DEVELOPMENT OF A FOOD SUPPLEMENT FOR DIABETES TYPE II MANAGEMENT WITH ENHANCED ANTIDIABETIC AND ANTIOXIDANT PROPERTIES FROM SELECTED LOCAL FOODS

HENGAME YOUSEFIFAR (B.Sc. Nutrition Science)

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Signed: _____ Date: _____

Hengame Yousefifar A56/82477/2015

Department of Food Science, Nutrition and Technology

This dissertation has been submitted with our approval as university supervisors:

Signed: _____ Date: _____

Dr. Catherine N. Kunyanga (PhD)

Department of Food Science, Nutrition and Technology

University of Nairobi

Signed: _____ Date: _____

Prof. Jasper K. Imungi (PhD)

Department of Food Science, Nutrition and Technology

University of Nairobi

UNIVERSITY OF NAIROBI

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It's my pleasure to inform you that, after the peer review, your paper,

Antidiabetic and Antioxidant Properties of Methanolic Extract for a Diabetic Supplement Developed from Drumsticks Leaves (Moringa oleifera), Garlic (Allium sativum) and Cinnamon (Cinnamomum verum) Powders

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Dedication

This dissertation is dedicated to my family for their support, care and encouragement throughout the entire study period.

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God bless you all

Contents

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Abbreviations and acronyms

AACE	American Association of Clinical Endocrinologists
ADA	American Diabetes Association
AFR	Africa
AGI	Alpha glucosidase inhibitor
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
BG	Blood Glucose
CBR	Case-Based reasoning
CHO	Carbohydrate
DM	Diabetes Mellitus
DPPH	1, 1- diphenyl-2-picryl hydrazyl
DSME	Diabetes self-management education
FAO	Food and Agriculture Organization of the United Nations
ICU	Intensive Care Unit
IDF	International Diabetes Federation
NCDs	Non communicable diseases
PDP	Principal display panel
PGPP	P-nitrophenyl-α-D-glucopyranoside
PUFA	Polyunsaturated fatty acid
RDA	Recommended Dietary Allowance
SD	Standard deviation
SPSS	Statistical Package for the Social Sciences
UON	University of Nairobi
USDA	United States Department of Agriculture
WHO	World Health Organization

General abstract

Diabetes is one of the chronic diseases that is growing in the world with a high prevalence and shocking rate particularly in countries with low and middle income. The disease is directly associated with nutrition and the type of diet consumed. Dietary management of the disease has been shown to reduce the incidence of diabetes. This study was designed to develop an antidiabetic supplement from low cost and easily accessible food ingredients for dietary management of type II diabetes. The food supplement formulation contains three main ingredients including Drumsticks leaves (*Moringa oleifera*), Cinnamon (*Cinnamomum verum)* and Garlic (*Allium sativum*). Nutrisurvey software was used for linear programming in order to formulate and determine the high nutritional quality and low calorie levels of the supplements. The formulated supplements were analyzed for chemical composition and nutritional quality using standard methods. Antidiabetic and antioxidant activity, were determined using α-amylase and α-glucosidase inhibition activity and DPPH (1, 1- diphenyl-2-picryl hydrazyl) assay. Sensory analysis was conducted using 12 panelists to evaluate the product's characteristics and acceptability for color, flavor, odor and overall appearance. The developed supplements were also analyzed for stability using accelerated shelf life studies for 6 months. The final cost of products was calculated in Ksh/grams. Data collected was analyzed statistically using SPSS software. Results showed that the supplements had high nutritional quality and low calorie content providing 14 to 16% RDA in 100g, low sodium from 6 to 9%, 29 to 42% protein, about 12% carbohydrate, 9 to 13% fat, 31% dietary fiber. At the same time, they were rich sources of vitamins such as 189 to >300% Vitamin A, 113 to 185% Vitamin B1, >300% Vitamin B2, 69 to 109% total folic acid and 90 to 139% Vitamin C. Meanwhile, all supplements had acceptable levels of minerals such as potassium 28 to 38%, calcium 121 to 162%, magnesium 74 to 111%,

phosphorous 28 to 37%, iron 161 to 270% and zinc 19 to 30% according to RDA for target groups. The methanolic extracts of supplements showed 95 to 99% α-amylase inhibitory activity with the highest percentage of 99% in supplement no.3 (70% drumstick leaves, 25% garlic and 25% cinnamon). Supplements showed 15 to 29% α-glucosidase inhibitory activity with no.3 exhibiting 29% as the highest activity. The antioxidant activity of the supplements ranged from 66 to 81% in different concentration and supplement no.2 (80% drumstick leaves, 10% garlic and 10% cinnamon) was the highest antioxidant with 81% scavenging activity in 30µg/ml concentration. In ethanolic extracts, the alpha-amylase inhibition activity results ranged between 45 to 54% and supplements no.1 (90% drumstick leaves, 5% cinnamon and 5% garlic) with 54% inhibition showed the highest activity. Supplement no.4 (50% drumstick leaves, 25% cinnamon and 25% garlic) with 48% enzyme inhibitory activity showed the highest result while the range was between 36 to 48%. Antioxidant activity percentage of supplements was ranged between 58 to 67% in different concentrations and supplement no.3 (70% drumstick leaves, 15% cinnamon and 15% garlic) in 20µg/ml with 67% activity indicated the highest radical scavenging activity. The sensory evaluation findings indicated that the most accepted supplement was no.1 (90% drumstick leaves, 5% cinnamon and 5% garlic). The supplement was stable for 12 months according to the shelf life studies. The cost analysis showed that the most cost-effective supplement powder is no.4 (50% drumstick leaves, 25% cinnamon and 25% garlic can be sold at 142Ksh/100g. These food supplements are low calorie and natural. They are available locally and affordable for middle and low income diabetic patients. At the same time, the easy usage of the indigenous powder as a functional food can play an effective roll to improve the life quality and nutritional status of diabetic patients. Additionally, they are highly antioxidant and antidiabetic with great health benefits.

CHAPTER ONE: INTRODUCTION

1.1. Background of the study

The main characteristic of Diabetes type II is high blood sugar levels. It is a non-communicable and metabolic disorder which can increase the risk of onset of life-threatening diseases. It can decrease the patient's life quality, increase the mortality and morbidity rate (Baena-Dı´ez *et al.*, 2016).

108 million individuals were estimated by WHO (World Health Organization) to be suffering from diabetes in 1980. This number was quadruplicated in 2014 (NCD, 2016). It is predicted that this high prevalence reaches to over 592 million in year 2035 (WHO, 2016). Diabetes influences 8.3% of adult individual's lives all over the world which is rising rapidly (IDF, 2013). The most probable interpretation for diabetes increment rate is lifestyle changes to more sedentary life, lower physical activity, and urbanization as well as changes in socioeconomic conditions in communities (Cho *et al*., 2018).

In case of poor BG (Blood Glucose) control, insulin resistance of body tissues and pancreatic β-cells dysfunction occurs. These are the major characteristics of Type II diabetes (Butler *et al.*, 2003; Ashcroft and Rorsman, 2012; Quan *et al.*, 2015). In the event of insulin resistance and β-cells dysfunction, the body fuel equilibrium changes in response to this overflow of nutrients (Nolan *et al.*, 2011). The continuous high levels of blood glucose can cause serious damages to vital body organs. Consequently, affects kidneys, nerves and eyes and limb amputation because of infection. It can result in vascular damage which seriously affects heart and ends up with heart attack, stroke and death (WHO, 2009; WHO, 2016). About 90% of diabetic patients are overweight and have a sedentary lifestyle. This increases the necessity of the body to insulin. Pandemic outbreak of diabetes is rising quickly in many territories especially in developing countries with low and middle income. In 2012, more than 80% of mortality rate that is 1.5 million cases took place in developing

countries due to diabetes type II. Diabetes is forecasted to be the seventh most leading reason for human death by the year 2030 (WHO, 2016).

The disease is more common among men rather than women while more women are suffering from diabetes than men. It is estimated that a twofold urbanization increase will take place in low and middle income countries in 30 years from 2000 to 2030. The considerable alteration in disease prevalence may develop among people under 65 globally (Wild, 2004). In 2015, 415 million individuals had diabetes in the universe and 75% of them used to live in developing countries while 8.8% of them were between the ages of 20 to 79 years. With the current trend continuation, 642 million cases will be stricken in 2040 that is 1 person in 10. This increment of diabetes will happen in areas with more economic development (IDF, 2015; Axelsson, 2017). Formerly diabetes was found rarely in Africa continent whilst the situation is worsening at the present time. The disease prevalence among the adults with 20 to 79 years of age is about 4.9%. The majority of them are below the age of 60. The highest proportion of 43.2% is the diabetics at the age of 40 to 59 years old. The statistics are predicted to increase to 41.5 million cases in year 2035 from 19.8 million patients in 2013. This is a 110% increment of the rapidly growing rate of diabetes type II. Evidently there is an association between the disease growing rate and economic development in Africa. The diabetes rate among regions with lower income is 4.4%. The rate rises to 5% in lower-middle income countries and 7% prevalence rate of diabetes in upper-middle income regions is observed (Guariguata *et al.*, 2014; IDF, 2015). In 2013, 7.3% of the people at the age of 20 to 79 years, with high risk factors to develop the disease were the patients with impaired glucose tolerance. In Africa continent, over 500,000 individuals were died due to diabetes in 2013. About 75% of the death cases for diabetes were under the age of 60 years. Unfortunately, over 50% of diabetes prevalence is undiagnosed with over 75% cases in low-income regions. The prevalence of the disease decreases to 46% in countries with upper-middle income (Peer *et al*., 2014). Kenya is a member of the IDF

(International Diabetes Federation) Africa region countries. It was estimated that Kenya has 478,000 diabetic patients in 2015 and 342,000 death cases due to diabetes. More than 51% of death cases ascribed to diabetes were below 60 years old in Africa region. The outcome of the combination of a few factors is under 60 year death. Factors such as translocation of the North African and Middle East countries into a more advanced environment and lifestyle without having sufficiently equipped health system. Consequently, the health system is not able to well manage the high rising rate of diabetic patients and retarded disease diagnosis (IDF, 2015).

The ingredients studied in the present research such as cinnamon and garlic are known as effective local foods in preventing and managing type II diabetes (Alkhatib *et al*., 2017). Drumstick leaves containing significantly high levels of total phenolics are demonstrated to exhibit potential antidiabetic and antioxidant properties (Kunyanga *et al*., 2012).

1.2. Statement of the problem

During the past decades, the incidence and prevalence of diabetes has constantly been on the rise. According to WHO in 2016, the prevalence rate of diabetes in middle and low income countries such as Kenya has shown a faster increment than the high income populations during the last 30 years. One of the major causes of premature death in almost all the countries is diabetes and its resulting complications due to uncontrolled blood sugar levels. IDF (2018) has estimated that the total economic costs of diagnosed cases of diabetes has raised from 245 billion dollars in 2012 to 327 billion dollars in 2017, with a 26% increment. More than 12% of health expenditure is allocated to diabetes treatment in all over the world. At the same time, 75% diabetic cases live in low and middle income countries. The main limiting factors for the majority of people in Kenya are the low rate coverage of health insurance and the heavy expenses of treatment. Due to the lack of sustainable dietary solutions for diabetic patients, efficient disease management is not easily

feasible. The study results offers a solution to a sustainable diabetes management for its culturally acceptability, accessibility, affordability and nutritionally adequacy while at the same time it is safe, healthy and natural. Furthermore, it can play a key role to improve the utilization of foods with high antidiabetic and antioxidant properties.

In less developed countries, the fundament of diverse nutrition in traditional food systems is food sources that are indigenously cultivated and traditionally consumed. Indigenous foods are rich in nutrients. They are usually consumed in small quantities comparing with the famed and popular foods that are made and utilized globally (Konuma, 2014). Foods which are locally available and low cost have the potential to effectively manage diabetes Type II when food intervention is necessary.

1.3. Justification of the study

Diabetes mellitus is highly associated with nutrition. The patient's diet can effectively retard and prevent the consequences of the unmanaged disease. One of the foremost diabetes mortality reasons are cardiovascular diseases; which is the main cause of over 50% of deaths among some nations (Esposito *et al*., 2017). The actual mortality rate due to diabetes, is hard to estimate because more than $1/3$ of the countries have no data sources at all. The other limiting factor is underestimation of death cases due to diabetes among nations that have data sources (McEwen, 2011).

The present treatments are capable of decreasing diabetes progress. The common microvascular complications due to high blood glucose levels are retinopathy, neuropathy, nephropathy and limb amputation. The principal risk factor of heart attack and stroke is the kidneys failure. This happens when the function of kidneys is weakened and the glomerular filtration shows a lower rate (Forbes and Cooper, 2013).

Metformin, Orlistat and Acarbose are antidiabetic medications. In addition, these drugs have shown preventive activities similar to nutraceuticals possessing the same possible attributes. Acarbose reduces the digestion and absorption pace of CHOs (Carbohydrates). Its action in an imitation of the natural factors in the same activities (McCarty, 2005). Immediate diversification of food basis and species of the crops to make a wider range and a more flexible system is inevitable. This coercion is because of unpredictability of the climate conditions. Traditional crops and indigenous foods production helps to preserve the environment as well as its further cultural and social acceptance. Various local foods are available together with traditionally acceptancy which can help to make a successful alteration in a well-organized food system. Documentation, recording and collecting the science on indigenous foods is crucially important. This knowledge contains all the specifications of locally available foods and agricultural science. The underutilized foods and traditional beliefs have to be included as well.

Giving prominence to the important role of the underutilized foods in their production areas at this time helps to ameliorate the consumption quantity. This will provide more opportunity for further crop production, too. This knowledge helps to further development of foods and also increases the population awareness (Konuma, 2014). Diabetes management with chemical medicines causes several complications. Consumption of natural foods and plants for diabetes treatment and prevention was common since before. They cause almost no side effects. The advantage of plant medicines and herbal treatment is the cost-effectiveness and affordable prices in comparison with the chemical drugs.

There exists a large variety of plants with preventive diabetes treatment properties. Many researches have been performed to determine the antidiabetic properties of different plants for the above mentioned reasons (Maurya, 2009).

1.4. Aim of the study

This study aims to contribute towards diabetes type II management and the complications associated with the disease.

1.5. Purpose of the study

The purpose of the study was to contribute towards reduction of long term hyperglycemia complications among diabetic patients through a food based intervention.

1.6. Objectives of the study

1.6.1. Main objectives

Development of a low-calorie food supplement with antidiabetic properties for diabetes type II management from indigenous and locally available ingredients.

1.6.2. Specific Objectives

1. To determine the nutritional quality and chemical composition of the food ingredients and developed supplements.

2. To formulate a low calorie supplement powder with antidiabetic and antioxidant activity from drumstick leaves, garlic and cinnamon.

3. To determine the antidiabetic activity of the food ingredients and formulated supplements by in-vitro methods.

4. To determinate the antioxidant activity of the food ingredients the formulated supplements.

5. To determinate the shelf life stability of the developed supplement.

6. To evaluate the acceptability of the formulated supplements.

1.7. Study Hypothesis

1. The food ingredients and formulated supplements have high nutritional quality and rich chemical composition.

2. The food ingredients and supplements are low calorie with high antidiabetic and antioxidant properties.

3. The food ingredients and formulated supplements have high antidiabetic properties.

4. The food ingredients and formulated supplements have high antioxidant properties.

5. The products have a long shelf life.

6. The formulated supplements are accepted by the test panelists.

1.8. Dissertation layout

Chapter 1 describes the background of the study and states the problem. It justifies the study of diabetes type II and determines the aim, purpose, main and specific objectives as well as the study hypothesis.

In chapter 2, the literature review, the global review of diabetes and the disease prevalence in Kenya, Africa and the world were referred to as well as the costs of the disease. Diabetes management consisting of the hospitalized patients, pharmacologic therapy, nutrition therapy and dietary management were mentioned. Health promoting properties of the ingredients (Drumstick leaves, garlic and cinnamon were reviewed). The knowledge gap was discussed in this chapter.

In chapter 3, the methodology in order to develop the low calorie and antidiabetic supplement powder from the ingredients was described and the nutrient content of the products was optimized by Nutrisurvey Software. Proximate analysis method was used to determine the moisture, lipid,

fibre, ash, protein, carbohydrates and energy content of the ingredients. Sensory evaluation of the food supplements was performed among 24 panelists. Each ingredient and product was cost estimated and the most cost effective supplement was determined. The shelf life of the products was determined. Packaging and labelling of the final product according to the market standards were mentioned at the end of this chapter.

In chapter 4, antidiabetic and antioxidant properties of the methanolic extract for a diabetic supplement from the above mentioned ingredients is explained. Sample collection, preparation and formulation of the supplements is shown in the process flow diagram for the product development. The methanolic extraction method is explained. The antidiabetic effect of the ingredients and the products was evaluated by alpha-amylase and alpha-glucosidase inhibition activity assays. Antioxidant activity of the ingredients and products was determined as well, using DPPH radical scavenging activity method, and the results were discussed.

In chapter 5, antidiabetic and antioxidant properties of the ethanolic extract were assessed using the methods mentioned in chapter 4, and the results were discussed.

Chapter 6 includes the general conclusions and recommendations.

CHAPTER TWO: LITERATURE REVIEW

2.1. Global Overview of Diabetes

Diabetes Type II is a set of metabolic disorders which is specified by high blood sugar levels. The unusual high glycemic level is the consequence of insulin hormone secretion deficiency, its function or both reasons. The persistent abnormal blood glucose will result in body tissues damage. This will cause dysfunction of the body organs and several body organs failure in turn such as blood vessels, heart, kidneys, eyes and nerves. A number of pathogenic procedures entail on disease development. The process consists of autoimmune pancreatic beta-cells damage which results in insufficient insulin to abnormalities which ends up in insulin function resistance (Axelsson, 2017). The principal reason of malformation in diabetic patients in metabolism of fat, carbohydrates and protein is insulin defective action on target tissues (Riaz, 2014). Insufficient insulin secretion leads to its inadequate functionality and sequentially to decline the body tissues response to it. The initial reason of high blood glucose in diabetic patients is not obviously distinguished. It can be due to insufficiency of hormone secretion, its deficient action or both simultaneously present in the body (ADA, 2004; ADA, 2014). Obvious indicators of elevated glucose level are polydipsia, polyphagia and polyuria. The patient will lose weight and suffer from blurred vision (Pavithra *et al*., 2018). According to ADA (American Diabetes Association, 2004; 2013; 2014) chronic elevated blood glucose levels may cause in sensitivity to definite infections and impaired growth rate. In case of unmanaged glucose levels, serious fatal consequences will threaten the patient's life. For instance, ketoacidosis and nonketotic hyperosmolar syndrome may end up to death. Long-term hyperglycemia consequences may affect vital body organs such as kidneys which will cause renal failure; Risk of retinopathy and blindness; probability of neuropathy, foot ulcer and infection which ends up in foot amputation. Autonomic neuropathy may lead to problems in heart rate, blood

pressure, gastrointestinal discomfort and sweating. Sexual dysfunction and genitourinary are complications made by chronic and long-term hyperglycemia (Maxwell, 1995). Diabetes promotes a raised risk of diseases such as atherosclerotic cardiovascular and peripheral arterial. There is also a greater chance of developing cerebrovascular disease (Pavithra *et al*., 2018). High blood pressure and dysfunction in metabolism of lipoprotein are common problems in diabetic patients (Horwitt, 1986; ADA, 2004; ADA, 2013; ADA, 2014).

