

**NUTRITIONAL VALUE, ANTI-NUTRIENT LEVEL AND  
EFFECT OF PROCESSING ON *HYPTIS SPICIGERA*  
LAMIACEAE SEED (BLACK BENISEED) AS HUMAN FOOD**

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AGRICULTURE AND TECHNOLOGY**

**2018**

**Nutritional value, Anti-nutrient Level and Effect of Processing on  
*Hyptis spicigera* Lamiaceae seed (Black beniseed) as Human Food**

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**A Thesis Submitted in Partial Fulfilment for the Degree of Master of  
Science in Food Science and Nutrition in The Jomo Kenyatta  
University of Agriculture and Technology**

**2018**

## **DECLARATION**

This thesis is my original work and has not been presented for a degree in any other university.

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## **DEDICATION**

To the memory of my late father Prof. Natalino Abe Banja and mother Joyce Maka Matatia.

My sisters Akulia and Tiyu and brothers Lomoro, Lomore and Abe.

To my beloved Husband Dr.Yatta Samuel Lukou and our lovely kids Ngerja, Gubang and daughter Warun.

## **ACKNOWLEDGEMENTS**

Thanks to God the almighty for protecting and guiding me all these years. My sincere and heartfelt gratitude goes to my supervisors Dr. Beatrice Nyanchama Kiage (PhD), and Dr. Judith Kanensi Okoth (PhD) for their tirelessly support and guidance which brought this work to an end. You were there for me and never discouraged by my mistakes. This indeed made me to work hard. Thank you and God bless you.

I would like to appreciate the help provided by the laboratory staff in the department of food science and technology, Dr Karanja, Mrs, Jessica Oruka, Mr.David Rasuga, Mr. David Volta and the others.

I would also appreciate the support from all my colleges from the Department of Food Science (JKUAT) for their constant encouragement and support to complete the work despite the hardships.

My Special thanks to DAAD who sponsored my Masters studies in Food Science and Nutrition. I also thank the University of Juba for giving me the chance to pursue my studies.

Finally, heartfelt gratitude to my husband Dr.Yatta Samuel Lokou for trusting and believing in me. To our lovely children, thanks for the emotional support.

Thank you and God bless you all abundantly. Amen

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## LIST OF ABBREVIATIONS

<b>ANOVA</b>	Analysis of variance
<b>AOAC</b>	Association of Official Analytical chemist.
<b>FAO</b>	Food and Agriculture Organization of the United Nation.
<b>FEWS NET</b>	Famine Early Warning Systems Network
<b>G</b>	Gram.
<b>GAM</b>	Global Acute Malnutrition.
<b>HIV/AIDS</b>	Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome.
<b>HPLC</b>	High-performance liquid chromatography
<b>IAC</b>	InterAcademy Council.
<b>IFPRI</b>	International Food Policy Research Institute.
<b>IgG</b>	Immunoglobulin G.
<b>IPC</b>	Integrated Food Security Phase Classification
<b>JKUAT</b>	Jomo Kenyatta university of Agriculture and Technology.
<b>Kg</b>	Kilogram.
<b>KSH</b>	Kenyan Shilling.

<b>NBHS</b>	National Baseline Household Survey.
<b>OCHA</b>	United Nations Office for the Coordination of Humanitarian Affairs
<b>RDA</b>	Recommended Dietary Allowance.
<b>RID-6A</b>	Reractive Index Detector
<b>SD</b>	Stardard Deviation
<b>TPD</b>	Tropical Plants Database
<b>USAID</b>	United Nations Agency for international Development.
<b>WFP</b>	World Food Programme.
<b>WHO</b>	World Health Organization.

