 500mg of the extract. The increase in HDL level of GP6 fed 1000mg *Hibiscus cannabinus* is significantly higher than the increase in the rats administered starndard drug. This suggests that the high content of nutrients and phytochemicals present in the *Hibiscus cannabinus* leaf extract (Tables 4.8, 4.10) must have worked in synergy to bring the high effect. This result supports the work of Sundarayanan *et al*. (2011) who reported that methanoic leaf extract of *Hibiscus cannabinus* showed presence of phytosterol, flavonoids and glycosides and that the methanoic extract of *Hibiscus cannabinus* leaf exhibited hypoglycemic activity in streptozotocin induced diabetic rats. The increase in HDL level by *Hibiscus cannabinus* leaf extract (30.77%) is significantly higher than the increase in all other groups fed various doses of the other vegetable extracts. This might be connected with the high content of vitamin C in *Hibiscus cannabinus* leaf extract (26.33mg/100g) more than other vegetables in the study (Table 4.8). Vitamin C is a strong antioxidant. Padayatty *et al.* (2003) reported in their work that vitamin C may help decrease total cholesterol as well as increase HDL levels in rats.

The effect of *Adansonia digitata* leaf extract on HDL level of the rats is not dose dependent as the group of rats fed 500mg of the extract showed increases (24.01%) that are higher than the group fed 1000mg of the extract (4.35%). This might be because of the high nutrient and antioxidant phytochemical content of *Adansonia digitata* leaf extract (Table 4.8), which makes it to be very potent at a low dose. The results also support the assertion that phytochemicals works better in low doses.

The effect by *Sesamum indicum* leaf extract on HDL of the rats is highly dose dependent because the rats fed 1000mg extract had higher increase (28.01%) than the groups fed 500mg (19.23%) of the

extract. This might be because the nutrient and phytochemical content of *Sesamum indicum* leaf in the study is moderate and as such was effective when the dose is high.

The effect of *Cassia tora* leaf extract on HDL is not dose dependent as the group fed 500mg of *Cassia tora* leaf extract had higher increase (24.01%) than those fed 1000mg (20.83%). This result might be due to the high content of tannins (7.07mg/100g and phytate (178mg/100g) (Table 4.8) in the leaf extract. These constituents are usually beneficial as antioxidant phytochemicals at lower doses but demonstrate antinutritional properties at higher doses. This might explain why *Cassia tora* leaf extract did not display high phytochemical effects as is shown on the effect of its high doses on the lipid profile of the rats in the study. Tannins act as beneficial antioxidants at lower doses. The tannins and phytate in the leaf extract might have worked better at the lower dose as antioxidant phytochemicals with other nutrients to bring the effect.

5.1.10 Low density lipoproteins (LDL).

The effect of *Hibiscus cannabinus* leaf extract on LDL is not dose dependent as the group fed 1000mg of the extract had lower decrease (31.49%) than the group fed 500mg (36.79%). The effect might be due to the high antioxidant phytochemicals (saponins, tannins and vitamin C) and other nutrients present in the leaf extract. The constituents must have worked in synergy to reduce the LDL levels at the low dose.

The effect of *Adansonia digitata leaf* extract on LDL levels of the rats is not dose dependent as the rats fed 500mg of the extract had higher decrease (43.51%) than the group fed 1000mg (38.53%) of the extract. The lower dose offered better effect even above the standard drug. This might be connected with good combination of antioxidant minerals (potassium and magnesium), vitamins (beta carotene, vitamin C, E and niacin) and phytochemicals (Tables 4.7, 4.8, 4.10) found in the methanol

extract of *Adansonia digitata* leaf. These constituents must have worked in synergy to reduce the LDL levels of the rats.

The effect of *Sesamum indicum* leaf extract on LDL levels of the rats is dose dependent as the group fed 1000mg of the extract had higher decrease (40.18%) than those fed 500mg (32.52%) of the extract. The result might be connected with the moderate levels of nutrients and phytochemicals present in the leaf extract which became effective as the dose and time increased (Table 4.15). The effect of Cassia *tora* leaf extract on LDL levels of the rats is dose dependent as the group of rats fed 1000mg of the extract had higher decrease (41.44%) than those fed 500mg (22.64%) . This might be connected with the good content of phytosterols, flavonoids and terpenes in the leaf extract. These constituents are known to protect the body by decreasing the risk of heart diseases, stroke and certain types of cancers (Nnam, 2011). The vegetable extracts of *Hibiscus cannabinus, Adansonia, digitata, Sasamum indicum and Cassia tora* leaves produced significant beneficial effects in the lipid profile in the treated diabetic rats that consumed rat chow and various vegetable extracts ($p > 0.5$). The vegetable extracts reduced total cholesterol, triglyceride and LDL and increased HDL of the rats.