In Africa continent, the prevalence of diabetic patients is closely related with economic development.

The rate of disease rises from 4.4% in lower income economies to 5% and 7% in lower middle and upper middle countries respectively (Peer *et al*., 2014).

Table 2.1.: Prevalence of diabetes in Kenya, Africa and globally

	Global (20-79) years	**Africa (20-79) years**	**Kenya (18-99) years in 1000s**
Adult population	4.84 billion	468 million	25,381,008
Diabetes prevalence (%)	8.8 [7.2-11.3]	3.3 [2.1-6]	1.83 [0.65-7.29]
Relative prevalence-age standardized (%)	-	4.4 [2.9-7.8]	2.75 [0.97-12.03]
No. diabetic patients	424.9 million [346.4-545.4 million]	15.5 million [9.8-27.8 million]	463.902 [163.96-1850.676]
No. of deaths attributed to Diabetes	4.0 million [3.2-5.0 million]	298.160 [196.089-533.916]	8078.84 [32857.299-2952.045]
Undiagnosed diabetes (%)	30-38	69.2	5-250,000 (20-79) years
No. of undiagnosed diabetic individuals	212.4 million	10.7 million [6.8-19 million]	167.004 [59.025-666.243]

Source: (*Diabetes Atlas-IDF*, 8th edition, 2017)

As economies develop, the life expectancy grows as well and the individuals with more risk factors of diabetes type II, will have greater chance to develop the disease, too. This can happen among

people with impaired glucose tolerance likewise. In 2017, 7.3% of people with the age of 20 to 79 years old were with impaired glucose tolerance. In 2013, 75% of diabetes caused death cases occurred in patients under 60 years old among the African countries which is estimated to be over 500,000 deaths.

Unfortunately, the prevalence of undiagnosed cases of diabetes in Africa continent is directly related with the society income. The less the income, the higher the prevalence is. This situation emphasizes the insufficient reaction of the local health systems whose responsibility is to supply accessible, inexpensive and optimum care for those suffering from diabetes (Peer *et al.*, 2014).

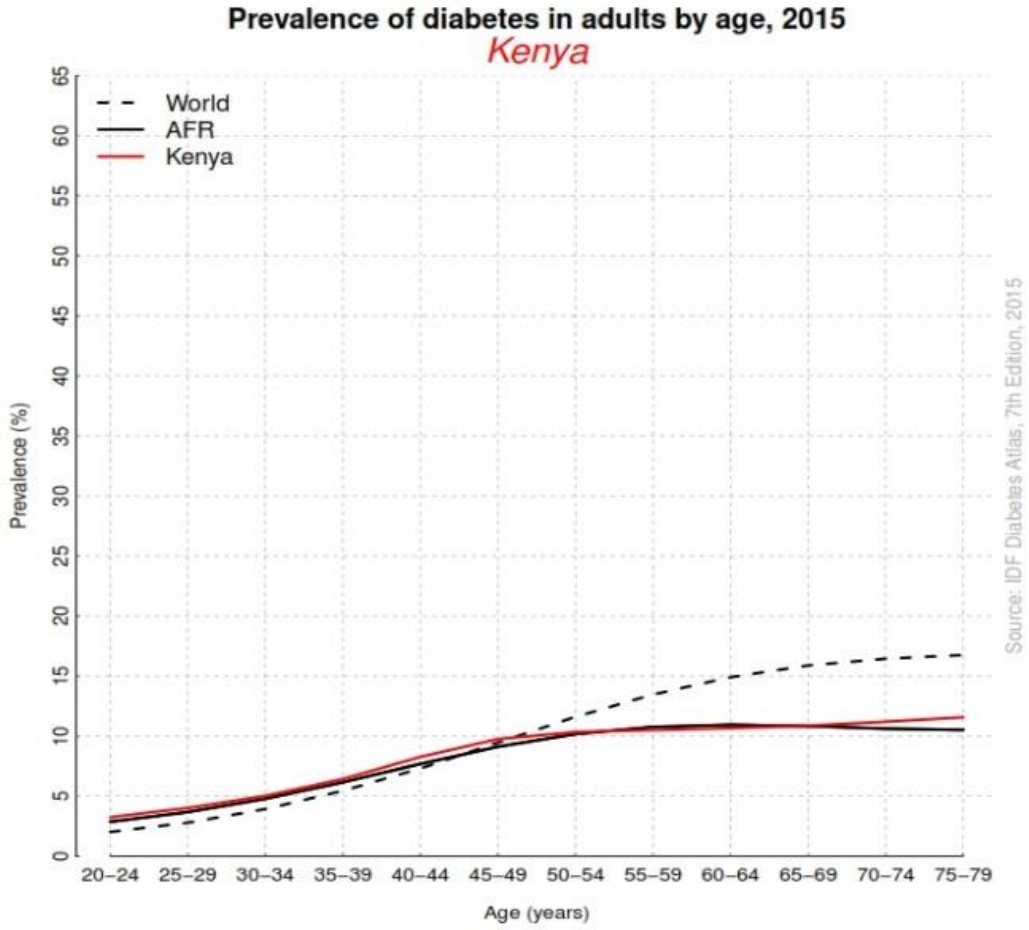

Figure 2.1.: Age-adjusted prevalence of diabetes in Kenya, 2015

Source: IDF Diabetes Atlas, 7th Ed., 2015

According to above figure, the prevalence of diabetes among people in different age groups is shown.

The distribution of diabetes mellitus is shown in different lines and colors for the world, Africa region and Kenya. The number of diabetic patients below 60 years is higher in many low and middle income countries in comparison with the world average. Meantime, a greater fraction of the high income individuals develop the disease over 60 years of age (IDF, 2015).

Patient costs of diabetes in public and private facilities according to Subramanian and others (2018), is summarized in the Table below:

Table 2.2.: Patient costs of diabetes in public and private facilities in Kenya - 2018

		Public facilities [a*] (US $)	Private facilities [a**] (US $)
Screening		4.95	19.00
Diagnosis		41.95	382.91
Management	Oral medication only	88.61	488.60
	Insulin only	186.40	541.22
	Both insulin and oral medication	234.44	675.85
Managing complications		20,871.86	73,863.91

*The proportions reported in the public facilities are on the basis of a study by Some *et al*., 2016.

**The proportions are based on expert opinion in the private facilities.

[a] The costs include physician consultations for the average admissions for managing hypoglycemia, medications and the patient, and. Stroke is not included.

2.2. Diabetes Management

Lifestyle modification in order to lose weight in obese patients, healthy food intake and Diabetes self-management education patient (DSME) are the principal approaches in diabetes management. The next step is to reach HbA1C 6.5% or lower in a safe way. Hypoglycemia and weight gain risk minimization, postprandial and fasting blood sugar control, medication and regular patient follow up are essential in diabetes management (Garber *et al*., 2015). Individuals with diabetes type II and

prediabetes frequent physical activity can reduce A1C, blood pressure, triglycerides and insulin resistance and as a result decreases the risk factors of cardiovascular diseases (Snowling and Hopkins, 2006; Colberg *et al*., 2016).

Frequent physical activity, weight loss and a healthy diet along with drug intake can manage blood glucose level in most diabetic patients. Well managed glucose level can reduce the risk of various consequent complications of diabetes (Philpott, 2016). Patients need to get medical care services from a diabetes expert coherent group including physicians and his/her assistant. Meanwhile, the presence of a nurse, a dietitian and a pharmacist is important. A mental health professional can support the patient while the patient himself plays an active key role in his treatment.

Diabetes management program is prepared with the given information from the patient and his family. Each member of the health care team helps to write the plan, too. Self-management education for diabetic patients as well as persistent support are the main elements in writing a good plan. In order to reach this goal, patients have to become capable of self-managing the disease applying diverse methods and strategies such as learning the skills of problem-solving for each and every component of the plan (ADA, 2015; ADA, 2016). Diabetes management plan and its goal must be tailored for each patient considering his preferences. When writing a plan, the age, working or school time schedule, food preferences and physical activity of the patient must be considered. Furthermore, existence of any complications due to the disease, health priorities, cultural and social situation or any medical status must be taken into account (ADA, 2015).

2.2.1. Diabetes Care in the Hospitalized patients

In the intensive care unit (ICU), ADA and AACE (American Association of Clinical Endocrinologists) recommend various blood glucose targets on impatient individuals (Moghissi *et al.*, 2009). According to their guidelines, the target blood glucose level is 140mg/dl<BG>180mg/dl. Blood sugar level higher than 180mg/dl and lower than 110mg/dl is not allowed. (Corsino *et al*.,

2017). Recently ADA has lessen the target BG lower than 180mg/dl and higher than 140mg/dl for surgery cases and general medicine (ADA, 2017).

For the non-ICU impatient individuals, ADA and AACE and also the Endocrine Society recommend a blood glucose level of less than 140mg/dl before taking a meal and lower than 180mg/dl randomly measured patients who take insulin (Moghissi *et al.,* 2009; Umpierrez *et al*., 2012; Handelsman *et al*., 2015).

In case of BG lower than 70-100mg/dl, the insulin dose must be decreased in order to hypoglycemia avoidance. In case of terminally ill cases or severe comorbidity conditions, BG rises to over 200mg/dl is accepted to avoid symptomatic BG (Umpierrez *et al*., 2012).

2.2.2. Pharmacologic Therapy for Type II Diabetes Management

The principal first line oral medicine for all ages is metformin (Viollet *et al*., 2012). Metformin is recommended in case of recently diagnosed patients for its low price, effectiveness and safety. It helps to manage the blood sugar and reduces the cardiovascular diseases risk (Holman *et al*., 2008). It is recommended that the patients who take metformin to be careful about vitamin B12 deficiency in case of comorbidity of anemia or neuropathy with diabetes type II (ADA, 2017). It is well tolerated with moderate side effects. Furthermore, the drug has low risk of hypoglycemia and weight gain. Metformin decreases the death rate among diabetic patients by reducing gluconeogenesis. In addition, this medicine activates the insulin receptor and improves insulin sensitivity. It decreases lipid levels of plasma to prevent cardiovascular diseases (Viollet *et al*., 2012). There might cause a mild weight loss in obese patients or overweight individuals (Inzucchi *et al*., 2014).

Insulin injection must be started without any delay if the diabetic patient fails to reach the glycemic target. With the objective to limit the atherosclerotic cardiovascular complications and mortality risk of not well managed disease, liraglutide or empagliflozin is prescribed in addition to standard care

(ADA, 2017). Insulin is the most recommended high blood glucose level controller in inpatient acute ill cases to achieve the target BG. In case of BG≥180mg/dl, insulin infusion is a must in order to keep BG lower than 180mg/dl (Rea *et al*., 2007; George *et al*., 2015).

In non-ICU patients for general medicine and surgery cases, subcutaneous insulin injection is recommended for the purpose of blood glucose level management (Umpierrez and Maynard, 2006; Hirsch, 2009).

Table 2.3.: Pharmacologic Therapy

Alterations in lifestyle must be the first therapy for patients with diabetes type II		
If lifestyle modifications have not helped to manage glycemic goals	→	-Metformin must be added (Recommended initial drug if not prohibited and tolerated)
Newly diagnosed patients with hyperglycemia, high A1C and marked signs and symptoms	→	Use insulin therapy alone or together with other agents
When highest tolerated doses of noninsulin monotherapy is not successfully manages A1C target after three months	→	Must add: -Basal insulin or -The second oral drug or -A GLP-1 receptor agonist
Insulin therapy must not be retarded. Insulin is eventually required for the progressive characteristic of diabetes type II		

Source: ADA, 2016.

2.2.3. Nutrition therapy for diabetes management

The most important challenge for a lot of diabetics is choosing the right food. ADA recommends not using a "one-size-fits-all" diet plan for every patient with diabetes as it is feasible and practical. ADA emphasizes on nutrition therapy as a key factor in diabetes management. Patients who are more interested in self-management and try to learn and collaborate with the health care group members are able to improve the plan more successfully. The treatment plan makes the diet program of the diabetic person (Inzucchi, 2012; ADA, 2014). There are certain nutritional recommendations for patients with

diabetes including: 1. It is accepted if various food patterns are consumed according to each individual's traditional, religious and cultural preferences or made by different ingredients from food groups. If a food pattern is advised to replace another, glycemic target, health beliefs and the patients' economic situation must be taken into consideration, too. 2. Replacement of low glycemic index diet with high glycemic index diet which helps to meliorate the glycemic control. 3. Recommendations must be given to the patients to wisely choose the healthier carbohydrates rather than the foods with added salt, sugar or fat. Healthy sources of carbohydrates are low-fat dairies, whole grains, legumes, fresh vegetables and fruits (Evert *et al*., 2014).

2.3. Dietary management of type II diabetes

According to the results obtained from recent studies indicates that dietary patterns, foods and nutrients play an important role in preventing and controlling DM (Diabetes Mellitus). The quality of dietary macronutrients like carbohydrates and fats is more important than the ingested quantity. The recommended diet for diabetic patients is to consume plenty of whole grains, nuts, vegetables and legumes. It is advised to limit the consumption of refined grains, meat (red and processed) and sugar containing drinks and alcohol. Studies have confirmed that healthy diet adherence can improve blood glucose and lipid levels management and decrease the risk of the disease. The general quality of diet can be well adapted with cultural and personal food preferences and needs to successfully manage and prevent diabetes (Sylvia *et al*., 2014).

2.4. Health promoting properties of ingredients

2.4.1. Drumstick leaves (*Moringa oleifera*)

Tropical, subtropical and semiarid areas are the main habitat of Drumstick trees which is widely spread in all over the world. Some countries cultivate the tree for its economic benefits such as Southeast Asia, India, South and Central America, Hawaii, Mexico and Africa continent. Drumstick

tree is called with names like “drumstick” for its unripe seed pod shape. It is nominated as “horseradish” owing to ground roots taste. It is also called as “ben oil tree” due to oil extraction from its seeds which are eaten by some individuals. Drumstick leaves are the edible parts of the tree regarded as a main food with considerable nutrient content (Thurber and Fahey, 2009; Mbikay, 2012; Razis *et al*., 2014). All parts of the tree like oil, bark, root, leave, sap, flower and seed has a special application in traditional medicine. Drumstick leaves are characterized by the favorable balance which can be found in fatty acids, amino acids vitamins and minerals (Moyo *et al*., 2011; Teixeira *et al*., 2014; Razis *et al*., 2014).

Furthermore, drumstick leaves have various antioxidants like phenolic compounds, flavonoids, carotenoids and ascorbic acid (Alhakmani *et al*., 2013; Vongsak *et al*., 2014; Stohs and Hartman, 2015).

2.4.2. Garlic (*Allium sativum*)

Use of herbal medicines is very common for their advantages such as little side effects. They are inexpensive and effective in treatment of several diseases. Recent researches have confirmed the importance of traditional herbal plants more than before. Garlic is one of most favorite herbs with antidiabetic properties (Mahesar *et al.*, 2010; Santaiah and Kamakshamma, 2018). Garlic from Liliaceae family is used widely in all over the world and have the ability to decrease the effect of several risk factors which can cause numerous diseases (Thomson *et al*., 2007). Garlic have various effective compounds which gives it high beneficial health properties. It exhibits anti-thrombotic properties (Augusti and Sheela, 1996; Anwar and Meki, 2003) and also acts as a strong antioxidant (Rees *et al*., 1993; Bakri and Douglas, 2005). It is well-known for its hypocholesterolaemic effects (Ali and Thomson, 1995; Adebiyi *et al*., 2018) and antibiotic activities (Kiesewetter *et al*., 1991; Benkerroum and Patel, 2018). This herb helps to lower blood glucose as well as blood pressure (Ali

et al., 2000; Banerjee and Maulik, 2002; Hassan *et al*., 2018). Studies have proved the anti-hyperglycemic effects of garlic in animal cases (Bordia *et al*., 1996; odo *et al*., 2018) and on diabetic patients (Kumar *at al*., 2013). Garlic helps to lower glucose levels due to its various compounds containing: Sulphur, allicin, alliin, S-allyl cysteine etc. (Santaiah and Kamakshamma, 2018). It has an anti-hyperglycemic effect due to S-allyl cysteine. This component is able to rise insulin in the body (Mashkoor *et al*., 2018).

2.4.3. Cinnamon (*Cinnamomum verum*)

One of the richest sources of polyphenols are spices with considerable antidiabetic and antioxidant effects. Cinnamon is a spice with a sweet, warm and woody aroma which is considered as a traditional medicine, used by people widely (Charles, 2013). Animal researches have proven the high hypoglycemic effects of cinnamon. In addition, it can also have an insulin mimic action or stimulator of glucose metabolism in cells (Gruenwald *et al.,* 2010; Unlu *et al*., 2010; Zu *et al*., 2010). Cinnamon bark's extract has shown inhibitory activity of alpha amylase secreted from pancreas and alpha glucosidase from intestine which is able to manage postprandial blood glucose level (Adisakwattana *et al*., 2011). Furthermore, it can control glucose intolerance (Anderson *et al*., 2004) and lower fasting blood sugar in type II diabetic patients and prediabetes (Davis and Yokoyama, 2011).

Cinnamon extract is confirmed to be effective in both controlling and prevention of hyperglycemia. As a result, the process of the disease progression can be controlled much better. The rate of deaths and illnesses depends on how the progression process is controlled (Anderson, 2015).

CHAPTER THREE: DEVELOPMENT OF A LOW CALORIE ANTIDIABETIC SUPPLEMENT POWDER FROM DRUMSTICK LEAVES (*MORINGA OLEIFERA*), CINNAMON (*CINNAMOMUM VERUM*) AND GARLIC (*ALLIUM SATIVUM*)

3.1. Abstract

Plants foods and natural products contain bioactive compounds which have been shown to possess both preventive effects and health benefits for chronic diseases such as diabetes with less or no side effects. Natural locally available ingredients being low calorie can manage diabetes type II especially for obese patients and diabetics. The study was designed to develop a food supplement with antidiabetic and antioxidant activity. Methanolic extracts were made from drumstick leaves, garlic and cinnamon and used for the analysis.