## ABSTRACT

Civil conflicts have aggravated the situation of food security in South Sudan and the problem reached record levels in 2016 and 2017. Wild plant seeds constitute an important part of human diet. *Hyptis spicigera* has some attributes that could make it a suitable candidate to address the problem of food insecurity in South Sudan. Although, the plant is well known for its insecticidal, herbal and medicinal properties, its seed could be used as food, because of its high nutritional content. The state of art of nutritional value, anti-nutrient value and effect on processing content of the seed is lacking in the literature. The objectives of the present study were to determine the nutritional composition of the seed; to determine the anti-nutrient levels of the seed; to evaluate the effect of processing on the nutrient composition of the seed and to evaluate the effect of processing on the anti-nutrient content of the seed. Ten kilograms of *Hyptis spicigera* seeds collected from local farmers in South Sudan and transported in closed box to Jomo Kenyatta University of agriculture and technology laboratory then kept in a cold room at 10°C until analysis was done. Standard methods (AOAC, 1995) were used for proximate analysis; anti-nutrients, oxalic acid was determined by HPLC; phytic acid by HPLC and tannins was determined by the Folin-Denis colorimetric method. The samples of *Hyptis* seeds were treated for different time periods for the soaked; soaked and germinated or boiled and roasted. Raw seed served as control. The treated and untreated seeds were milled into flour and their proximate composition and anti-nutrient content were analyzed. Data were subjected to analysis of variance; means were separated by Duncan multiple range tests at 5% significance level using Genstat Release 14.1 Software. The nutrient composition and anti-nutrient analysis were reported on dry weight basis. The nutritional composition of the untreated seed were: moisture 4.71%; ash 2.82; fat 34.1%; crude fibre 18.2%; protein 16.0%; carbohydrate 24.1%; iron 2.72 mg/100g; calcium 252.6 mg/100g; zinc 2.52 mg/100g and magnesium 297.9 mg/100g. The anti-nutrient content of the untreated seeds were: tannins 332.0 mg/100g; oxalates 434.8 mg/100g and phytates 390.4 g/100g. Different processing techniques significantly ( $p \leq 0.05$ ) increased the nutrient content of the seed but at the same time significantly reduced the anti-nutrient content of the seeds. Germination and roasting improved the nutritional content of the seed (carbohydrates, fat and fibre) when compared to soaking and boiling treatments. Hence, anti-nutrient treatments reduced the anti-nutrient content (tannins, oxalates, and phytates) of the seed, with germination registering the best reduction in tannins and oxalates. In conclusion, roasting and germination appears to be a promising processing method of enhancing the proximate composition of *Hyptis spicigera* Lam seed and reducing its anti-nutrient level. Its utilization could be recommended to fight against micronutrient deficiencies in the community.

Keywords: Effect of processing, Determination, Nutrient, Anti-nutrient, *Hyptis spicigera* Lamiaceae seed.

# CHAPTER ONE

## INTRODUCTION

### 1.1 Background

Food security and nutrition is Africa's first development agenda with greater commitment to ending hunger, achieving food security and advancing optimal nutrition for all Africans (FAO, 2017<sub>a</sub>). Africa still suffers from a multiple burden of malnutrition as defined by under nutrition with rising levels of non-communicable diseases, and micronutrient (vitamins and minerals) deficiencies (FAO, 2017<sub>b</sub>). Though some progress has been made in reducing malnutrition, famine is still a tragic reality in parts of South Sudan (FAO, 2017<sub>a</sub>). Among the 3.9 million people, nearly one in every three is severely food insecure in South Sudan and more than one million people are acutely malnourished (OCHA, 2016).

Plant seeds form an important part of human diets and are usually regarded as nutritious (Zeghichi et al., 2003). Its significance especially in the diet of the population in developing countries is increasing for several reasons (Kuku *et al.*, 2014). They can be described as good source of "famine food" (Okpashi et al., 2013). Seeds have nutritive and calorific values which make them necessary in diets. They are good source of edible oils and fats Agatamor (2006). The seeds are also potential raw materials for local industries, especially, in the oleochemical and animal feeds industries (Kuku *et al.*, 2014).

Seeds have very different and complex chemical compositions that are nutritionally grouped as macronutrients, micronutrients, and other components, which include phytochemicals such as phenolic antioxidants, that have demonstrated potential beneficial health properties (Parry, 2006). Many plant products have antioxidants such as flavonoids, tannins, coumarins, curcuminoids, xanthons, phenolics, and (Jeong *et al.*, 2004).

However plants foods are also associated with anti-nutrients, this have been found to interfere with nutrient utilization and hence can be a cause of malnutrition (Kuku *et al.*, 2014). Nonetheless, it has been reported that these anti-nutritional factors in food may be partially inactivated by processing methods such as boiling, soaking, fermenting or sprouting (Kuku *et al.*, 2014). Given that heat processing has a detrimental effect on the nutritional and functional properties of foods, it is important that after processing ,scientific studies are done to ensure that nutritional and other useful properties are still of significant value and that anti-nutritional components have been reduced considerably (Arinola and Adesina, 2014).