5.2 Conclusion

Hibiscus cannabinus, Adonsonia, digitata, Sesamum indicum and Cassia tora leaves have much potentials for human food and for management of diabetes mellitus. The vegetables contained high levels of antidioxidant vitamin, minerals and phytochemicals that could be useful in the management of diabetes and other non communicable diseases. The antinutrient and toxicant levels of the vegetables were within the safe limits prescribed by WHO/FAO. The methanol extracts of *Hibiscus cannabinus, Adonsonia, digitata, Sesamum indicum and Cassia tora* leaves in various doses reduced blood glucose concentrations and improved lipid profile in alloxan induced diabetic rats. The

vegetables could therefore be used in preparation of diets for management of diabetes and some other diet related non ï communicable diseases because of their rich nutrient and antioxidant phytochemical constituents.

5.3 Recommendations

The following Recommendations were made based on the results of the study:

1 The results of this work would be useful to both nutritionists and dietitians to counsel individuals on the efficacy of the vegetables and their extracts for managing diabetes.

2 Human studies are needed to confirm the results obtained in the study.

3 Doses of 500 and 1000mg/kgbody weight of *Hibiscus cannabinus, Adonsonia, digitata, and Cassia tora*leaf extracts need further investigation with humans.

4 Studies to determine the portion sizes of the vegetables to maintain desired effects on humans are strongly recommended.

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Acta Cirurgica Brasileira .On-line version ISSN 1678-2674

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APPENDIX: PICTURES

100g Hibiscus cannabinus

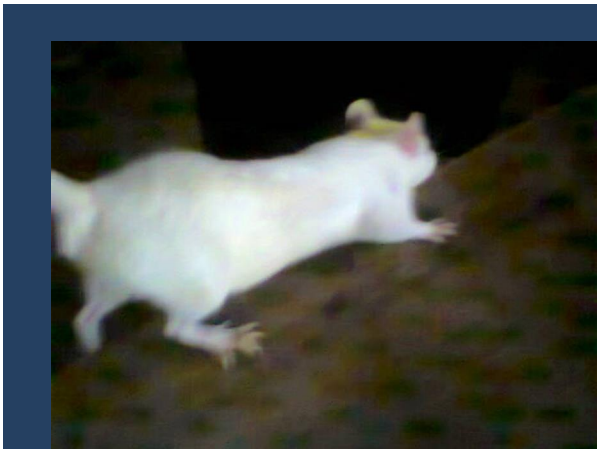
100g Adansonia digitata

100g Sesamum indicum

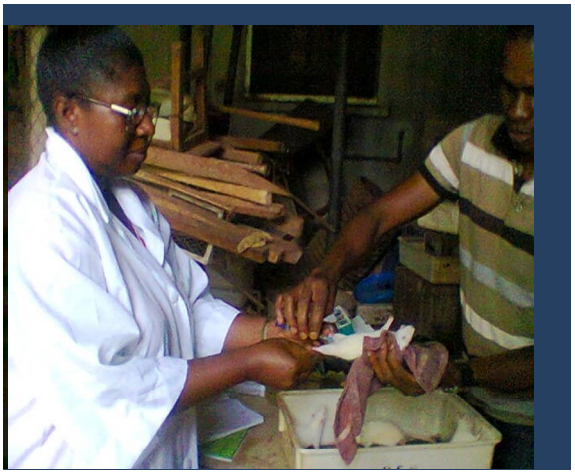
100g Cassia tora

Bags of the vegetables used n the nutrient analyis

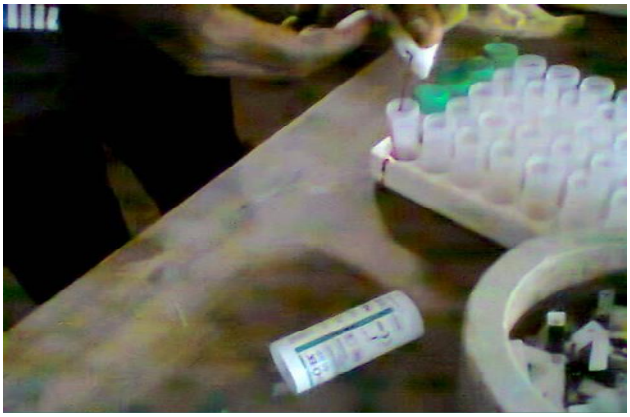
The researcher anaylizing the samples



Healthy abino rat used in the research



The researcher inducing diabetes on a rat



Collection of blood samples from the rat