The chemical composition including protein, carbohydrate, fat, fiber, ash and moisture content of the food ingredients and in the supplement powders were measured using standard methods. Linear programming model was used to blend the different proportions of the food ingredients and in selection of an optimized supplement powder in quantity of vitamins and minerals according to RDA (Recommended Dietary Allowance). The four selected formulated supplements were blended in different proportions: S1: Dried drumstick leaves powder 90% + cinnamon powder 5% + garlic powder 5%. S2: Dried drumstick leaves powder 80% + cinnamon powder 10% + garlic powder 10%. S3: Dried drumstick leaves powder 70% + cinnamon powder 15% + garlic powder 15%. S4: Dried drumstick leaves powder 50% + cinnamon powder 25% + garlic powder 25%). The supplements were also evaluated for sensory attributes using 24 panelists, consisting of 12 Kenyans and 12 Iranians. A sensory evaluation was used to determine consumer acceptability based on the attributes of color, appearance, taste, aroma and overall acceptance of each powder to assess the most accepted powder. Results showed that the supplements had high nutritional quality and low

calorie content providing 14 to 16% RDA in 100g, low sodium from 6 to 9%, 29 to 42% protein, about 12% carbohydrate, 9 to 13% fat, 31% dietary fiber. At the same time, they were rich sources of vitamins such as 189 to >300% Vitamin A, 113 to 185% Vitamin B1, >300% Vitamin B2, 69 to 109% total folic acid and 90 to 139% Vitamin C. Meanwhile, all supplements had acceptable levels of minerals such as potassium 28 to 38%, calcium 121 to 162%, magnesium 74 to 111%, phosphorous 28 to 37%, iron 161 to 270% and zinc 19 to 30% according to RDA for target groups.

The sensory evaluation findings indicated the most accepted supplement regarding color, appearance and overall acceptance attributes was supplement no. 1 containing 90% drumstick leaves, 5% cinnamon and 5% garlic with the mean score of 5.58 in overall acceptance, 6.13 in both color and appearance, 4.33 in taste and 4.54 in aroma. The supplement was stable for 12 months according to the shelf life studies. The cost analysis shows that the most cost-effective supplement powder can be sold at 142 Ksh/100g.

The developed food supplements are locally available, indigenous and natural. The calorie content is low which makes it a suitable food for diabetic type II patients with overweight. The price of the final product is affordable for low and middle income diabetic patients. In addition, its powder form makes it easy to use. The high antioxidant and antidiabetic properties can play a great role in managing hyperglycemia in patients and avoid the long term consequences of the disease, improving their life quality. Furthermore, health level and nutritional status of the patients will improve by consumption of the supplement.

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3.2. Introduction

The data presented in WHO Global Report on Diabetes in 2016, points out that better management of diabetes will lessen disease complications, reduce the burden on health systems and improve life quality level of patients living with diabetes. According to this report, healthy diet including enough dietary fiber intake of at least 20 g per day and consumption of polyunsaturated fatty acids instead of saturated fat along with regular physical activity, can both decrease diabetes type II risk and increase glucose uptake and insulin sensitivity (WHO, 2016).

Management of diabetes with synthetic drugs such as Acarbose and Miglitos is associated with some disadvantages such as gastro intestinal problems including flatulence and diarrhea, modest HbA1c efficacy and frequent dosing schedule with moderate cost (Inzucchi *et al*., 2015).

Diabetes type II as a metabolic disorder causes pathogenic situations such as oxidative stress and inflammation which in turn ends up in insulin resistance and various long time complications (Bahadoran *et al.*, 2013). According to Bahadoran and others (2013), the increasing number of widespread presence of diabetes complications proposes that the present medication are not successful in managing diabetes and therefore applying further treatments such as nutraceuticals and functional foods can help to manage the disease more efficiently.

It was estimated that there was 425 million adults between 20 to 79 years with diabetes worldwide in 2017 which is expected to reach to 629 million by 2045 (Cho *et al.*, 2018). International Diabetes federation (2017) Africa region reported 458.900 cases with diabetes in Kenya. Dr. Gojka Roglic, the leader of WHO global work on diabetes in 2012 declared that 1% of death cases in Kenya is directly related to diabetes, while most people with diabetes die because of consequences of the untreated disease such as cardiovascular complications like heart attack (WHO, 2014). Diabetes prevalence estimated to be 460 cases in 10 thousand population Kenya in 2014 (Githinji *et al*.,

2018). It is much like 750,000 people and 20,000 deaths per year. About 10.7% live in urban areas and 2.7% are rural indwellers. This information is based on regional estimations and is probably underestimated. More than 60% of people who are diagnosed with diabetes in Kenya, are referred to health care facilities for unrelated complaints. Around 14% of the country's population is estimated to have impaired glucose; Kenya national strategy for the prevention and control of non-communicable diseases, 2015-2020 (Joseph *et al*., Ministry of Health, 2015).

The challenges in type II DM management are mostly classified to: the need to upgrading the current available therapies to achieve the optimum glycemic, lipid and blood pressure control which leads to reduction of the risk and retardation disease complications. The patients with diabetes need to be trained for self-management and improving their adherence to medicinal interventions and lifestyle changes. Improvement of the health care services for the patients in chronic situation is a need (Blonde, 2005). There are other challenges which delay the early initiation of insulin usage such as the fear of hypoglycemia, injection, weight gain, patient's time limitation for education and raised need of blood glucose self-monitoring (Brown and LeRoith, 2010). WHO global coordination mechanism on the prevention and control of non-communicable diseases Working Group on how to realize governments' commitments to engage with the private sector for the prevention and control of NCDs (Non communicable diseases), assessed the progress which is defined in accompaniment of policy briefs, recognized common challenges and barriers specially in middle and low income countries in order to improve the action areas. The challenges are: 1. Insufficient political and public awareness and comprehension about the function of private sector. 2. Competition between the health funding priorities in global and national level. 3. Insufficient capacity and supporting regulation in order to legislate. 4. Contradiction between the drivers and the objectives. 5. Insufficient data to assist action, monitoring and setting the target such as dietary patterns. 6.

Inadequate infrastructures, capacities and capabilities to involve and monitor and manage the private sector activities (WHO, 2015). In Kenya, many diabetic patients and their families have economic problems to continue the treatment expenses. Consequently, some patients who are not able to adhere the treatment will be led to a high risk of end organ damage and amputation. Kenya household economic survey reports that 46% of the country's population lives on less than one dollar per day and can't afford the necessary expensive and advanced expenses to care diabetic patient's complications. when the situation gets worse, the patient and his family will be forced to sell their meager assets to pay for treatment and this will end up to poverty. DM also has an impact on the people of most productive ages in the population (Kenya National Diabetes Strategy 2010-2015, 2010).

Many studies have proved that polyphenols in plants such as phenolic acids, flavonoids and other phytochemical can effectively manage diabetes and are able to prevent the long time complications of the disease (Bahadoran *et al.*, 2013).

Obese diabetic patients need to lose weight by a low calorie diet. According to Dworatzek *et al.* (2013), maintaining or increment of protein intake is necessary in order to prevent lean muscle loss due to inadequate intake of protein. Every existing information about locally available foods and all their characteristics must be amassed and documented. It is fundamental to collect the knowledge about foods that are underutilized and practices on agriculture area. This information should contain the traditional beliefs as well. Emphasizing on the importance of underutilized food species in regions where they are being produced at the present time along with making use of opportunities in order to increase the production and consumption of indigenous foods, can improve the consumption levels. The data will help to promote development of the product and also will raise the awareness (Konuma, 2014).

In the present study, the three ingredients and four supplements have potential for use in the supplement formulation with high antidiabetic and antioxidant activity. At the same time, the supplements contain low calorie levels to help the overweight patients lowering their excessive weigh. The supplement is also hypothesized to contain high nutritional and sensory quality.

This study was designed to explain the possibility of utilizing Drumstick leaves and two spices Garlic and Cinnamon to develop a supplement with antidiabetic and antioxidant activity.

The food supplements with indigenous and locally available ingredients are natural. The final price of the products is low and affordable for middle and low income patients suffering from diabetes type II. Additionally the powder form of the supplements makes it easy to use. They contain a high antidiabetic and antioxidant property which helps to play a great role in promoting the life quality of diabetic patients as well as their health and nutritional status.

Recent researches have shown that dried leaves of drumstick blended with other foods were successfully incorporated in many different traditional recipes. The mixture was acceptable among the tested groups. The anti-hyperglycaemic properties were confirmed as well (Ali *et al*., 2017). Recent findings show that addition of cinnamon to other food components for a long period of 210 days, have not been altered in organoleptic attributes and its proximal composition (Antigo *et al*., 2017). Another study has suggested that drumstick as the based spice in a mixture with other spices can be used as an accepted spice by households. This study demonstrated that the proximate composition and organoleptic properties of spices is increased by adding drumstick powder (Balogun *et al*., 2017). Drumstick as a wondering plant is known for its rich sources of minerals, vitamins and protein (Anjorin *et al*., 2010; Wakil and Kazeem, 2012). During shelf life, garlic acts as an obstacle against spoilage bacteria due to its high antimicrobial and antioxidant properties (Horita, 2016).

3.3. Methodology

3.3.1. Preparation of samples

The samples were collected based on their potential to possess high antidiabetic and nutritional properties. Sample ingredients including 5kg fresh Drumsticks leaves (*Moringa oleifera*) purchased from Meru County, 0.5kg fresh Garlic (*Allium sativum*) purchased from Nakumat supermarket and 250g Cinnamon (*Cinnamomum verum*) sticks purchased from Healthy Life shop. Drumstick leaves were dried at 60 ^{o}C-65 ^{o}C in air oven for 3.5 hours. The fresh garlic and cinnamon barks were cleaned, washed, size reduced and dried for 6 and 4 hours respectively at 60 ^{o}C – 65 ^{o}C. All three samples milled by a home blender (National blender, model MJ-176NR, Japan) and sieved to obtain a very soft powder (particle size: 0.125mm).

3.3.2. Supplement formulation

Linear programming model was used to optimize and formulate the blends (http://www.nutrisurvey.de/). Each sample was prepared after weighing, mixing and labeling. The formulations included: Ingredients: I1: 100% dried drumstick leaves powder. I2: 100% cinnamon powder. I3: 100% garlic Powder. Supplements: S1: 90% dried drumstick leaves powder + 5%cinnamon Powder + 5%garlic powder. S2: 80% dried drumstick leaves powder + 10% cinnamon Powder + 10% garlic powder. S3: 70% dried drumstick leaves powder + 15% cinnamon powder + 15% garlic powder. S4: 50% dried drumstick leaves powder + 25% cinnamon Powder + 25% garlic powder.

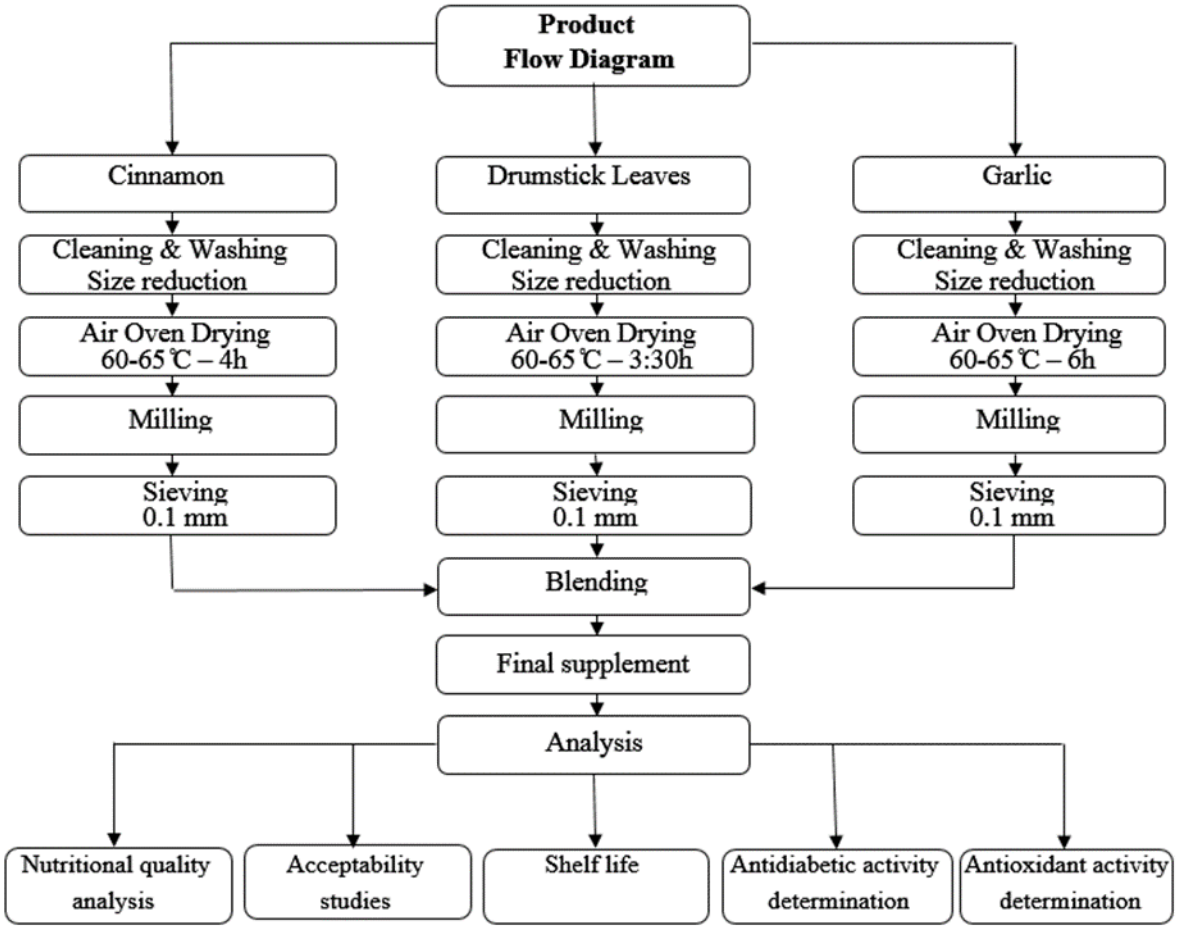


Figure 3.1.: Process flow development of low calorie antidiabetic supplement

3.3.2.1. Nutrient content optimization

Linear programming model was also used to determine supplement formulations with high nutritional quality and also low calorie levels of each food ingredient in different proportions and selection of the final product. Linear programming technique was applied by using Nutrisurvey Software. As the nutrient values of dried *Drumstick* leaves and *Cinnamomum verum* was not found in the software, the data obtained from proximate method analysis was added to the food table. Then the data was completed adding USDA (United States Department of Agriculture) reference tables (USDA, 2015).

3.4. Analytical method

Proximate Analysis conventionally consists of moisture, fat, ash, fiber, protein and nitrogen-free determination analysis (AOAC, 2008). The analysis is also called "Weende proximate analyses" and is initially used for materials which are going to be applied in formulating a food diet as a source of energy or crude protein (total nitrogen) and finished food. The analysis plays the role of a control to check the characteristics found in formulation process.

Protein, carbohydrate, fat, fiber, ash, moisture of the three ingredients was determined using proximate analysis method at the food chemistry laboratory, UON (University of Nairobi). Calculating the difference, the carbohydrate content was determined. Using Atwater's conversion factors the energy value was calculated from fat, protein and carbohydrate contents for each ingredient.

3.4.1. Moisture determination

AOAC (Association of Official Analytical Chemists) method 952.10, oven drying. 2g of each food ingredient was weighed in a dish accurately. The dish and contents were put in the air-oven and were maintained at 105^{o} and was dried for three hours. After cooling in a desiccator, were weighed. The dish was returned to the oven and dried for another 30 minutes. Again cooled in a desiccator and weighed. The moisture content of the sample calculated in percentage using the following formula:

$$\%moisture = \frac{loss}{sample\ weight} \times 100$$

3.4.2. Crude Lipid determination

To determine the lipid percentage, petroleum ether is used to extract the fat from the food ingredient and calculated as the percentage of the sample weight before evaporation of the solvent (Olvera-Novoa, 1994).

AOAC method 920.39, also known as Soxhlet extraction method was used with some modifications. 5g of each food ingredient weighed and put into an extraction thimble, covered with cotton wool and placed into the sohxlex extractor. Petroleum ether (200ml) was heated in tared flat bottom flask and connected to the sohxlet extractor for 8 hours. After evaporation of the solvent, residue was dried in air-oven at 105^0C for 1 hour. Crude fat contented by the following formula:

$$\%fat = \frac{(conical\ flask + oil) - empty\ conical\ flask\ weight}{sample\ weight} \times 100$$

3.4.3. Crude fiber determination

The fiber content of each food ingredient is determined by digestion in sodium hydroxide and sulphuric acid solution and residue is calcined. The percentage of the fiber is calculated by the weight difference before and after calcination.

AOAC method 991.43 was used to determine the fiber content of the food ingredients. Each food ingredient (4g) was weighed accurately into a graduated 600ml beaker. Some boiling distilled water and 25ml of 2.04N H2S04 solution added. The volume was made up to 200ml by adding boiling distilled water. Then was maintained this volume during boiling time, 30 minutes, on a hot plate. A Büchner funnel was used to filter the content of the beaker. It was slightly covered with some glass wool. The residue was washed 3 times with boiling distilled water. The residue and glass wool was transferred to the beaker. Small amount of boiling distilled water and 25ml of 1.73N KOH solution was added. The volume was made up to 200ml with more boiling distilled and maintained this while

boiling for 30 minutes on a hot plate. Glass wool was applied as filter. Then it was filtered and washed again. The washing process of the residue with ethanol was repeated for more three times. Both the glass wool and the residue were moved into a porcelain dish regarding its quantity. Drying process continued for 2 hours at 105 ^{0}C in the air oven. Left in desiccator to cool down and weighed. The dish and content were then ignited at 550 ^{0}C to constant weighed. Cooled in a desiccator and weighed. To calculate the fibre content, the following formula we used:

$$\%\ \boldsymbol{fiber} = \frac{(\boldsymbol{Crucible\ +wool\ +\ sample}) - (\boldsymbol{crucible\ +\ wool})}{\boldsymbol{sample\ weight}} \times \mathbf{100}$$

3.4.4. Ash determination

Ash quantity in food is determined by calcination in this method. The inorganic amount and total mineral in each sample is represented by ash.

AOAC 900.02A method, also known as muffle furnace. About 2g of each food ingredient was weighed accurately into a tared porcelain crucible. The process was started with a bunsen burner on low flame and continued in a muffle oven at 500 ^{o}C until a light grey or white ash of constant weight was obtained. The ash content of the sample was calculated by the following formula:

$$\%\ ash = (\frac{(crucible + ash) - cruicible\ weight}{sample\ weight}) \times 100$$

3.4.5. Crude protein determination

The standard way of determining nitrogen was used with some modifications. It evaluates the total nitrogen content of each sample. The procedure has three principal steps: 1. Digestion- each sample is digested in H_2SO_4 with the help of a catalyst (kjeldahl catalyst tablet). It ends in exchange of nitrogen to ammonia (NH_3). 2. Distillation- ammonia is distilled into the trapping solution (NHcl). 3. Titration- ammonia is titrated by a standard solution (NaOH) to be quantified (AOAC, 2008).