*Hyptis spicigera* Lamiaceae is a plant that belongs to the family of Lamiaceae. The mature aromatic herb is about one meter tall, with an inflorescence terminal where the seeds are positioned and packed in batches of quadruplets or even more in the existing flowers Ladan *et al.*, (2010). The seeds are tiny, either brown or black in color and are very light clustered in a group of 4, 5 or even more and are encased in every flower which makes up the inflorescence. This plant is also called Black Beni-Seed or Sesame. It is found around Senegal to Western Cameroon, possibly native to Brazil, and now

widely naturalized in tropical Africa and Asia (Ladan *et al.*, 2011). *Hyptis spicigera* Lam is also commonly found in the bush lands of Southern Sudan, and Western Kenya (Othira *et al.*, 2009).

In Central and Eastern Africa and in Guinea, *Hyptis spicigera* Lam is cultivated and the oleaginous seeds are eaten like sesame (Noudjou *et al.*, 2007). Essential oils from aromatic plants are being used as flavoring agents in food (Ladan *et al.*, 2011). The plant's seeds and leaves are good vegetables eaten due to its spicy aroma (Ladan *et al.*, 2011). *Hyptis spicigera* Lam (or locally named Kinu) has also been used as food in South Sudan especially among the Kakwa tribe from Yei (South Sudan), who grind roasted or raw seeds of *Hyptis spicigera* Lam and the paste is cooked with either dry meat and vegetables or added to porridge for lactating mothers and complementary feeding. Agea *et al.* (2013) reported that *Hyptis spicigera* Lam seeds are roasted and pounded using mortar and pestle, then used for preparing other food. *Hyptis spicigera* seed has the potentials to address food security because it is a drought tolerant plant, resists to insects and has a long shelf life of 10-12 months (Agea *et al.*, 2013).

Research has been done on the physical and chemical properties of the oil extracted from the *Hyptis spicigera* Lam seed as well as its medicinal and nutritional utility Ladan *et al.* (2010). However, there is limited information on the nutritional value, anti-nutrient level and effect of processing on *Hyptis spicigera* Lam seed in South Sudan.

*Hyptis spicigera* Lam (Kinu) is a promising plant seed which needs more research on both its raw and processed state. Investigating the effect of processing on the nutritional and the anti-nutritional components of *Hyptis spicigera* seed will help to increase the

awareness of not only this seed but also its nutritional capabilities. In view of the important attributes of *Hyptis spicigera* Lam seed, both in terms of its nutritional composition and its ability to resist drought, it is necessary to carry out research that will help to optimize its benefits especially in South Sudan where food insecurity and malnutrition levels are high.

However, information on nutritional value, anti-nutrient level, and effect on processing content of the seed is lacking in the literature. Based on this background, the study was initiated to investigate the nutritional composition and effect of different processing conditions on anti-nutritional factors to improve the nutritional composition of *Hyptis spicigera* seed.

## **1.2. Problem Statement**

Food insecurity constitutes a major threat in the world's contemporary societies with both short and long-term impacts on human survival and well-being (Szabo, 2016). Food insecurity has increased dramatically in South Sudan since the civil conflict started in December 2013, reaching record levels in 2016 and 2017 (IPC, June 2017). It is estimated 6.0 million (50% of the population) of the South Sudanese were expected to be severely food insecure in June-July 2017, compared to 5.5 million (45% of the population) people in May 2017 (IPC, 31 May 2017). Famine is projected to further deteriorate at the height of the lean season in July 2017 with the number of food insecure people increasing to 5.5 million (47% of the population (IPC, June 2017). In South

Sudan, food insecurity is driven by conflict and insecurity, which have severely disrupted livelihoods, trade and agricultural production (FAO, 2017).

Moreover, the sharp devaluation of the local currency has inflated food prices, and transport costs are high because of insecurity along trade routes (FAO, 2017). Indeed, malnutrition affects households throughout the entire region of Southern Sudan and has not shown any sign of improvement over the past years. Causes point to the lack of proper nutrition, micronutrient deficiencies, poor hygiene and sanitation, customs and beliefs leading to negative infant and young child feeding practices, inequitable distribution of food, and finally, seasonal climatic changes (Harvey & Rogers-Witte, 2007).