AOAC method 955.04 also known as Kjeldahl method. 0.5g of each sample weighed in a nitrogen-free filter paper, folded and placed in a kjeldahl flask with antibumping pumice. One kjeldahl catalyst tablet and 20ml of conc. H_2so_4 was added. The mixture was heated in a fume cupboard to boil until a clear solution is obtained and continued for 1 hour. Then cooled and distilled water added to ¾ of the flask. Some drops of phenolphthalein was added. In a 400ml conical flask containing 25ml of 0.1N Hcl solution, some drops of methyl orange indicator was added under the outlet of the distillation unit. The kjeldahl flask was added to the distillation unit. An average of 15.9 ml of 40% NaOH solution was added to the kjeldahl flask to change the color of the solution. Distillation continued until a drop of distillate does not react with nessler's reagent. The distillate was back titrated with 0.1N NaOH solution. The crude protein content calculated using the following formula:

$$\%\text{Protein} = \frac{14 \times 6.25 \times 100 \times 0.1}{\text{Sample}}$$

3.4.6. Carbohydrate determination

To determine the carbohydrate content of each food ingredient according to AOAC (2008), the formula below was used:

%Carbohydrate = 100% - (protein + Fiber + ash + fat + moisture)

3.4.7. Energy determination

The energy content of each food ingredient is determined using the formula below:

$$Energy\ content\ (\frac{cal}{100g}) = (protein \times 4) + (carbohydrate\ \times 4) + (fat\ \times 9)$$

3.5. Sensory evaluation of the food supplement

24 panelists consisting of 12 Iranians and 12 Kenyans were randomly selected. The powders were prepared in the above mentioned proportions using the lab scale and mixed in separate containers. It was presented to the panelists using white porcelain plates to show the color and appearance of each powder clearly. Each plate was served coded with symbols like: (□ ○ ◊ △). The panelists were asked to fill in the questionnaire carefully. The first table was about the attribute of the supplements like color and appearance. The second table was about other attributes such as taste, aroma/odor and the overall appearance. They were asked to enter the room one by one and leave the place after filling the questionnaire. The purpose of the evaluation was explained for each panel member individually briefly and asked to taste the supplements before completing the second table. The data collected from sensory evaluation were calculated as the means of the panelists' scores for each attribute and used to select the most accepted supplement powder (Lawless and Heymann, 2010).

3.6. Product cost estimation

Case-Based reasoning (CBR) method was used in order to estimate the product cost. The method uses the information from previous products with similar attributes to estimate the new product's cost (Niazi *et al.*, 2006). The present prices of each ingredient were determined by inquiry emails to manufacturers in order to estimate the new product prices. Other costs including labor, electricity, work space rental fees, transportation, packaging, labeling and tax were estimated. Finally a profit of 10% was added to the final product price.

3.7. Shelf life

It is significantly important to determine the shelf life of food products in order to satisfy the consumer acceptance on appearance, organoleptic properties, quality and its safety. The studied food supplements are classified as the medium-to-long shelf life products because of the thermal

process received for drying. Dried, canned and frozen foods are known as the most stable foods (Phimolsiripol and Suppakul, 2016). Accelerated method as an appropriate and standard way of shelf life determination was used in this study at 55 ^{o}C for 6 days (1 day representing 1 month). The supplements were stored in the air oven to simulate the encountered conditions in a short time period (Kilcast and Subramaniam, 2000; Phimolsiripol and Suppakul, 2016).

Simulation refers to methods that are able to evaluate the stability of food products according to the data obtained in shorter time period in comparison with the real shelf life (Steele, 2004; Phimolsiripol and Suppakul, 2016).

3.8. Packaging

Packaging the food products means protecting the food against the deteriorative consequences of the outer conditions in the environment such as moisture, temperature, light, microorganisms, pressure and etc. It facilitates the consumer with easy usage and saves his time and provides the choice of selecting the quantity of the product (Yam *et al.*, 2005; Marsh and Bugusu, 2007). The appropriate package for the product was chosen to be the plastic ziplock bags of 100g sealed by the electronic ziplock bag sealer to provide a cost effective product for the consumer while it can protect the product for a long shelf life and safe as FDA regulations for food products standards.

3.9. Labelling

The only way that a consumer or a patient have to obtain further information about a product in order to make his decision to buy on not, is the food label. Recent studies have proved that the consumer's reactions and the objective characteristics of the food label are related together (Cavicchi, 2008; Bialkova and Van Trijp, 2010; Di Pasquale, 2011; Grunert, 2011; Veneziani *et al.*, 2012; Vianelli and Marzano, 2012; Siriex *et al*., 2013). The food label gives information to the

consumer about the product attributes like weight, texture, ingredients, etc. which is limited due to the package size and regulations (Bacarella *et al*., 2015).

According to FDA food labeling guides, the product's label format and information were designed to be as follows:

A) On principal display panel (PDP): Name of the food product and the brand, Net quantity statement (weight of food in the package in grams and ounce units)

B) On the other display panel (PDP): Nutrition Facts table, ingredient list in descending order of dominance, name and address of the manufacturer, packer, or distributor, allergy labeling and country of origin statement must be conspicuous (FDA, 2013).

3.10. Statistical methods

All the analyses were carried out in triplicate (n=3). All the obtained data was shown as means of standard error of deviation (±SEM). Analysis of variance was determined at 5% level of significance. Statistical analysis was done by SPSS (Statistical Package for the Social Sciences) software (version 23).

3.11. Results and discussion

3.11.1. Development of supplements

Different proportions of the three ingredients was assessed and the 4 supplements with the highest nutritional values providing more than 100%/100g of the RDA values of vitamins A, B1, B2, B6, C, acid folic and minerals such as Calcium and Iron were selected as following:

The nutrient content and optimization of supplement 1 including 90% *Drumstick* dried leaves powder + 5% *Cinnamomum verum* powder + 5% *Allium sativum* powder is shown in Figure 3.2.

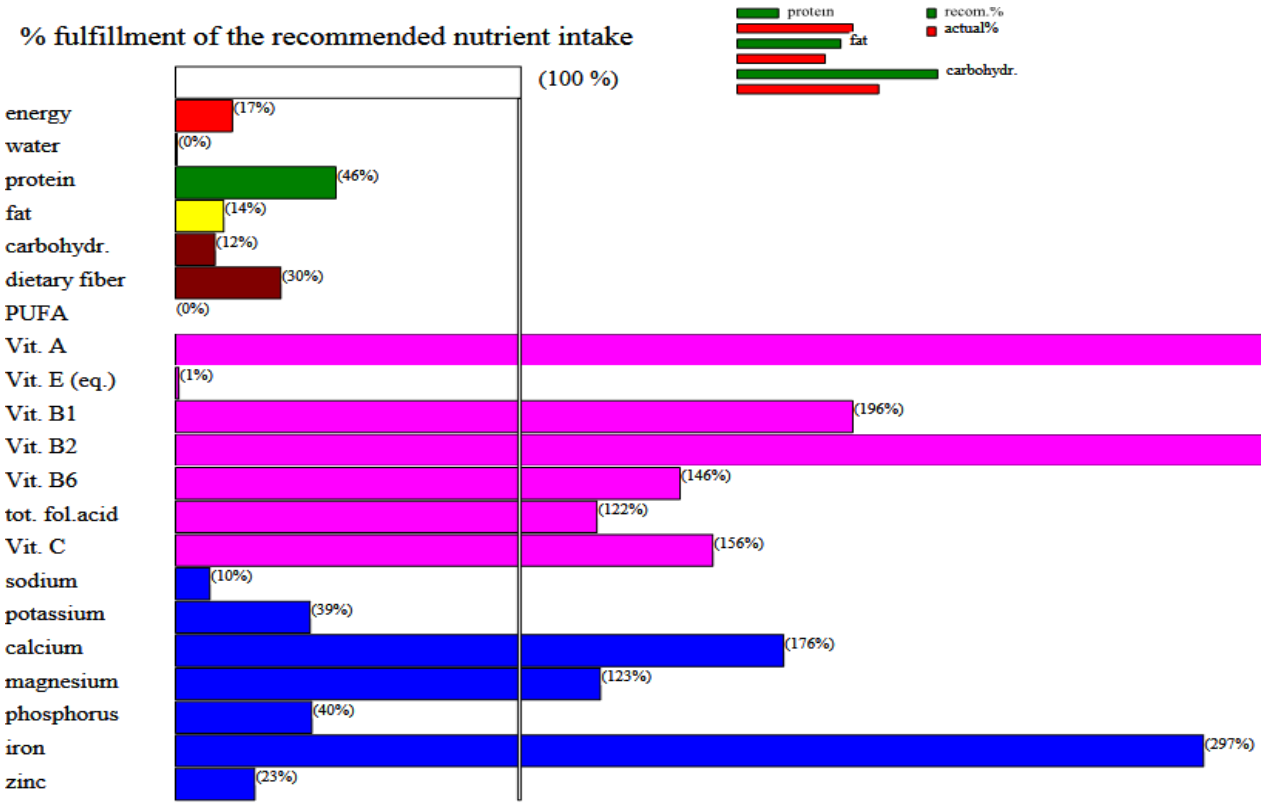


Figure 3.2.: Nutrient content optimization of the supplement powder (S1)

The nutrient content and optimization of supplement 2 including 80% *Drumstick* dried leaves powder + 10% *Cinnamomum verum* powder + 10% *Allium sativum* powder is shown in Figure 3.3.

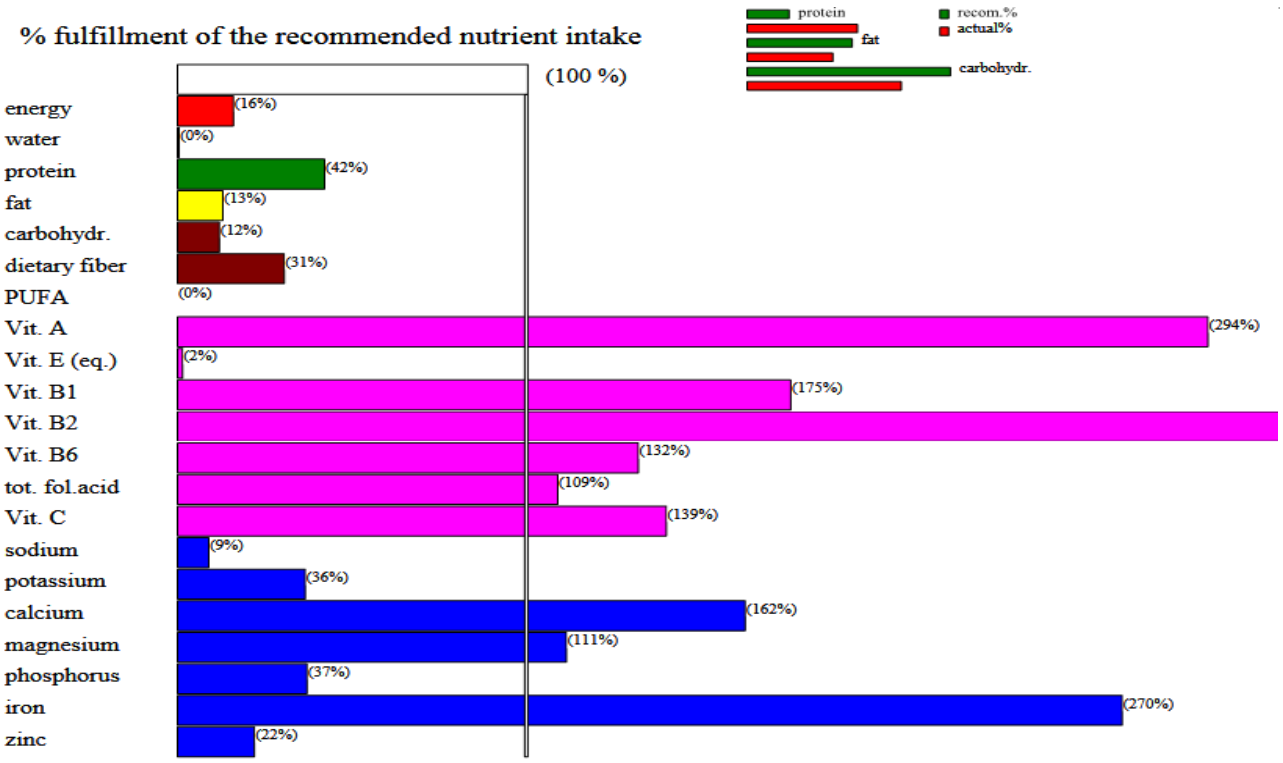


Figure 3.3.: Nutrient content optimization of the supplement powder (S2)

The nutrient content and optimization of supplement 3 including 70% *Drumstick* dried leaves powder + 15% *Cinnamomum\ verum* powder + 15% *Allium sativum* powder is shown in Figure 3.4.

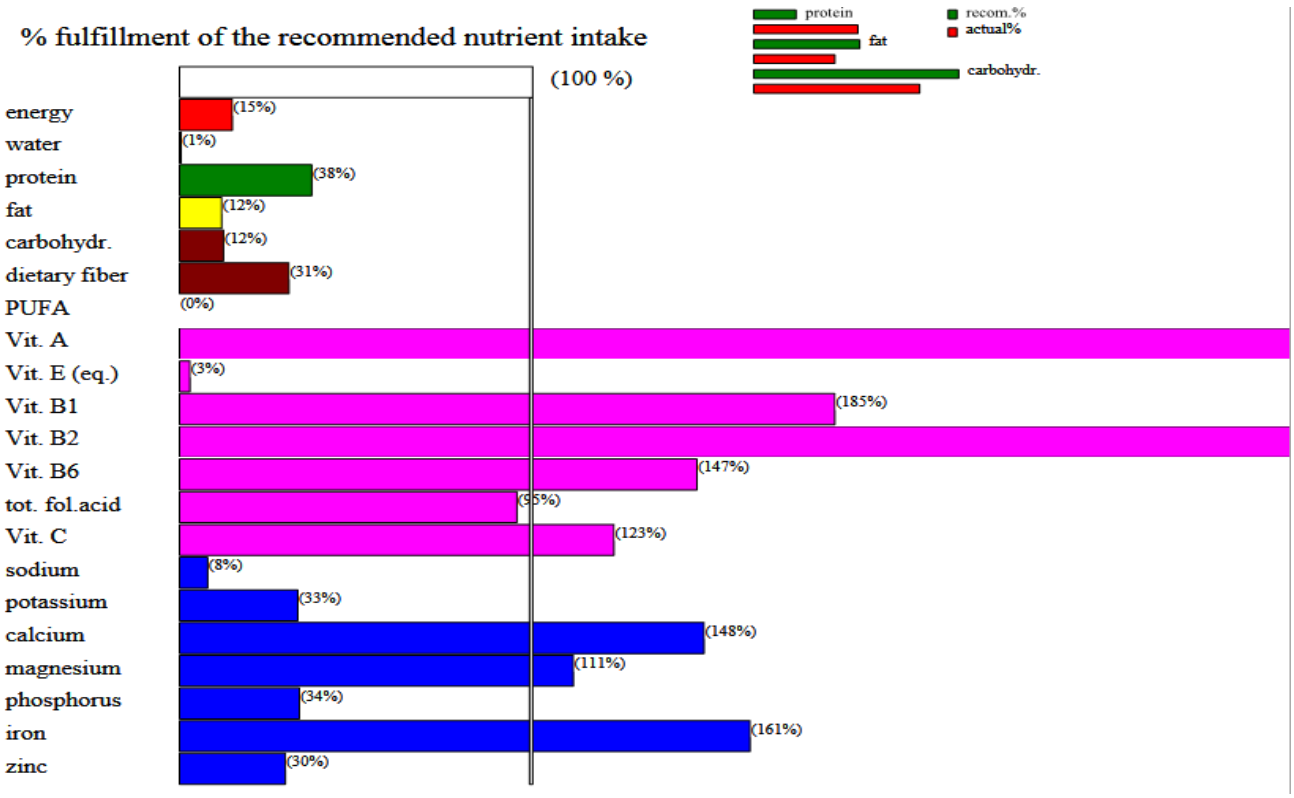


Figure 3.4. : Nutrient content optimization of the supplement powder (S3)

The nutrient content and optimization of supplement 4 including 50% *Drumstick* dried leaves powder + 25% *Cinnamomum verum* powder + 25% *Allium sativum* powder is shown in Figure 3.5.

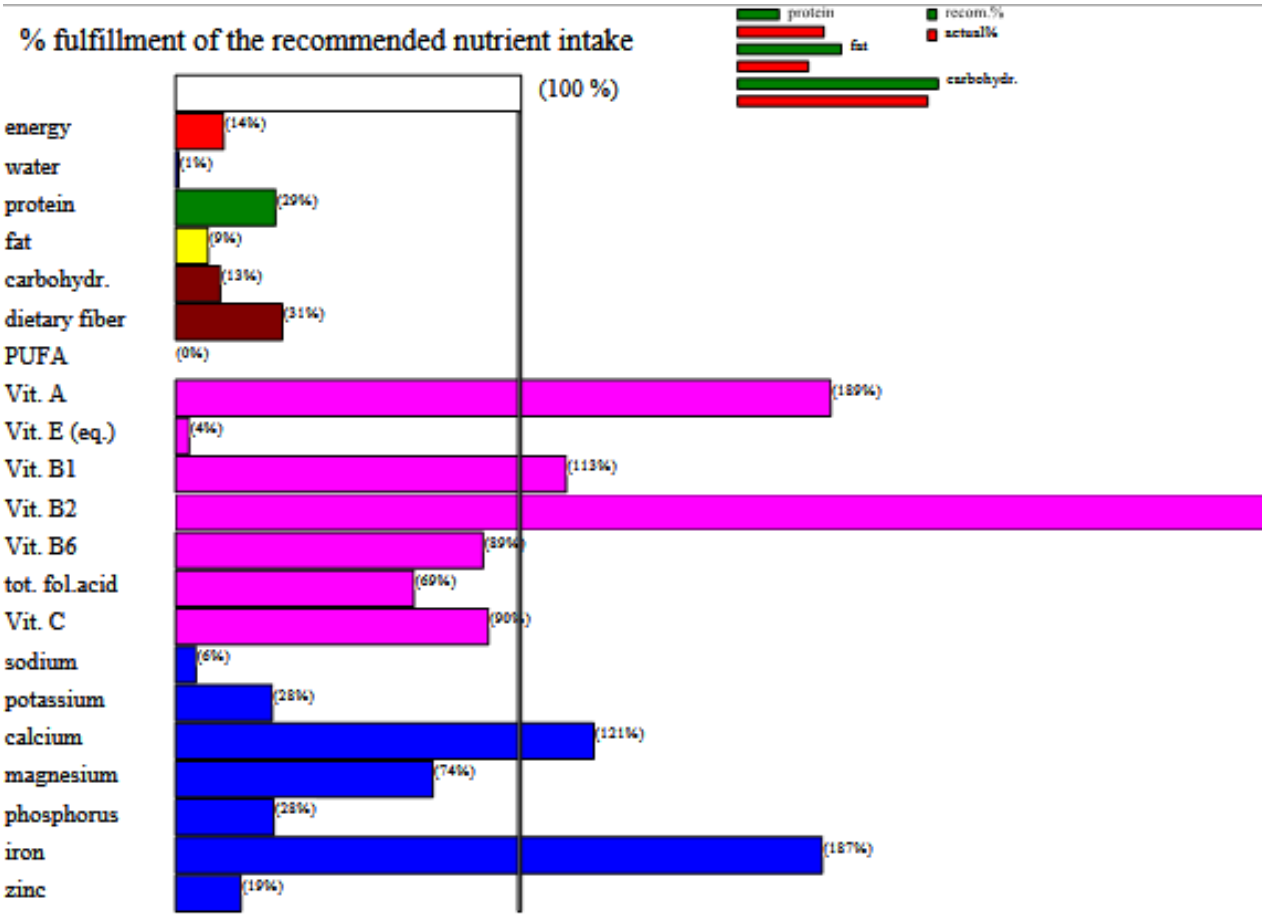


Figure 3.5. : Nutrient content optimization of the supplement powder (S4)

The analysis shows that all the four supplements are rich sources of protein, vitamins and minerals. Significant content of vitamins A, B1, B2, B6, C, Folic acid were observed as shown in Table 3.1. Minerals such as calcium, Iron and Magnesium were shown to be capable of providing more than 100%/100g RDA of body needs.

Table 3.1. Nutrient composition of the developed supplements

	Supplement 1	**Supplement 2**	**Supplement 3**	**Supplement 4**
Energy	14%	15%	16%	16%
Water	1%	1%	0%	0%
Protein	29%	38%	42%	42%
Fat	9%	12%	13%	13%
Carbohydrates	13%	12%	12%	12%
Dietary fiber	31%	31%	31%	31%
PUFA	0%	0%	0%	0%
Vitamin A	189%	>300%	294%	294%
Vitamin B1	113%	185%	175%	175%
Vitamin B2	>300%	>300%	>300%	>300%
Vitamin B6	89%	147%	132%	132%
Total folic acid	69%	95%	109%	109%
Vitamin C	90%	123%	139%	139%
Sodium	6%	8%	9%	9%
Potassium	28%	33%	36%	36%
Calcium	121%	148%	162%	162%
Magnesium	74%	111%	111%	111%
Phosphorus	28%	34%	37%	37%
Iron	187%	161%	270%	270%
Zinc	19%	30%	22%	22%

100% is Recommended Dietary Allowance (RDA)

3.11.2. Chemical composition of food ingredients used in supplement formulation

The results for food ingredients obtained from proximate analysis and energy content calculation are shown in Table 4.1. The highest energy content was observed in Drumstick leaves with 350Kcal/100g in comparison with the two other ingredients. Drumstick contained 31% protein and 11% lipid as the highest ingredient while garlic powder showed the highest content of 30% fiber. Meanwhile, cinnamon powder has the highest carbohydrate content (60%) among the ingredients and 11.88% moisture. The richest ingredient in total mineral ash content was shown to be cinnamon with 12%. Supplement no. 4 with 307 Kcal/100g is the lowest calorie supplement.

Table 3.2.: Proximate composition of the food ingredients and formulated supplements*

Food ingredients and formulated supplements	Energy Kcal/100g	Protein %	Lipid %	Fiber %	Carbohydrates %	Ash %	Moisture %
I1: Drumstick leaves powder (Moringa Oleifera leaves)	349.54 ± 0.40	30.55 ± 0.31	10.67 ± 0.04	9.04 ± 0.08	32.82 ± 0.22	10.34 ± 0.04	6.58 ± 0.16
I2: Cinnamon (Cinnamon Verum powder)	289.07 ± 1.73	2.30 ± 0.08	4.61 ± 0.24	17.56 ± 0.10	59.58 ± 0.11	4.06 ± 0.01	11.88 ± 0.13
I3: Garlic (Sativum powder)	242.30 ± 1.51	14.39± 0.11	0.92 ± 0.06	30.31 ± 0.21	44.11 ± 0.48	3.72 ± 0.02	6.55 ± 0.28
S1: Drumstick leaves powder 90% +Cinnamon Powder5% +Garlic powder 5%	341.11±1.01	28.33±0.48	9.89±0.06	10.52±0.13	34.70±0.03	9.63±0.04	6.83±0.23
S2: Drumstick leaves powder 80% +Cinnamon Powder 10% +Garlic powder 10%	332.33±0.02	26.11±0.43	9.37±0.49	12.01±0.13	36.61±0.22	9.04±0.05	7.10±0.2
S3: Drumstick leaves powder 70% +Cinnamon Powder 15% +Garlic powder 15%	324.26±0.36	23.89±0.37	8.29±0.07	13.49±0.12	38.51±0.13	8.39±0.05	7.36±0.16
S4: Drumstick leaves powder 50% +Cinnamon Powder 25% +Garlic powder 25%	307.58±0.07	19.59±0.39	6.71±0.09	16.47±0.11	42.33±0.02	7.11±0.04	7.89±0.08

* Values are means ± standard error of mean (n=3)

3.11.3. Sensory evaluation

The results from 24 panelists who completed the sensory evaluation questionnaire on four different food supplement proportions showed that the color, appearance and the overall acceptance of sample no. 1 coded with symbol △ and composition (90% drumstick + 5% cinnamon + 5% garlic) was the most accepted. The more the deep green color of the drumstick percentage is present in the sample, the more it was accepted by the panelists. While the most favorite taste was observed to be

sample no. 3 coded with symbol ○ and composition of (70% drumstick + 25% cinnamon + 25% garlic). At the same time, the most favorite aroma was sample no. 4 with the highest content of cinnamon (25%) and garlic powder (25%) mixed with 50% dried drumstick leaves. The sensory evaluation findings indicated the most accepted supplement regarding color, appearance and overall acceptance attributes was supplement no. 1 (S1) containing 90% drumstick leaves, 5% cinnamon and 5% garlic with the mean score of 5.58 in overall acceptance, 6.13 in both color and appearance, 4.33 in taste and 4.54 in aroma.

Table 3.3. Sensory attributes of developed supplements

	Color	Appearance	Taste	Aroma	Overall acceptance
S1	6.13 ± 1.227	6.13 ± 1.424	4.33 ± 1.685	4.54 ± 1.693	5.58 ± 1.349
S2	6.0 ± 0.978	6.04 ± 0.999	4.88 ± 1.227	4.42 ± 1.558	5.17 ± 1.373
S3	5.50 ± 1.022	5.33 ± 1.129	5.21 ± 1.25	4.38 ± 1.555	4.92 ± 1.176
S4	4.38 ± 1.907	4.25 ± 1.8	4.96 ± 1.922	4.96 ± 2.074	4.75 ± 1.539

* Number of panelists n=24

The composition of the most accepted and selected food supplement shows that the supplement is a very good food source of several nutrients. Therefore, it can play an important role as a supplement in providing high amounts of protein, vitamins and minerals. Furthermore, the pleasant color, appearance while being ready and easy to consume, can help to successful in being a rich supplement for the whole population especially the diabetic type II patients. According to the results all the supplements are highly accepted for being healthy and natural coming from its light green color. The taste of cinnamon and the appetizing aroma of garlic were highly noticed among the panelists.

3.11.5. Product cost estimation

According to cost estimation tables (tables 3.3, 3.4 and 3.5), supplement no. 4 with 50% Drumstick +25% +25% Cinnamon is the cheapest (the most cost-effective) product shown in Table 3.5. The

final price of supplement no. 4 is 142 Ksh/100g which is affordable for low income type II diabetic patients.

Table 3.4.: Cost estimation

Food ingredients and developed supplements		
Food ingredient 1: (100% Drumstick)	1680 Ksh/Kg**	
Food ingredient 2: (100% Cinnamon)	550 Ksh/Kg	
Food ingredient 3: (100% Garlic)	525 Ksh/Kg	
Supplement 1: (90%D + 5%G + 5%C)*	156.575 Ksh/100g	
Supplement 2: (80%D + 10%G + 10%C)	145.15 Ksh/100g	
Supplement 3: (70%D + 15%G + 15%C)	133.725 Ksh/100g	
Supplement 4: (50%D + 25%G + 25%C)	110.875 Ksh/100g	
Materials (Ksh)		
Electric zip lock bag sealing machine impulse heat sealer 200mm × 2	3,875	7,750
Digital food scale × 2	1,000	2,000
Manual electrical date codes printing machine × 2	4,000	8,000
Utensils	5,000	5,000
Maintenance - 1%	147	147
Total[***]		**22,897**
Total cost per package 100g		**0.62**
Other costs (Ksh)		
Labor × 2	26,000	
Electricity	2,500	
Workspace rental fee	10,000	
Transportation (from workspace to distributer twice a month)	5,000	
Labeling (3,000 printed labels)	300	
Zip lock bags (3,000 bags)	1,950	
Total costs (3,000 packages)	45,750	
Total costs (100g package)	**15.25**	
VAT (16%)	2.44	
Total cost including VAT****	**17.69**	

*D: Drumstick leaves powder. G: Garlic powder. C: Cinnamon powder

**Prices for ingredients in bulk (100kg)

***Estimated annual depreciation cost

****Predicted monthly production total costs for 300 kg (3,000 packages of 100g)

Table 3.5.: Final product price estimation

Supplements	**Product price Ksh/100g**	**Total price* Ksh/100g**
Supplement 1 (90% Drumstick + 5% Garlic + 5% Cinnamon)	174.885	**192**
Supplement 2 (80% Drumstick +10% Garlic + 10% Cinnamon)	163.46	**180**
Supplement 3 (70% Drumstick +15% Garlic + 15% Cinnamon)	152.035	**167**
Supplement 4 (50% Drumstick +25% Garlic + 25% Cinnamon)	129.185	**142**

*The final price of each supplement = Estimated ingredient's cost + estimated monthly machinery and utensils cost + other production costs + 10% profit

Table 3.6.: Food ingredients and formulate supplements cost estimation summarized

(Ksh/100g)	Supplement 1 (90%M+5%G+5%C)	Supplement 2 (80%M+10%G+10%C)	Supplement 3 (70%M+15%G+15%C)	Supplement 4 (50%M+25%G+25%C)
Ingredients	156.575	145.15	133.725	110.875
Machinery and utensils annual depreciation	0.62	0.62	0.62	0.62
Other costs	17.69	17.69	17.69	17.69
Final product price	**192**	**180**	**167**	**142**

* Values are Mean ± SD (Standard deviation). Mean values with different superscripts are significantly different at $P \leq 0.05$ in each column.

3.11.6. Shelf life

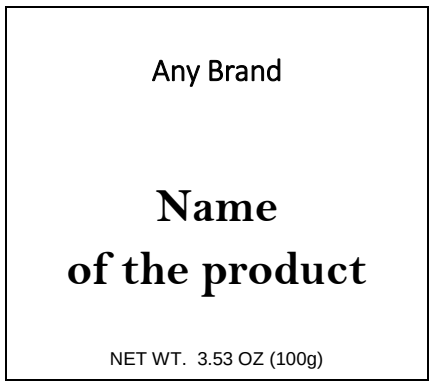
The organoleptic properties such as taste, odor, texture and appearance of all supplements were monitored during the shelf time test. The samples kept at 50 - 55 ^{o}C for 12 hours and checked every one hour. Each day at 55 ^{o}C is equal to one month storage of the product at standard conditions at 25 ^{o}C. No significant changes were observed in organoleptic properties of the samples. The best date before one year after production date was determined as the appropriate shelf time of the supplements.

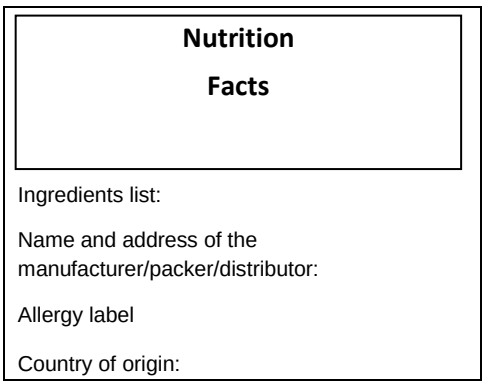
3.11.7. Packaging

The appropriate package for the product was chosen to be the plastic ziplock bags of 100g sealed by the electronic ziplock bag sealer to provide a cost effective product for the consumer while it can protect the product for a long shelf life and safe as FDA regulations for food products standards.

3.11.8: Labeling

The general label designed for the product is as the following picture:





CHAPTER FOUR: ANTIDIABETIC AND ANTIOXIDANT PROPERTIES OF METHANOLIC EXTRACT FOR A DIABETIC SUPPLEMENT DEVELOPED FROM DRUMSTICKS LEAVES (*MORINGA OLEIFERA*), GARLIC (*ALLIUM SATIVUM*) AND CINNAMON (*CINNAMOMUM VERUM*) POWDERS

4.1. Abstract

Diabetes Mellitus is a progressive and chronic metabolic disorder which is defined by hyperglycemia. The disease is a result of insufficient insulin hormone secretion or its action. One of the methods to control blood glucose level is to inhibit the action of α-glucosidase and α-amylase enzymes with the aim of slowing down or decreasing hydrolyze of carbohydrates and therefore management of hyperglycemia in diabetes type II patients. This will help to avoid long and short term consequences of the disease. Plants containing great amounts of phenolic compounds are natural, accessible and cheap sources of α-glucosidase and α-amylase inhibitors. At the same time, the present food supplements made up of natural ingredients has potential to possess antioxidant activity as well. The present research evaluated the antidiabetic effects and antioxidant activity of the methanolic extract of the supplement powders developed from three food ingredients including Drumsticks Leaves (*Moringa oleifera*), Garlic (*Allium sativum*) and Cinnamon (*Cinnamomum verum*) with different percentages with the purpose of determination of the anti-glycemic and antioxidant properties of the supplement to manage blood glucose levels of type II diabetes. The inhibition activity of alpha amylase enzyme is ranged between 97 to 99% for the food ingredients and 95 to 99% for the supplements. The value of inhibition percentage for drumstick leaves is 98.36% ± 0.09. Values observed for cinnamon and garlic were 99.2% ± 0.27 and 97.52% ± 2.05 respectively. The inhibition activity of the supplement no. 3 containing 70% dried drumstick leaves powder + 15% cinnamon powder and 15% garlic powder shows the highest inhibiting

activity of 99.2%. The α-glucosidase enzyme inhibition activity for drumstick leaves is 33.64%, cinnamon 26.73% and garlic 22.64% while the supplements activity are ranged between 15.55 to 28.69%. Supplement no. 3 has the highest inhibition activity of 28.69%. Radical scavenging activity of methanolic extracts was performed using 3 different concentrations of 10, 20 and 30µg of each sample extract. In this research, the activity of drumstick leaves in 10µg/ml show 74.65%, 79.67% in 20µg/ml and 76.17% in 30µg/ml. The obtained values for cinnamon are 85.98% in 10µg/ml, 90.33% in 20µg/ml and 89.81% in 30µg/ml while the radical scavenging activity of garlic is 72.57% in 10µg/ml, 67.92% in 20µg/ml and 63.84% in 30µg/ml. DPPH radical scavenging activity of the supplements are ranged from 66.96 to 71.90% in 10µg/ml, 65.62 to 80.04% in 20µg/ml and 73.16 to 80.96% in 30µg/ml as shown in Table 4.4. Supplement no. 2, containing 80% dried drumstick leaves powder, 10% cinnamon powder and 10% garlic powder in 30µg/ml concentration shows the highest radical scavenging activity, 81%, in comparison with other supplements in different concentrations. The research proved that the formulated supplements have high antidiabetic and antioxidant properties. Therefore, the products can be consumed by type II diabetic patients as a safe, low calorie, natural and low-price supplement to manage hyperglycemia and prevent the long and short time consequences of the unmanaged disease and accordingly retard and decrease complications during lifetime.

4.2. Introduction

The epidemic of diabetes type II, as a public health global crisis threatens population's economies and health specially developing nations. The crisis accelerates by more sedentary lifestyle, rapidly urbanization and nutrition habits changes. Obesity and overweight increment helps the current situation gets even worse globally (HU, 2011).

Diabetes is continuously increasing in the world not only in rich populations but also in countries with middle income (Chan, 2016). DM as a serious public health problem is one of the four NCDs diseases that the leaders in the world have targeted. During the past decades, the incidence and prevalence of the disease have been on a rise constantly. It was estimated that the prevalence of diabetes cases have been elevated since 1980 with 108 million to 422 million patients in 2014 with the high raise of 8.5% which is predicted to reach over 592 million in 2035. In 2012, a total number of 3.7 million deaths were reported caused by diabetes. The prevalence rate in middle and low income countries has shown a faster increment than the high income populations during the last 30 years (World Health organization, 2016). Glycemic management can decrease the rate of consequences of diabetes like cardiovascular problems, neuropathy, nephropathy, retinopathy, limb amputation and death. Each 1% decrement of HbA1C, can effectively lower about 35% in microvascular problems and 25% death rate related to diabetes type II (ADA, 2018). Metformin is the most known and first line medicine to treat diabetes which is accompanied with side effects such as gastrointestinal discomfort, vomiting, nausea and diarrhea (Kim *et al*., 2012; McCreight, Bailey and Pearson, 2016; Bonnet and Scheen, 2017). Meglitinides, gliclazide and glimepiride as sulfonylureas, can cause hypoglycemia and weight gain (Tran *et al*., 2015). Medicine's Agency (EMA) has suppressed the authorization for marketing Thiazolidinediones (e.g. pioglitazone) because of its cardiovascular risks (National Institute for Health and Clinical Excellence, 2009). Tran and others (2015) reported the increased risk of bladder cancer and heart failure with Thiazolidinediones intake. Well-timed commencement and suitable intensification of insulin injection is a challenge in DM management (Bajaj, 2018). Insulin qua a blood glucose level in DM treatment, herewith the fear and pain on frequent injections, difficulty of the regimens and worries about the self-management failure are considered as the obstacles in insulin therapy initiation (Fonseca and Haggar, 2014).

There are many ways of treating and managing type II diabetes. Nowadays, there is more attention on natural options rather than the chemical and synthetic drugs to both treating and managing the disease. Combined herbal supplementation is one of the best methods which can be helpful to control blood glucose levels, possessing great health benefits while being cost effective for both diabetics and healthy people. There are various types of natural supplements which can be used to manage diabetes type II. Drumstick leave is a rich source of nutraceuticals with high biological capacity to effect on chronic diseases such as diabetes, cancer, inflammation, microbial infections, etc. (Udechukwu, 2018). *Cinnamon verum* is a potential healing factor with several useful effects in managing diabetes type II (Ranasinghe *et al*., 2012). Garlic, a traditional herbal remedy with various health benefits can effectively manage glucose levels and act as an antioxidant (Tsai *et al*., 2012).

This study sought to analyse the potential of drumstick leaves, garlic and cinnamon utilization in developing a low calorie supplement with high antidiabetic and antioxidant activity for type II diabetes management.

4.3. Materials and methods

4.3.1. Chemicals

Methanol extra pure (Loba Chemie, batch no.: LB020707), Hydrochloric Acid 35.4% (Lot no.: A164061506), Formic acid 98% (Lot no.: A154251501), Petroleum ether (Fisher scientific UK, Code: P/1760/17, Lot: 121048).

4.3.2. Ingredients

The three food ingredients used to formulate the present food supplements were chosen for their known anti-diabetic properties including drumsticks leaves, garlic and cinnamon shown in Table 4.1.