### **1.3. Justification**

Sorghum is the main staple food of South Sudan and the local production is not sufficient to meet the overall demand (FEWS NET, 2007). As a result, supplies of other staple grains are imported from neighboring countries, mainly Uganda and Sudan (WFP, 2014). As stated in Harvey and Rogers-Witte (2007), the quality of the diet in Southern Sudan is generally poor and the majority of the populations rely on sorghum or maize and either cow or goat milk. These staple foods are usually consumed throughout the year, with seasonal disparities. To a lesser extent, the other foods are also eaten depending upon ecological zone and seasonal availability (FEWS NET, 2007).

The availability of the non-staple foods varies greatly throughout Southern Sudan, but the majority of diets are not sufficiently diversified. Nutrition surveys have also reported higher rates of malnutrition during specific periods of the year, including the “hunger

period” of March through September. Further Global Acute Malnutrition (GAM) prevalence is often double the WHO cut-off for emergencies of 15% (Harvey & Rogers-Witte, 2007).

Seeds are good sources of protein, fats, carbohydrate and minerals (Bello et al., 2008); they could be used to alleviate malnutrition and food insecurity. Therefore, wild seeds produce like *Hyptis specigera* Lam, if its nutritional value is fully ascertained, will improve food security in South Sudan, since the plant is wild and easily found. Moreover, many people in South Sudan still depend on agriculture for existence and if the plant is found to be of high nutritional value, it will be cultivated by farmers for consumption and commercial use, thereby contributing to low levels of malnutrition especially in children.

The plant *Hyptis specigera* is almost grown and found in the wild in most parts of the country even in the settlements of the pastoralists. Secondly, harvesting and the post-harvest processing of the plant does not require extensive labor and sophisticated machines which may mean that everybody would be in a position to grow it. It can also be processed and availed to consumption in urban area thereby creating employment and building the economy. Processing will encourage farmers to grow it.

Therefore, with the current food situation in Southern Sudan, the utilization of a seed such as *Hyptis specigera* Lam is timely since it grows in cultivated farmlands as weeds [Ladan et al., \(2011\)](#), and may be utilized to address food and nutrition insecurity. In the course of growing, the plant does not require herbicides to be applied as the plant itself destroys unwanted herbs (Ladan et al., 2010). This means that there will be no worry

about parasitic herbs and need to buy the herbicides. Unlike the seeds of other plants, the seeds of *Hyptis specigera* Lam are always in the market everywhere and in all seasons making it available and accessible everywhere and to everybody and hence may respond better to food emergencies.

Therefore, with all these advantages the utilization of the plant may be very popular. Hence there is a need to determine the potential nutritional contribution of this seed as a human food.

To the knowledge of the author no comprehensive report on the nutritional composition and anti-nutrient content of *Hyptis specigera* Lam seed. Therefore, given detailed information on the nutritional values of this plant, the information could be included in the Nutritional Data Base in South Sudan's Ministry of Health. Hence, could be used in projects by the relevant governmental and non-governmental organizations concerned with the nutritional status of the South Sudanese population.

## **1.4 Objectives**

### **1.4.1 Main Objective**

This was to determine the nutrient composition, anti-nutrient level and effect of processing on the nutrient and anti-nutrient content of *Hyptis spicigera* Lam seed.

### **1.4.2 Specific Objectives**

These were as follows:

1. To determine the nutritional composition of *Hyptis spicigera* seed.
2. To determine the anti-nutrient levels of *Hyptis spicigera* seed.

3. To evaluate the effect of processing (soaking, germination, boiling and roasting) on the nutrient composition of *Hyptis spicigera* seed.
4. To evaluate the effect of processing (soaking, germination, boiling and roasting) on the anti-nutrient content of *Hyptis spicigera* seed.

### **1.5 Hypothesis**

The study tested the following null hypothesis:

1. Nutritional composition and nutrient content of *Hyptis spicigera* Lamiaceae are not significantly affected by processing treatments (soaking, germination, boiling and roasting).
2. Processing treatments (soaking, germination, boiling and roasting) does not significantly affect the anti-nutrient content of *Hyptis spicigera* seed.

## **CHAPTER TWO**

### **LITERATURE REVIEW**

#### **2.1 Overview of Food Security**

Food security describes a situation in which people do not live in hunger or fear of starvation (Nyangweso *et al.*, 2007). There are 852 million people worldwide who are chronically hungry due to extreme poverty (FAO,2003), while up to 2 billion people lacked food security intermittently due to varying degrees of poverty (McNeill, 2011).