4.4. Sample collection and preparation

Samples were collected including drumstick leaves, cinnamon and garlic. The fresh drumstick leaves were collected from Chiromo campus, University of Nairobi, Nairobi, Kenya. The cinnamon barks and fresh garlic were purchased from Open air markets, Westlands, Nairobi, Kenya.

The drumstick leaves were collected directly from the tree, washed and rinsed with distilled water, dried in air Oven (Memmert GmbH+Co.KG, D-91126 Schwabach FRG, Germany. type: UE 500, F.-Nr.: c504.0495) at 60-65 ^{o}C for 3:30 hours. Then milled by home miller (VON Hotpoint, model no.: HB251KW, China). A 125 micron sieve was applied to obtain a fine powder. The fresh garlic was cleaned and size reduced, dried in air oven for 6 hours at 60-65 ^{o}C. Milled and sieved through 125 micron sieve. The cinnamon barks washed, dried in air oven at 60-65 ^{o}C for 4 hours, milled and sieved through 125 micron sieve. The process flow diagram for product formulation is shown in Figure 4.1.

Table 4.1: Food ingredients and supplements proportions used for the formulation

Supplement no.	Food ingredients (%)
I1	Dried Drumstick leaves powder - 100%
I2	Cinnamon powder - 100%
I3	Garlic Powder - 100%
S1	Dried Drumstick leaves powder 90% - Cinnamon Powder 5% - Garlic powder 5%
S2	Dried Drumstick leaves powder 80% - Cinnamon Powder 10% - Garlic powder 10%
S3	Dried Drumstick leaves powder 70% - Cinnamon Powder 15% - Garlic powder 15%
S4	Dried Drumstick leaves powder 50% - Cinnamon Powder 25% - Garlic powder 25%

4.5. Methanolic extract preparation

Petroleum ether (125ml) was added to 1.25g in 1:10 (w/v) ratio of each sample in order to remove the fats. The samples were kept in normal water bath (Memmert, type: WB 22, F.Nr.: 1505.0320,

Memmert GmbH+Co.KG, Germany) for 30 minutes at 50-60̊ C. The samples placed in centrifuge (Model Supra 22K, serial no.: 4241227, Human lab instruments co., Korea) at 13,000rpm for 5 minutes. All the supernatants were removed. The residue was air dried overnight. Each defatted sample (1g) was extracted by sequentially adding 10ml of HCL-methanol (1ml/100ml) and then by adding 10ml of HCL-methanol-water (1ml/79ml/100ml) and 10ml of HCL-methanol-water (1ml/49ml/100ml) in the water bath for 30 min at 50-60 ^{o}C. The samples were centrifuged for 5 min at 13,000rpm. The supernatants were pooled together after each extraction. Each sample extract was eluted by adding 10ml of methanol-water. Methanol was removed by a rotary vacuum evaporator at 337mbar pressure for 30-35 min at 40 ^{o}C each sample. Then samples were freeze dried by lyophilizer for 24 hours at -10 ^{o}C, +35mbar. The residue was weighed; the total dry yield of the extract calculated. Each sample extract was re-dissolved in water:methanol:formic acid (47.5:47.5:5, v/v/v) solution the ratio of 1mg of extract per milliliter of solvent and used for further tests.

4.6. Supplement formulation

Linear programming model was used to blend the different proportions of the food ingredients and in selection of an optimized supplement powder in quantity of nutrients including vitamins and minerals according to RDA.

The four selected formulations selected in different proportions including S1: Dried Drumstick leaves powder 90% + Cinnamon Powder 5% + Garlic Powder 5%. S2: Dried Drumstick leaves powder 80% + Cinnamon Powder 10% + Garlic Powder 10%. S3: Dried Drumstick leaves powder 70% + Cinnamon Powder 15% + Garlic Powder 15%. S4: Dried Drumstick leaves powder 50% + Cinnamon Powder 25% + Garlic Powder 25%). The samples were prepared and labeled as shown in Table 4.1.

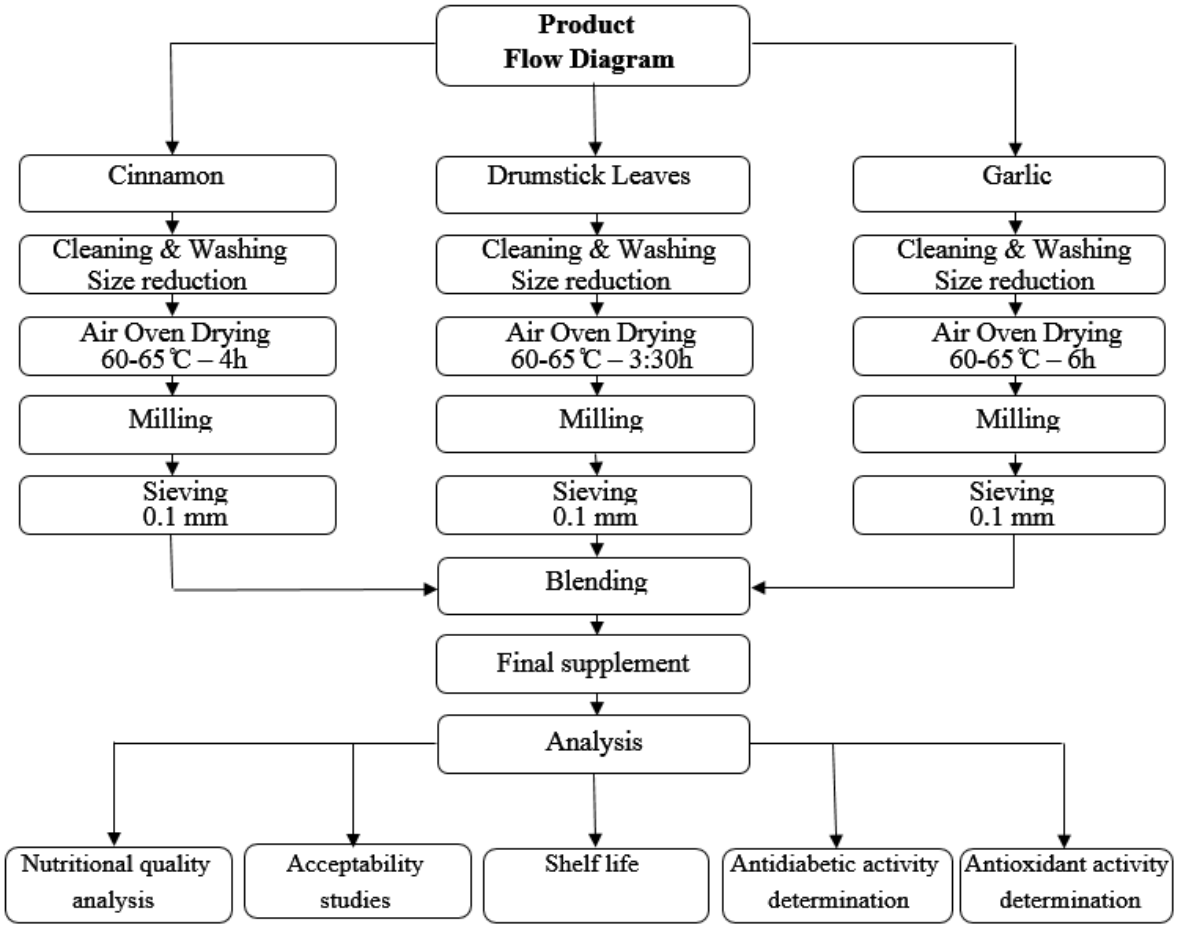


Figure 4.1.: Process flow development of low calorie antidiabetic supplement

4.7. Antidiabetic effect

4.7.1. α-Amylase inhibition activity assay

In order to measure alpha amylase inhibition activity of each ingredient and the supplements, Worthington (1993) method was used. The methanolic extract of each sample (500 µl) was added to 500 µl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006M NaCl) which contained Hog pancreatic α-amylase (EC 3.2.1.1) (0.5 mg/ml) and were incubated for 10 min at 25°C. The mixture of Sodium phosphate buffer 0.02M + Sodium chloride 0.006M (pH 6.9) and 500µl starch solution 1% (1g starch + 100ml distilled water) was prepared and regularly added up to all test tubes.

Thereafter, incubation for 10 minutes at ambient temperature (25° C), the reaction came to a standstill after adding 1ml color reagent (dinitrosalicylic acid). Afterwards, the tubes were incubated for 5 minutes at 95 ^{o}C water bath and left to cool at room temperature. Adding distilled water (15ml), the reaction was diluted. Using the spectrophotometer (JENWAY 6305), absorbance recorded at 540 nm. Readings were recorded and compared with the control containing buffer in place of samples. The percentage of enzyme inhibitory of the aqueous extracts was calculated according to the following formula:

$$\text{Inhibition (\%)} = (A_{\text{control}} - A_{\text{sample}}) \div A_{\text{control}} \times 100$$

4.7.2. Alpha-glucosidase inhibition activity assay

Phosphate buffer 0.1M (100 µl) at pH 6.9 consisting alpha-glucosidase solution (1unit/ml) and 50 µl of each sample (100µg/ml) were transferred to the 96-well flat-bottom microtitration plate and then pre-incubated for 10 min at 25 ^{o}C. Frequently was added to each well 50µl of 5 mM PGPP (P-nitrophenyl-α-D-glucopyranoside) solution in 0.1M phosphate buffer pH 6.9. Afterwards, the reaction mixture was incubated for 5 min at 25 ^{o}C. The absorbance readings were recorded at 405nm using Eliza reader (ref. no.: 51118170). The values were compared with the control that had 50 µl buffer solution in place of sample extract. Alpha-glucosidase inhibition activity was calculated in percentage using formula:

$$\text{Inhibition (\%)} = (A_{\text{control}} - A_{\text{sample}}) \div A_{\text{control}} \times 100$$

4.8. Antioxidant activity

4.8.1. DPPH radical scavenging activity

Sanchez-Moreno *et al.* (1998) method with some modifications was used to evaluate the antioxidant activity of each methanolic extract. DPPH methanolic solution 500 µl (0.0157g/200 ml methanol) was added to 3 different concentrations of 10, 20 and 30 µg/ml of each sample extract. After

incubation at 25 ^{o}C for 30 mins, the readings were measured at 515nm with spectrophotometer. The tested sample's radical scavenging activity was calculated as a decline in the absorbance of DPPH radical scavenging activity:

$$\text{DPPH radical scavenging activity (\%)} = (1\text{-}A_{\text{sample}} \div A_{\text{negative control}}) \times 100$$

4.9. Statistical analysis

Each test was repeated in triplicate (n=3). The results presented as mean ± SD (Standard deviation). Data analysis was done by two-way ANOVA (Analysis of Variance) to specify the significant differences of supplements. SPSS (version 23) was used to perform statistical analysis.

4.10. Results and discussion

4.10.1. Antidiabetic activity

4.10.1.1. Alpha-Amylase inhibition activity assay

In the present research, α-amylase enzyme inhibition activity of the methanolic extracts of Drumsticks Leaves, Garlic and Cinnamon and the formulated supplements were studied.

The enzyme inhibition activity percentage of the methanolic extracts tested in this study, is shown in Table 4.2. The inhibition percentage was ranged between 97 to 99% for the three food ingredients and 95 to 99% for the supplements. The value of inhibition percentage for drumstick leaves was 98.36% ± 0.09, cinnamon 99.2% ± 0.27 and garlic 97.52% ± 2.05. The inhibition activity of supplement no. 3, containing 70% dried drumstick leaves powder + 15% cinnamon powder and 15% garlic powder showed the highest value of 99.2% ± 0.72. There was overall significant difference between the ingredients and supplements (p-value < 0.05). Ingredients no. 1, 3 and supplement no. 4 have no significant difference in (p-value > 0.05).

Table 4.2. Alpha-amylase activity of the methanolic extracts of food ingredients and developed supplements

Food ingredients and supplements	α-amylase inhibition activity (%)*
I1- Dried Drumstick leaves powder - 100%	$98.36^{a} \pm 0.092$
I2- Cinnamon powder - 100%	$99.20^{b} \pm 0.271$
I3- Garlic Powder - 100%	$97.52^{c} \pm 2.052$
S1- Dried Drumstick leaves powder 90% - Cinnamon Powder 5% - Garlic powder 5%	$98.73^{d} \pm 0.890$
S2- Dried Drumstick leaves powder 80% - Cinnamon Powder 10% - Garlic powder 10%	$95.21^{abcdef} \pm 1.589$
S3- Dried Drumstick leaves powder 70% - Cinnamon Powder 15% - Garlic powder 15%	$99.20^{e} \pm 0.722$
S4- Dried Drumstick leaves powder 50% - Cinnamon Powder 25% - Garlic powder 25%	$97.95^{f} \pm 0.890$

*The values in the table are means of the triplicates ± SD

- The values are significantly different at (p-value < 0.05), in case of difference in superscript letters

The recent researches have proved the high ability of α-amylase inhibition activity as a natural alternative for synthetic drugs in type II diabetes management. Drumstick leaves have shown remarkable α-amylase inhibitory activity due to its high phenolic contents (Jimoh, 2018). Verspohl and others (2005) and Sudha and others (2011) have also described α-amylase inhibition activity of both cinnamon and garlic as a blood glucose reducers and insulin level increasers.

There are number of therapeutic ways of diabetes type II management such as insulin demand reduction, insulin secretion stimulation, insulin action improvement at tissue level and controlling the action of breakage of disaccharides and oligosaccharides (Funke and Melzing, 2006). One of the effectual ways of diabetes management is to control hyperglycemia through decelerating the digestion of consumed carbohydrates by inhibiting the CHO degrading enzymes. This can notably decrease postprandial blood sugar level after each food intake (Tundis *et al*., 2011).

α-amylase enzyme acts as a catalyzer at the first stage in starch hydrolysis to maltose. At the next step maltose breaks to glucose by α-glucosidase enzyme's action as the catalyzer. Accordingly, retardant action of α-amylase in starch digestion, can effectively control hyperglycemia after each meal in diabetic patients (Brayer *et al*., 1995; Tundis *et al*., 2011).

4.10.1.2. α-Glucosidase inhibition activity of food ingredients and supplements

In this research methanolic extracts were evaluated for α-glucosidase enzyme inhibition activity, which was between 22 to 33% for the three ingredients. Drumstick leaves showed 33.64%, cinnamon 26.73% and garlic 22.64% inhibition activity and the supplements were ranged from 15.55 to 28.69%, shown in Table 4.3. Supplement no. 3 demonstrates the highest activity with 28.69% inhibition amongst the other supplements. There was no overall significant difference between the ingredients and supplements (p-value > 0.05). No significant difference in (p-value > 0.05) was between Supplements no. 1, 2 and 3.

Table 4.3. α-Glucosidase enzyme inhibition activity of the methanolic extract of food ingredients and supplements

Food ingredients and supplement	α-glucosidase inhibition activity (%)*
I1- Dried Drumstick leaves powder - 100%	$33.64^{a} \pm 0.551$
I2- Cinnamon powder - 100%	$26.73^{b} \pm 0.622$
I3- Garlic Powder - 100%	22.64 ± 1.333
S1- Dried Drumstick leaves powder 90% - Cinnamon Powder 5% - Garlic powder 5%	$28.34^{c} \pm 1.945$
S2- Dried Drumstick leaves powder 80% - Cinnamon Powder 10% - Garlic powder 10%	25.86 ± 3.108
S3- Dried Drumstick leaves powder 70% - Cinnamon Powder 15% - Garlic powder 15%	28.69 ± 11.370
S4- Dried Drumstick leaves powder 50% - Cinnamon Powder 25% - Garlic powder 25%	$15.55^{abcd} \pm 8.179$

* The values in the table are means of the triplicates ± SD

-The values are significantly different at (p-value < 0.05) where superscripts are shown by different letters

Previous researches have shown that one of the effective curative ways in controlling blood glucose level in DM is to manage postprandial hyperglycemia by alpha glucosidase enzyme inhibition. This enzyme catalyzes the action of carbohydrates hydrolyzing; therefore its inhibition will end up to retard glucose absorption and avoids hyperglycemia. Many research works have been done through recent years to find effective natural AGI (Alpha glucosidase inhibitor) to substitute the synthetic medicines in order to avoid the side effects like diarrhea, stomach pain, flatulence and bloating. Plants secondary metabolites are rich phytochemical sources like phenolic compounds, anthocyanins, flavonoids, terpenoids, glycosides and alkaloids having appreciable inhibitory activity against α-glucosidase enzyme (Bukhari *et al*., 2017).

Dried drumstick leaves powder can effectively inhibit the action of α-glucosidase enzyme and therefore has the potential to act as a natural food source to manage type II diabetes. The thermic effect of drying process can degrade the composition of chemical compounds (Natsir *et al.*, 2018). Cinnamon contains phenolic phytochemicals which inhibits the action of α-glucosidase, the carbohydrate hydrolyzing enzyme (Brown *et al.*, 2017). Garlic possesses antidiabetic properties through its inhibition of alpha-glucosidase and alpha-amylase accompanied by the ability of lipid peroxidation prevention in heart and pancreas which explains its high antioxidant ability (Oboh *et al.*, 2018). Development of a food supplement with potential of AGI which can work as a functional food containing phenolic compounds to well manage diabetes with a low price and locally available ingredients, was the aim of the present research.

4.10.2. Antioxidant activity

Methanolic extracts were evaluated for DPPH radical scavenging activity. The experiment was performed in 3 different concentrations of 10, 20 and 30µg/ml of each sample extract. In this research, the activity of drumstick leaves in 10µg/ml showed 74.65%, 79.67% in 20µg/ml and

76.17% in 30µg/ml. The inhibition values for cinnamon were 85.98% in 10µg/ml, 90.33% in 20µg/ml and 89.81% in 30µg/ml while the radical scavenging activity of garlic was 72.57% in 10µg/ml, 67.92% in 20µg/ml and 63.84% in 30µg/ml. Radical scavenging activity of the supplements were ranged between 66.96 and 71.90% in 10µg/ml, 65.62 to 80.04% in 20µg/ml and 73.16 to 80.96% in 30µg/ml as shown in Table 4.4. Supplement 2, with 80% dried drumstick leaves powder, 10% cinnamon powder and 10% garlic powder in 30µg/ml concentration showed the highest radical scavenging activity 81%, comparing the other supplements in different concentrations. There was significant difference (p-value < 0.05) between 10 and 30µg/ml in Supplement no. 2.

Table 4.4. Antioxidant activity of the methanolic extract of food ingredients and developed supplements

Food ingredients and supplement samples	DPPH radical scavenging activity (%)*		
	10µg/ml	20µg/ml	30µg/ml
I1- Dried Drumstick leaves powder - 100%	74.65 ± 3.54	79.67 ± 5.93	76.17 ± 2.06
I2- Cinnamon powder - 100%	85.98 ± 1.12	90.33 ± 2.53	89.81 ± 5.01
I3- Garlic Powder - 100%	72.57 ± 3.03	67.92 ± 6.74	63.84 ± 11.26
S1- Dried Drumstick leaves powder 90% - Cinnamon Powder 5% - Garlic powder 5%	70.01 ± 2.55	65.62 ± 10.43	74.24 ± 3.17
S2- Dried Drumstick leaves powder 80% - Cinnamon Powder 10% - Garlic powder 10%	71.90 ± 4.77	76.69 ± 3.98	80.96 ± 1.31
S3- Dried Drumstick leaves powder 70% - Cinnamon Powder 15% - Garlic powder 15%	66.96 ± 9.52	80.04 ± 15.50	73.16 ± 1.13
S4- Dried Drumstick leaves powder 50% - Cinnamon Powder 25% - Garlic powder 25%	71.64 ± 7.76	73.13 ± 10.14	73.16 ± 10.60

* The values in the table are means of the triplicates ± SD

Drumstick leave is a rich source of phenolic antioxidant compounds like quercetin, gallic acid, kaempferol and flavonoids which enables the human body to improve antioxidant system of free radical scavenging ability (Santos *et al*., 2012; Fitriana *et al*., 2016). Many studies have approved

antidiabetic and antioxidant activity of cinnamon along with its antimicrobial, anti-inflammatory, antifungal, mosquito larvicidal, antimycotic, anticancer, nematicidal, antitermitic and insecticide properties (Benzie and Strain, 1999; Bruni *et al*., 2004; Cherioconi *et al*., 2005; Butt *et al*. 2009; Shahid *et al*., 2018). Garlic is known for its various health benefits and antioxidant properties (Galano and Francisco-Marquez, 2009). It is proved that garlic extracts are potential natural antioxidants (El-Hamidi and El-Shami, 2015).

DPPH radical scavenging activity is a very common method to evaluate the antioxidant activity of all liquid or solid food samples to measure the capability of the free radical scavengers of food compounds or hydrogen donors. DPPH is used to assess the overall antioxidant capacity of a food sample to specify a special antioxidant component (Parasad *et al*., 1995). The modified method of Sanchez-Moreno *et al*. (1998) was used in this study.

CHAPTER FIVE: ANTIDIABETIC AND ANTIOXIDANT PROPERTIES OF ETHANOLIC EXTRACT FOR A DIABETIC SUPPLEMENT DEVELOPED FROM DRUMSTICKS LEAVES (*MORINGA OLEIFERA*), GARLIC (*ALLIUM SATIVUM*) AND CINNAMON (*CINNAMOMUM VERUM*) EXTRACTS

5.1. Abstract

Diabetes type II is a set of syndromes with incessant hyperglycemia owing to different metabolic disorders such as Krebs cycle, synthesis and release of insulin, gluconeogenesis, cholesterol synthesis. Plants are natural sources of compounds that have been well-known for their effectiveness in treating several diseases since early times. Public believes about medicinal plants have helped the researchers to investigate and explore more in this area. This research was carried out in order to determine the antidiabetic and antioxidant properties of the supplements formulated from different proportions of three plants which are well known for their high anti-hyperglycemic and antioxidant properties like drumstick leaves, garlic and cinnamon. The four selected formulations selected in different proportions included S1: Dried drumstick leaves powder 90% + cinnamon powder 5% + garlic powder 5%. S2: Dried drumstick leaves powder 80% + cinnamon powder 10% + garlic powder 10%. S3: Dried drumstick leaves powder 70% + cinnamon powder 15% + garlic powder 15%. S4: Dried drumstick leaves powder 50% + cinnamon powder 25% + garlic powder 25% shown in Table 5.1. The ethanolic extracts of the ingredients and the supplements were prepared using the standard method. Alpha-amylase and alpha-glucosidase enzymes inhibition action of the supplements and the ingredients was used to determine the antidiabetic activity while the antioxidant activity was determined using DPPH assay. Alpha amylase inhibitory activity of ingredients ranged between 41 to 53% and the highest activity of supplements observed in S1 and S3 with 53.651% ± 0.149 and 53.055% ± 0.298 while the highest

alpha glucosidase enzyme inhibition was found in S4 containing 50% dried drumstick leaves powder, 25% cinnamon powder and 25% garlic powder. α-glucosidase activity of the ingredients was between the range of 43 to 85%. S3, with 70% dried drumstick leaves powder, 15% cinnamon powder and 15% garlic powder in 20µg/ml concentration demonstrated the highest radical scavenging activity in comparison with other supplements in different concentrations. Plants with antidiabetic properties play a key role in managing hyperglycemia and thus can help to prevent short and longtime complications of diabetes type II. Comprehending this important role, the results of this research can be suggested as a potential natural supplement which is a nutritive and therapeutic food with an affordable price and accepted by the patients to manage diabetes type II.

5.2. Introduction

One of the most important chronic diseases in the world is diabetes mellitus. It is a caused by pancreas deficiency when enough insulin is not produced or patient's body is not able to use the secreted insulin. If diabetes is not managed, high blood glucose level occurs and will cause serious damages to main body organs such as nerves, eyes, kidneys and blood vessels (WHO, 2017). Medical systems are still faced with challenges to manage diabetes with no side effects (Chakraborty and Rajagopalan 2002; Kameswararao *et al*., 2003; Geethalakshmi *et al*., 2010).

Post-prandial hyperglycemia control by retarding glucose absorbance is a key method in DM treatment. α-amylase and α-glucosidase are enzymes which hydrolyze carbohydrates in digestive system and their inhibition will retard digestion period and decrement of glucose absorption rate. This will result in preventing rapid blood glucose rise (Rhabasa-Lhoret and Chiasson, 2004). The main goal of diabetes management is to preserve both fasting blood sugar level and post-prandial near normal range (Matsui *et al*., 2007). In high income countries, patients suffering from chronic

diseases like diabetes, tend to consume plant medicine from natural sources due to less adverse effects in comparison with synthetic drugs (Klepser and Klepser, 1999; WHO, 2002; Hamdan and Afifi, 2004). In comparison with chemical drugs, plants with medical properties are low price with less side effects that can efficiently manage type II diabetes while are affordable in lower income countries (Kooti *et al*., 2016) and are prescribed more frequently (Li *et al.*, 2004).

Polyphenols with phenol rings are a wide class of phytochemicals (Hanhineva *et al*., 2010). Natural foods like legumes, fruits, cereals, vegetables and beverages contain hundreds of various polyphenols (Pandey *et al*., 2009; Scalbert *et al*., 2005; Xiao *et al*., 2015). Dietary polyphenols are capable of ameliorating sugar hemostasis in β-cells, liver and small intestine. Results obtained from recent researches have shown that dietary polyphenols can lower the risk of diabetes type II (Kim *et al*., 2016).

The two enzymes, α-amylase and α-glucosidase are important in blood glucose level increment. These enzymes catalyze the action of α-glucose secretion from the non-reducing end of the substrate (Sidhu *et al*., 2014). After carbohydrate intake, blood glucose raise decreases by enzyme inhibitors (Abirami *et al*., 2014). These enzymes are located in the epithelium of small intestine and absorb glucose. The enzymes catalyze oligosaccharides cleavage into monosaccharides to be absorbed (Kumar *et al*., 2011). When the enzymes are inhibited in small intestine, the oligosaccharide cleavage rate reduces. The digestion process of the consumed carbohydrates in the small intestine moves to the lower part and consequently makes the digestion process to delay. The total absorbed sugar rate reduces and postprandial blood glucose declines (Kimura *et al*. 2004; Lebovitz, 2005; Kumar *et al*., 2013).

An important benefit of plant foods is the capability of free radicals scavenging. This can decrease the incidence of diabetes and other chronic diseases. Lifestyle and dietary moderations in order to improve antioxidant supply prevent oxidative stress efficiently (Dal and Sigrist, 2016). Plant sources in a normal diet provide antioxidant compounds of different classes and have various properties chemically and physically (Sravani and Paarakh, 2012). DPPH free radical scavenging method is the most accepted, easy, fast and low-price to determine the antioxidant activity of foods and drugs (Kirtikar and Basu, 2006). DPPH assay determines the capability of food compounds as free radical scavenger to specify their antioxidant activity. DPPH at 517 nm has a high absorption for possessing a single electron (Alamed *et al*., 2009). Immediately after an antioxidant compound as a proton donor is mixed with DPPH radical as the reducing factor, is reduced to DPPH-H (Miliauskas *et al*., 2004). The solution is changed from purple to yellow which depends on the ability of hydrogen donation of antioxidant food compounds. DPPH Reduction by an antioxidant compound is shown as below (Hangun-Balkir and McKenney, 2012):

$$DPPH + A\ (antioxidant) \rightarrow DPPH\text{-}H + A^{\bullet}$$

DPPH possess an odd electron and in reaction with antioxidant food compounds, at 517nm gives the maximum absorption. When DPPH reacts with antioxidant at the presence of a hydrogen donor, it reduces to DPPHH (Harborne, 1998). Decolorization from purple to yellow results from DPPH to DPPHH form which depends on the captured electrons number (Ghosh, 1998). The more the reducing power, the more the decolorization will be (Wagner *et al*., 1996). In case of mixing a DPPH solution with a hydrogen donor compound, the reduced form (DPPH) will increase. This reaction causes a color transformation from purple to yellow (Handa *et al*., 2006).

This study sought to analyze the potential of drumsticks leaves, garlic and cinnamon in developing a low calorie supplement with high antioxidant and antidiabetic activity for type II diabetes management.

5.3. Methods and materials

5.3.1. Chemicals

Petroleum ether (Fisher scientific UK, Code: P/1760/17, Lot: 121048), Methanol extra pure (Loba Chemie, batch no.: LB020707), Hydrochloric Acid 35.4% (Lot no.: A164061506), Formic acid 98% (Lot no.: A154251501).

5.3.2. Ingredients

The three food ingredients used to formulate the present food supplement were chosen for their known anti-diabetic properties which included drumsticks leaves, garlic and cinnamon.

5.4. Sample collection and preparation

Samples collected included Drumstick Leaves, Garlic and Cinnamon. The fresh drumstick leaves were collected from Chiromo campus, University of Nairobi, Nairobi, Kenya. The Cinnamon barks and the fresh Garlic were purchased from Open air markets, Westlands, Nairobi, Kenya.

The Drumstick leaves were collected directly from the tree, washed and rinsed with distilled water, dried in hot air Oven (Memmert GmbH+Co.KG, D-91126 Schwabach FRG, Germany. type: UE 500, F.-Nr.: c504.0495) at 65 ^{o}C for 4 hours. Then milled by home miller (VON Hotpoint, model no.: HB251KW, China) and sieved through 125 micron sieve to obtain a fine powder. The fresh Garlic was cleaned and size reduced, dried during 6 hours in hot air oven at 65 ^{o}C. Milled and sieved through 125 micron sieve. The Cinnamon barks washed, milled and sieved by a 125 micron sieve. Then all of the samples were prepared and labeled as Table 5.1:

Table 5.1.: Food ingredients and proportions used in formulation of food supplements percentages

Supplement no.	Food ingredients (%)
I1	Dried Drumstick leaves powder - 100%
I2	Cinnamon powder - 100%
I3	Garlic Powder - 100%
S1	Dried Drumstick leaves powder 90% - Cinnamon Powder 5% - Garlic powder 5%
S2	Dried Drumstick leaves powder 80% - Cinnamon Powder 10% - Garlic powder 10%
S3	Dried Drumstick leaves powder 70% - Cinnamon Powder 15% - Garlic powder 15%
S4	Dried Drumstick leaves powder 50% - Cinnamon Powder 25% - Garlic powder 25%

5.5. Supplement formulation

Linear programming model was used to blend the different proportions of the food ingredients and in selection of an optimized supplement powder in quantity of nutrients including vitamins and minerals according to RDA.

The four selected formulations selected in different proportions included: S1: dried drumstick leaves powder 90% + cinnamon powder 5% + garlic powder 5%. S2: dried drumstick leaves powder 80% + cinnamon powder 10% + garlic powder 10%. S3: dried drumstick leaves powder 70% + cinnamon powder 15% + garlic powder 15%. S4: dried drumstick leaves powder 50% + cinnamon powder 25% + garlic powder 25%).

5.6. Ethanolic extract preparation

Adding 125ml petroleum ether, in 1:10 (w/v) ratio to 1.25g of each sample the fats were removed. The samples were kept in normal water bath (Memmert, type: WB 22, F.Nr.:1505.0320, Memmert GmbH+Co.KG, Germany) at 50-60° C for 30 minutes. The tubes were placed in centrifuge (Model

Supra 22K, serial no.: 4241227, Human lab instruments co., Korea) at 13,000rpm for 5 minutes. All the supernatants were removed and the residue was kept to be air dried overnight. Each sample was extracted by adding 10 ml of aqueous ethanol 50% for 30 minutes and centrifuges at 13,000rpm for 5 minutes. The same procedure was repeated for 6 times after each extraction. All the supernatants were pooled/collected together after each extraction into falcon tubes and were made up to a known volume of 32.5ml with ethanol. The solvent removal was done using rotary vacuum evaporator.

At 175mbar pressure for 35-40 minutes at 40 ^{o}C for each sample. All the samples were frozen overnight at the temperature of -80 ^{o}C freezer. Afterwards they freeze dried in lyophilizer (Christ alpha I-6, Medizinischer apparatebau, type 1052) at +35mbar pressure and -10 ^{o}C for 24 hours. The weight of the residue was measured. The extract's total dry yield calculated. Each extract was re-dissolved in ethanol:formic acid (97.5:2.5, v/v) solution in the ratio of 1mg/ml. All the extracts were kept in fridge for further tests.

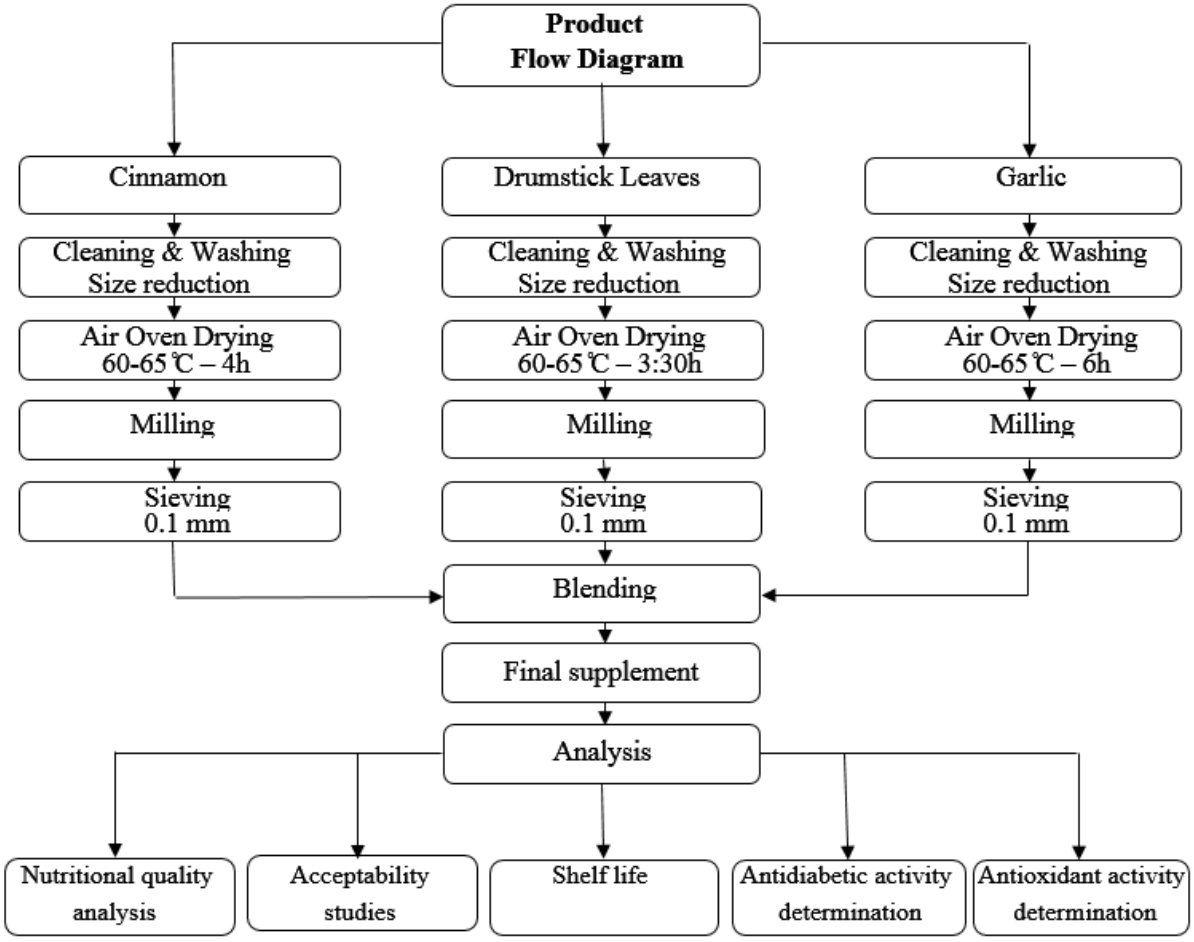


Figure 5.1: Process flow diagram for the product development

5.7. Antidiabetic effect

5.7.1. α-Amylase inhibition activity assay

α-Amylase inhibition activity of each ingredient and the supplements was determined by Worthington (1993) method. The ethanolic extract (500 µl) of each sample was prepared. Sodium phosphate buffer 0.02 M (500 µl) (pH 6.9 with 0.006M NaCl) comprising 0.5 mg/ml Hog pancreatic α-amylase (EC 3.2.1.1) were preserved for 10 min at 25°C. Then, 500 µl of starch solution 1% in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006M NaCl) was added to each tube frequently. The reaction mixtures were incubated at 25°C for 10 min and stopped by adding 1.0 ml dinitrosalicylic acid color reagent. Thereafter, the mixture was incubated in water bath at boiling

temperature for 5 min and cooled to ambient temperature. Adding distilled water (15ml), the reaction was diluted. Using the spectrophotometer (JENWAY 6305), absorbance was read at 540 nm. The values were compared with the control possessing buffer in place of sample extract. The inhibition percentage extracts were calculated by the formula below:

$$\text{Inhibition (\%)} = (A_{\text{control}} - A_{\text{sample}}) \div A_{\text{control}} \times 100$$

5.7.2. α-Glucosidase inhibition activity assay

Phosphate buffer 0.1M (100 µl), pH 6.9 consisting α-glucosidase solution (1unit/ml) and 50 µl of each extract sample (100µg/ml) were transferred to a 96-well flat-bottom microtitration plate and pre-incubated for 10 min at 25 ^{o}C. To each well frequently was added 50µl of 5 mM p-nitrophenyl-α-D-glucopyranoside (PGPP) solution in 0.1 Mphosphate buffer pH 6.9. The reaction mixture incubated for 5 min at 25 ^{o}C. Afterwards, the readings of Eliza reader (ref. no.: 51118170) at 405nm were recorded. The obtained data were compared with the control containing 50 µl buffer solution instead of sample extract. The values of enzyme activity was calculated according to below formula:

$$\text{Inhibition (\%)} = (A_{\text{control}} - A_{\text{sample}}) \div A_{\text{control}} \times 100$$

5.8. Antioxidant activity

5.8.1. DPPH radical scavenging activity

The antioxidant activity of each methanolic extract was evaluated according to Sanchez-Moreno *et al.* method (1998) with some modifications. DPPH ethanolic solution (500 µl) (0.0157g/200 ml methanol) was added to 3 different concentrations of 10, 20 and 30 µg of each sample extract. After incubation at 25 ^{o}C for 30 mins, the readings were measured at 515nm with spectrophotometer. The tested sample's radical scavenging activity was calculated as a decline in the absorbance of DPPH radical scavenging activity:

DPPH radical scavenging activity (%) = (1-A sample ÷ A negative control) × 100

5.9. Statistical analysis

Each test was performed in triplicate (n=3). The values presented as mean ± SD. Data analysis was done using two-way ANOVA to specify the significant differences between each supplement using the pure ingredient as the control. SPSS (version 23) was used to perform statistical analysis.