According to McNeill (2011), the definition of food security adopted by the FAO is consistent with the principle that everyone has a right to adequate food, to be free from hunger and to enjoy the general human dignity enshrined in the International Bill of Human Rights. It also encompasses the criterion of affordability and acknowledges that food production does not always equate with food security – if food is available in fields or in stores, but people cannot afford to acquire it, then their food security is jeopardized. The dimension of affordability is further reiterated in a definition developed by the World Bank (1986) and subsequently adopted by USDA (McNeill, 2011).

#### **2.2. Dimensions of Food Security**

The three core pillars or determinants of food security based on various definitions have emerged namely: food availability, access and utilization (Opara, 2013).

### **2.2.1 Food Availability**

This refers to the physical availability of food through local production, imports, and handout (such as food aid). A wide range of factors can affect food availability, from production index to good postharvest management which maintains quality and food safety, to incidence of reduce losses; hence, adequate availability of food does not translate into food security at all levels, from individual to country and global level (Opara, 2013).

### **2.2.2 Food Access**

Food access could be physical access in the market or economic access (purchasing power) at the household level. Hence, the ability to spend on food (through income) is a good measure of access to food (Opara, 2013). Physical access to food in the market could also be affected by the availability (or lack) of infrastructure such as good road networks, transport, and postharvest handling and storage facilities (Opara, 2013). It is important to emphasize that more food production does not necessarily mean more food for those who need it. Most experts would agree that the largest part of the production increase has to come from yield increases (Boon, 2007).

### **2.2.3. Food Utilization**

As a core determinant of food security, it refers to the consumption of food (in quantity and quality) that is sufficient to meet the calorie (energy) and nutrient requirements, with optimal uptake of nourishment (Pinstrup-Andersen, 2009). In this context, nutritional

status is considered to be an outcome of food intake and health status. The importance of food utilization and factors affecting its effectiveness in ensuring food security was alluded to by Pinstrup-Andersen (2009).

### **2.3 Food Insecurity**

Globally, food insecurity has been described quite simply as the absence of food security, although a number of international definitions seek to conceptualize food insecurity in terms of a range of other factors - including war, terrorism, corruption and environmental degradation - conceptualizations emanating from industrialized western countries tend to correlate food insecurity with relative poverty and inadequate resources, including income (McNeill, 2011).

An example of this approach recognizes food insecurity at households level “at times, uncertain of having or unable to acquire, enough food for all household members because they had insufficient money and other resources for food”. In essence then, food insecurity in affluent nations can occur with or without hunger where adequate, nutritious and safe food cannot be acquired in socially acceptable ways - usually a result of inadequate income (McNeill, 2011).

#### **2.3.1 Root Cause of Food Insecurity**

The root cause of food insecurity in developing countries is the inability of people to gain access to food due to poverty (Council. I, 2004). While the rest of the world has made significant progress towards poverty alleviation Barrett (2008), Africa, in particular Sub-Saharan Africa continues to lag behind (Maji et al., 2012). Projections

show that there will be an increase in this tendency unless preventive measures are taken (Onwuemenyi, 2007, 2008). Many factors have contributed to this tendency including the high prevalence of HIV/AIDS; civil war, strife and poor governance; frequent drought and famine; and agricultural dependency on the climate and environment (Kolapo, 2008).

Food security on the continent has not improved since 1970 and the proportion of the malnourished population has remained within the 33 to 35 percent range in Sub-Saharan Africa (Fawole *et al.*, 2015). The prevalence of malnutrition within the continent varies by region. It is lowest in Northern Africa (4%) and highest in Central Africa (40 %) (Mwaniki, 2006).

In South Sudan the underlying causes are insecurity, displacement, poor access to services, extremely poor diet (in terms of both quality and quantity), low coverage of sanitation facilities and deplorable hygiene practices are underlying the high levels of acute malnutrition.

#### **2.4 Food Insecurity in South Sudan**

The food security situation in South Sudan has been inadequate for decades particularly due to the independence-related conflict and the lack of development (Oxfam,2014).The situation of food security further worsened by the consequences of the current civil conflict, which erupted in December 2013, including the on-going economic deterioration throughout the country, resulting in a dramatic widespread escalation of food insecurity. In the year 2017 it was reported that 70 % of the population were

food insecure, with 14 %t severely food insecure, a slight increase from the values registered one year before; only 20 percent of the households were found to have acceptable food consumption.