5.10. Results and discussion

5.10.1. Antidiabetic activity

5.10.1.1. α-Amylase inhibition activity assay

In the present study, the inhibition activity of α-amylase enzyme of ethanolic extract of drumsticks leaves, garlic and cinnamon and the supplement products made from these plants is studied.

The percentage of alpha amylase enzyme inhibition activity of the ethanolic extract tested in this study is shown in Table 5.2. The inhibition activity of alpha amylase enzyme in the three main ingredients ranged from 41 to 52%. The percentage of inhibition in supplements ranged from 45 to 54%. The value of inhibition percentage for drumstick leaves is 52.687% ± 0.745, for cinnamon and garlic are 41.206% ± 8.535 and 52.831% ± 0.105 respectively. The inhibition activity of S1 with 53.651% ± 0.149 showed the highest enzyme inhibitory activity.

The test results demonstrated a good ability of α-amylase inhibition activity as a natural alternative for synthetic drugs in type II diabetes management. Drumstick leaves as Jimoh (2018) demonstrated, has remarkable α-amylase inhibitory activity due to its high phenolic contents. Verspohl *et al*. (2005) and Sudha *et al*. (2011) have also described α-amylase inhibition activity of both cinnamon and garlic as a blood glucose reducer and insulin level increaser.

There are some therapeutic ways of diabetes type II management such as insulin demand reduction, insulin secretion stimulation, insulin action improvement at tissue level and controlling the action of breakage of disaccharides and oligosaccharides (Funke and Melzing, 2006). One of the effectual ways of diabetes management is to control hyperglycemia by slowing down the digestion of consumed carbohydrates by inhibiting the CHO degrading enzymes. This can notably decrease postprandial blood sugar level after each food intake (Tundis *et al*., 2011).

Alpha amylase enzyme performs an important function as the first stage in starch hydrolysis. Hydrolyzing the starch to maltose is catalyzed by α-amylase enzyme. At the next step maltose breaks to glucose by alpha glucosidase enzyme as the catalyzer. Accordingly, retardant action of alpha amylase in starch digestion, can effectively control hyperglycemia after each meal in patients with diabetes mellitus (Brayer *et al.*, 1995; Tarling *et al.*, 2008).

Table 5.2.: α-amylase enzyme inhibition activity of the ethanolic extract of food ingredients and developed supplements

Food ingredients and formulated supplements	α-amylase inhibition activity (%)*
I1- Dried Drumstick leaves powder - 100%	52.682 ± 0.738
I2- Cinnamon powder - 100%	41.206 ± 8.535
I3- Garlic Powder - 100%	52.831 ± 0.105
S1- Dried Drumstick leaves powder 90% - Cinnamon Powder 5% - Garlic powder 5%	53.651 ± 0.149^{a}
S2- Dried Drumstick leaves powder 80% - Cinnamon Powder 10% - Garlic powder 10%	45.702 ± 2.906^{ab}
S3- Dried Drumstick leaves powder 70% - Cinnamon Powder 15% - Garlic powder 15%	53.055 ± 0.298^{b}
S4- Dried Drumstick leaves powder 50% - Cinnamon Powder 25% - Garlic powder 25%	51.067 ± 2.114

*The values in the table are means of the triplicates ± SD

- The values with different letter superscripts are significantly different at (p-value < 0.05)

5.10.1.2. α-Glucosidase inhibition activity assay

The α-glucosidase enzyme inhibition activity of the ethanolic extracts in this research for drumstick leaves exhibited 43.174% ± 3.061, cinnamon and garlic 50.943 % ± 0.943 and the supplements were ranged from 35 to 48%. The highest inhibition activity is found in supplement no. 4 containing 50% dried drumstick leaves powder, 25% cinnamon powder and 25% garlic powder, which is shown in Table 5.3.

Previous researches have shown that one of the effective curative ways in controlling the blood glucose level in DM type II is to manage postprandial hyperglycemia by alpha glucosidase enzyme inhibition. This enzyme catalyzes the action of carbohydrates hydrolyzing; therefore its inhibition will end up in retardation of glucose absorption and avoids hyperglycemia (Shai *et al*. 2010; Rouzbehan *et al*., 2017). Many research works have been done through recent years to find effective natural inhibitors of alpha glucosidase (AGI) to substitute the synthetic medicine to avoid the side effects like diarrhea, stomach pain, flatulence and bloating. Plants secondary metabolites are rich phytochemical sources like phenolic compounds, anthocyanins, flavonoids, terpenoids, glycosides and alkaloids having appreciable inhibitory activity against α-glucosidase enzyme (Bukhari *et al*., 2017). The aim of the this research was to develop a food supplement with the potential of AGI which can work as a functional food containing phenolic compounds to well manage diabetes with a low price and locally available ingredients for diabetics.

Table 5.3.: α-glucosidase enzyme inhibition activity of the ethanolic extract of food ingredients and developed supplements

Food ingredients and formulated supplements	α-glucosidase inhibition activity (%)*
I1- Dried Drumstick leaves powder - 100%	43.174 ± 3.061
I2- Cinnamon powder - 100%	85.001 ± 0.121
I3- Garlic Powder - 100%	50.943 ± 0.943
S1- Dried Drumstick leaves powder 90% - Cinnamon Powder 5% - Garlic powder 5%	35.825 ± 5.476^{a}
S2- Dried Drumstick leaves powder 80% - Cinnamon Powder 10% - Garlic powder 10%	47.125 ± 2.777^{a}
S3- Dried Drumstick leaves powder 70% - Cinnamon Powder 15% - Garlic powder 15%	40.869 ± 6.791^{b}
S4- Dried Drumstick leaves powder 50% - Cinnamon Powder 25% - Garlic powder 25%	48.333 ± 1.667^{b}

*The values in the table are means of the triplicates ± SD

- The values whose superscripts are different letters, are significantly different at (p-value < 0.05)

5.10.2. Antioxidant activity

The DPPH radical scavenging activity of ethanolic extracts was performed in 3 different concentrations of 10, 20 and 30µg of each sample extract. In this research, the activity of drumstick leaves in 10µg/ml show 64.76%, 66.847% in 20µg/ml and 66.666% in 30µg/ml. The values for cinnamon are 61817% in 10µg/ml, 70.141% in 20µg/ml and 66.329% in 30µg/ml while the radical scavenging activity of garlic is 58.433% in 10µg/ml, 56.332% in 20µg/ml and 52.949% in 30µg/ml. DPPH radical scavenging activity of the supplements are ranged from 64.462 to 66.056% in 10µg/ml, 64.241 to 67.159% in 20µg/ml and 57.759 to 66.005% in 30µg/ml as shown in Table 6.4. Supplement 6, with 70% dried drumstick leaves powder, 15% cinnamon powder and 15% garlic powder in 20µg/ml concentration shows the highest radical scavenging activity comparing the other supplements in different concentrations.

DPPH radical scavenging activity is a very common method to evaluate the antioxidant activity of all liquid or solid food samples to measure the capability of the free radical scavengers of food compounds or hydrogen donors. DPPH is used to assess the overall antioxidant capacity of a food sample to specify a special antioxidant component (Parasad *et al*., 1995). The modified method of Sanchez-Moreno *et al*. (1998) was used in this study.

Table 5.4.: Antioxidant activity of the ethanolic extract of food ingredients and developed supplements

Food ingredients and formulated supplements	DPPH radical scavenging activity (%)*		
	10µg/ml	20µg/ml	30µg/ml
I1- Dried Drumstick leaves powder - 100%	64.76 ± 3.426	66.847 ± 0.978	66.666 ± 2.662
I2- Cinnamon powder - 100%	61.817 ± 1.594	70.141 ± 1.133	66.329 ± 8.985
I3- Garlic Powder - 100%	58.433 ± 0.898	56.332 ± 1.369	52.949 ± 4.583
S1- Dried Drumstick leaves powder 90% - Cinnamon Powder 5% - Garlic powder 5%	64.552 ± 0.460	65.810 ± 1.536	66.005 ± 4.537
S2- Dried Drumstick leaves powder 80% - Cinnamon Powder 10% - Garlic powder 10%	65.680 ± 1.521	64.241 ± 1.347	62.102 ± 0.566
S3- Dried Drumstick leaves powder 70% - Cinnamon Powder 15% - Garlic powder 15%	66.056 ± 1.744	67.159 ± 2.536	61.156 ± 2.503
S4- Dried Drumstick leaves powder 50% - Cinnamon Powder 25% - Garlic powder 25%	64.462 ± 1.775	64.566 ± 2.572	57.759 ± 4.304

*The values in the table are means of the triplicates ± SD

-There was no significant difference between the supplements in different concentrations.

Ethanolic extract of drumstick shows a high antioxidant activity which is associated with the existence of polyphenolic compounds such as flavonoids (Goswami and Singhai, 2016). During the last decade, herbs and spices have received a great attention for their antioxidant compounds (Omar *et al*., 2007; Embuscado, 2015). Cinnamon possessing cinnamic acid, gallic acid, cinnamaldehyde and quercetin is considered as a spice rich in phytochemicals with noticeable antioxidant properties (Muhammad and Dewettinck, 2017). It is recommended that cinnamon for its long list of beneficial

effects is considered as a functional food for human body that might be a main part of individual's daily diet (Udayaprakash *et al*., 2015). Garlic is very well known for its antioxidant properties containing steroids and flavonoids (Narendhirakannan and Rajeswari, 2010). Liu *et al*. (2014) concludes that phenolic compounds and sulfhydryl present in garlic are responsible compounds for its antioxidant activity.

CHAPTER SIX: GENERAL CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

The results obtained in this research approve that the developed supplements have achieved both the main and the specific objectives of the study.

The analysis showed that all the four supplements are rich sources of protein, vitamins and minerals. Great quantities of vitamins A, C ,B1, B2, B6, Folic acid and minerals such as Iron, Calcium and Magnesium which provides more than 100%/100g RDA of body needs.

The formulated supplements have antidiabetic properties which are able to manage hyperglycemia in type II diabetic patients, and prevent the long and short time consequences of the unmanaged disease during the patient's lifetime.

The formulated supplements showed high antioxidant properties which can control the oxidative stress that is strongly linked to the development of diabetes.

The study has confirmed the long shelf-life of the supplements as an important factor in the market.

The studies on the panelists' show that the supplements are accepted for their organoleptic attributes such as color, appearance, taste, aroma and overall acceptance.

The supplements have potential health benefits and are inexpensive with affordable prices for the patients with Type II diabetes living in low and middle income populations. The supplements are functional foods that are safe, natural, available and accessible. This can assist diabetic patients to improve their quality of life, nutritional status and health level.

6.2. Recommendations

Diabetes is a chronic disease increasing rapidly all over the world annually. Therefore, there is a need for training the patients to self-care, self-management and self-monitoring the blood glucose levels by recording and keeping the data as a daily habit.

With the improving facilities of mobile phones and internet access, it is suggested that an integrated system to be designed in order to keep in touch with the patients regularly giving advices and update them with the recent findings. This can improve the patient's adherence to disease controlling guidelines. Obviously this will improve the quality of their life and life expectancy of diabetics.

It is recommended to determine the effective dosage of the supplements according to body weight, potential adverse effects and therapy duration. It is also recommended to prevent diabetes development especially among the individuals with risk factors before the onset of the disease.

The encouraging results in this research can be carried out on animal and human cases for further future investigations to approve the exact effects on diabetes type II management in vivo.

More research is needed to determine the quantity and the quality of the phenolic compounds of the developed supplements. Antimicrobial properties of each supplement can be determined in further studies as well. New blends developed from other indigenous plant sources ingredients is a good idea to manage or treat malnutrition and other diseases, too.

The supplements can be commercially produced for the market as a functional and healthy food for both diabetic patients and healthy people to improve their health level. Due to the high nutritional composition and the low price of the supplements, it can also play an effective role in treatment of vulnerable and malnourished populations with specified dosage in forms of capsules, tablets or

teabags supported by the stakeholders as a sustainable solution towards zero hunger in the world especially in Africa.

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Appendix 1: Scorecard sensory analysis

Please taste each sample powder and √ how much you like or dislike each characteristic. You can taste the sample more than once if needed.

Sample A	**Appearance**	**Aroma**	**Taste**	**Sweetness**	**Texture/ mouth-feel**
I liked the supplement powder a lot					
I liked the supplement powder a little					
Neither like nor dislike					
I disliked the supplement powder a little					
I disliked the supplement powder a lot					
Sample B	**Appearance**	**Aroma**	**Taste**	**Sweetness**	**Texture/ mouth-feel**
I liked the supplement powder a lot					
I liked the supplement powder a little					
Neither like nor dislike					
I disliked the supplement powder a little					
I disliked the supplement powder a lot					
Sample C	**Appearance**	**Aroma**	**Taste**	**Sweetness**	**Texture/ mouth-feel**
I liked the supplement powder a lot					
I liked the supplement powder a little					
Neither like nor dislike					
I disliked the supplement powder a little					
I disliked the supplement powder a lot					

Appendix 2: PARTICIPANT INFORMATION AND CONSENT FORM

ADULT CONSENT FOR ENROLLMENT IN THE STUDY

(To be administered in English or any other appropriate language e.g. Kiswahili translation)

Title of Study: Development of a food supplement for diabetes type II with enhanced antidiabetic properties from *Moringa leaves, Allium sativum* and *Cinnamomum verum*

Principal Investigator\and institutional affiliation: Hengame Yousefifar Kermani \ University of Nairobi

University Supervisors: Dr. Catherine Kunyanga, PhD – Department of Food Science, Nutrition and Technology, University of Nairobi. Contact no.: 254-722 873 357
Prof. Jasper K. Imungi, PhD – Department of Food Science, Nutrition and Technology, University of Nairobi. Contact no.: 254-721 468 181

Laboratory Technician and Assistant: Mr. Jeremiah Mthika - Department of Food Science, Nutrition and Technology, University of Nairobi. Contact no.: 254-722 918781

Introduction:

I would like to tell you about a study being conducted by the above listed researcher. The purpose of this consent form is to give you the information you will need to help you decide whether or not to be a participant in the study. Feel free to ask any questions about the purpose of the research, what happens if you participate in the study, the possible risks and benefits, your rights as a volunteer, and anything else about the research or this form that is not clear. When we have answered all your questions to your satisfaction, you may decide to be in the study or not. This process is called 'informed consent'. Once you understand and agree to be in the study, I will request you to sign your name on this form. You should understand the general principles which apply to all participants in a nutritional research: i) Your decision to participate is entirely voluntary ii) You may withdraw from the study at any time without necessarily giving a reason for your withdrawal.

We will give you a copy of this form for your records.

May I continue? YES / NO

This study has approval by The Kenyatta National Hospital-University of Nairobi Ethics and Research Committee protocol No. _____

WHAT IS THIS STUDY ABOUT?

The researcher mentioned above is evaluating a natural food supplement product which can decrease the long term hyperglycemia complications among diabetic patients and improve their health and nutritional status with the purpose of finding out its sensory acceptability by the panel members.
Participant in this research study is asked questions to evaluate and describe the product characteristics and acceptancy.

There will be approximately 12 panelists in this study randomly chosen. We are asking for your consent to consider participating in this study.

WHAT WILL HAPPEN IF YOU DECIDE TO BE IN THIS RESEARCH STUDY?

If you agree to participate in this study, the following things will happen:

You will be interviewed by a trained interviewer in a private area where you feel comfortable answering questions. The interview will last approximately 20 minutes. The interview will cover topics such as the physical properties and the taste of the product.

After the interview has finished, no procedures is necessary.

We will ask for a telephone number where we can contact you if necessary. If you agree to provide your contact information, it will be used only by people working for this study and will never be shared with others. The reasons why we may need to contact you is in case of the necessity to repeat it again.

ARE THERE ANY RISKS, HARMS DISCOMFORTS ASSOCIATED WITH THIS STUDY?

We will keep everything you tell us as confidential as possible. We will use a code number to identify you in a password-protected computer database and will keep all of our paper records in a locked file cabinet. Also, answering questions in the interview may be uncomfortable for you. If there are any questions you do not want to answer, you can skip them. You have the right to refuse the interview or any questions asked during the interview.

It may be embarrassing for you to have _____. We will do everything we can to ensure that this is done in private. Furthermore, all study staff and interviewers are professionals with special training in these evaluation.

In case of a complication related to this study, contact the study staff right away at the number provided at the end of this document. The study staff will treat you for minor conditions or refer you when necessary.

WHAT IF YOU HAVE QUESTIONS IN FUTURE?

If you have further questions or concerns about participating in this study, please call or send a text message to the study staff at the number provided at the bottom of this page.

For more information about your rights as a research participant you may contact the Secretary/Chairperson, Kenyatta National Hospital-University of Nairobi Ethics and Research Committee Telephone No. 2726300 Ext. 44102 email uonknh_erc@uonbi.ac.ke.

The study staff will pay you back for your charges to these numbers if the call is for study-related communication.

WHAT ARE YOUR OTHER CHOICES?

Your decision to participate in research is voluntary. You are free to decline participation in the study and you can withdraw from the study at any time without injustice or loss of any benefits.

CONSENT FORM (STATEMENT OF CONSENT)

Participant's statement

I have read this consent form or had the information read to me. I have had the chance to discuss this research study with a study counselor. I have had my questions answered in a language that I understand. The risks and benefits have been explained to me. I understand that my participation in this study is voluntary and that I may choose to withdraw any time. I freely agree to participate in this research study.

I understand that all efforts will be made to keep information regarding my personal identity confidential.

By signing this consent form, I have not given up any of the legal rights that I have as a participant in a research study.

I agree to participate in this research study:	**Yes**	**No**
I agree to provide contact information for follow-up:	Yes	No

Participant printed name: _____

Participant signature / Thumb stamp _____ Date _____

Researcher's statement

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has willingly and freely given his/her consent.

Researcher's Name: Hengame Yousefifar **Date**: 1.12.2016

Signature:

Role in the study: Principle investigator

For more information contact 0701427634 from Monday to Friday from 8:00am to 5:00pm.

Witness Printed Name

Name: Mohammad Kermani **Contact information: 0701414704**

Signature /Thumb stamp: **Date**: 1.12.2016