The local currency devaluation had its share in the general economic downturn with the consequence that cereals prices shot up in late 2015 and reached record levels in mid-2017 in which crop production was the lowest since the conflict began due to the loss of cultivated area because of conflict insecurity in productive areas of South Sudan with the consequence that cereal deficit of under 500 000 tonnes, worse than the previous year's deficit of about 380 000 tonnes (FAO,2018). Agriculture is almost entirely dependent on rainfall and hence the variability of rainfall in terms of amount and distribution is usually the major factor in determining crop production. Moreover, there are usually considerable variations in rainfall from year to year and from location to location within the same year. For example, in low-lying areas, flooding/water logging is a common occurrence, while many areas, especially those towards the northern border with the Sudan and in the southeast corner of the country, are susceptible to prolonged dry periods.

Crop production is mostly conducted by smallholder farmers on small plots of land cultivated by hand. The family size is five-seven persons that belong to larger family aggregations, reflecting the polygamous nature of most communities. Despite an abundant availability of land throughout the country, the area cultivated by households has previously, been limited by a combination of (a) the size of the household labour force and/or the ability of households to provide in-kind payment (essentially food/beer)

for the mobilization of traditional working groups (nafeer); (b) the lack of efficient tools and farm power for land clearing and ploughing; and (c) security of access (FAO, 2018).

## **2.5 *Hyptis spicigera* Lamiaceae**

### **2.5.1 Classification and names of *Hyptis spicigera* Lamiaceae**

*Hyptis spicigera* (Lamiaceae) is an aromatic annual plant with opposite and lanceolate leaves (Agbafor and Akubugwo, 2007). The plant in this family has socio-economic value in flavoring, cosmetics, perfumery, confectionery and medicinal preparations (Magness *et al.*, 2006). The family comprises of 250 genus and has approximately 6970 species (Falcao and Menezes, 2003).

Lamiaceae family is widely spread, especially in the tropical and subtropical regions; they grow abundantly in the Mediterranean areas where it is possible to find the vast majority of the genus constituents (Barbosa *et al.*, 2013). Currently, the family comprises of about 7200 species, the largest within the order is Lamiales, with 240 genera, divided among seven sub-families. The genus *Hyptis*, which belongs to this family, consists of approximately 400 species that occur naturally in tropical and subtropical regions: Nigeria, Thailand, India and the southern part of the United States to Argentina (Barbosa *et al.*, 2013).

*Hyptis spicigera* Lamiaceae is generally known as Black beni-seed or Black sesame (Ladan *et al.*, 2013). It is commonly known as bushmint in English (Uraku *et al.*, 2015). *Hyptis spicigera* plant is locally known as, “Kinu” by Kakwa tribe from Yei, Southern

Sudan. It is also called Nino and Nina in dinka and Luo languages respectively (Gullick, 2000). The plant is also known by other synonyms as *Hyptis lophantha* Mart. ex Benth; *Hyptis americana* (Aubl.) Urb ; *Nepeta americana* Aubl and *Mesosphaerum spicigerum* (Lam.) Brummitt *et al.*, (1992).

According to the USDA (2014) the plant is classified as below:

Table 2.1: Classification of *Hyptis spicigera*

Class position	<i>Hyptis spicigera</i>
Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Super division	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Asteridae
Order	Lamiales
Family	Lamiaceae/Labiatae – Mint family
Genus	<i>Hyptis</i> Jacq. – bushmint
Species	<i>Hyptis spicigera</i> Lam. – marubio

Source :( USDA, 2014)

### 2.5.2 Distribution of *Hyptis spicigera* Lamiaceae

Plants of the *Hyptis* genus are found in the tropical regions of the world; mainly in Africa and America (Conti *et al.*, 2011). *Hyptis spicigera* from the family Lamiaceae is one of the five species of the genus that occur in west Tropical Vegetation of Africa, America, the West Indies and Mascarene Islands (Onayade *et al.*, 1990). The plant is

found around Senegal to western Cameroon, possibly native to Brazil, now widely naturalized in tropical Africa and Asia as well as northern Nigeria and Asia (Piozzi *et al.*, 2009; Ladan *et al.*, 2013). *Hyptis spicigera* is also widespread in tropical North and South America as well as part of West Africa (Conti *et al.*, 2011).

### **2.5.3 Description of *Hyptis spicigera* Lamiaceae**

The plant has very tiny brown or black seeds clustered in groups of fours, fives or even more, which are encased in each flower that make up the inflorescence and the terminal inflorescence is dense cylindrical or ovoid with cylindrical spike up to 9 cm long (Ladan *et al.*,2011). The plant is an annual weed which grows naturally in roadsides, waste and damp places as well as in cultivated farmlands as a weed (Ladan *et al.*, 2011).



Source:( Tropical Plants Database, 2018).

**Fig 2.1: *Hyptis spicigera* Lamiaceae plant**

### **2.6 Phytochemical content of *Hyptis spicigera* Lamiaceae**

Plants provide phytochemicals which are useful in pharmaceuticals, cosmetics, agrochemicals, and flavor and fragrance production amongst other uses (Falcao and Menezes, 2003). The most diffused utilization of species belonging to the *Hyptis* genus is related to their essential oils, obtained in industrial scale, from several species and

such oils are largely used in cosmetic flavoring agents and as insecticides (Falcao and Menezes, 2003).

More than 25 species of the *Hyptis* genus have been subjected to chemical investigations, resulting in the isolation of several classes of compounds (Barbosa *et al.*, 2013). The phytochemical investigations conducted within *Lamiaceae* species have identified many classes of secondary metabolites derived from the acetate and shikimate pathways as well as small molecules from mixed biosynthesis (Falcão and Menezes, 2003). In addition to that, a lot of information has been reported in a review about the biological activities and related pharmacology (Falcao and Menezes, 2003).

### **2.7 Uses of *Hyptis spicigera* Lamiaceae**

Generally, the whole plant is used in traditional stores to protect cowpea against damage by *Callosobruchus* species and it is locally used as a mosquito repellent by burning of the whole plant (Ladan *et al.*, 2011). The leaves are hung on the walls of the henhouse and farmers also make an infusion of pounded leaves of this herb soaked in water for 6 to 12 hours, which is then sprayed in the hen house to control fleas and lice (Salifou *et al.*, 2013).

The seeds are used for oil production and the leaves are eaten as vegetables. (Ladan *et al.*, 2011). The seed yields a yellow, edible drying fixed oil which has been used in railway workshops in Senegal in place of linseed oil and the reported percentage yields of the fixed oil vary between 18% and 37%. Analyses of the fixed oils obtained from seeds of *Hyptis spicigera* Lamiaceae plants grown in both Nigeria and Sudan showed a

high content of linolenic acid (Onayade *et al.*, 1990). Numerous types of the Lamiaceae family are utilized as a part of cooking because of the flavor and taste. Other than that, the members of the family are a resource of aromatic essential oils as well as ornamental plants (Barbosa *et al.*, 2013).

The seeds and the leaves are eaten as vegetables because they are spicy nature (Iadan *et al.*, 2010). The seed is cultivated and eaten like sesame in central and eastern Africa and Guinea; it is roasted, and stored in large quantities by some tribes and used in stews and gravies in Sudan and central Africa (Onayade *et al.*, 1990). The plant has been used as food especially among the Kakwa tribe from Yei in Southern Sudan (Kakwa org.,2015).

### **2.8 Medicinal properties of *Hyptis spicigera* Lamiaceae**

*Hyptis spicigera* (family:Labiata),is an important medicinal plant used in treatment of gastrointestinal disturbances, wounds, skin infections and insect bites (Rogelio *et al.*,2001; Wekasa *et al.*,2011). The plant part commonly used traditionally are the leaves, these leaves are crushed and applied on the head to relieve colds and headaches (Ladan *et al.*, 2011). The Bajju and Tyapp people of Southern Kaduna state, Nigeria, make use of the inflorescence (where the seeds are packed) to cure headaches by sniffing it (Ladan *et al.*, 2011).

Generally, the plant has been used in the treatment of headaches, coughs, diarrhea, constipation, warts, worms, kidney malfunctions, in bath water or tea, as eupneic, or expectorant to treat bronchial infections and other disorders (Agbafor and Akubugwo, 2007).It is also used as a remedy for stomachache and as a source of flavouring for

pharmaceuticals (Kouninki *et al.*, 2005; Othira *et al.*, 2009). The leaves of *Hyptis spicigera* plant are boiled and are taken as infusions for malaria (Asase and Yeboa, 2012). Moreover, decoction of its flowers and fruits is drunk as revigorant; powdered flowers and fruits are used as an anti-migraine drug; the flower is stuck in the nostril as a rapid method of combating headaches, colds, and catarrh (Onayade *et al.*, 1990).

The use of some plants for medicinal purpose, in the traditional treatment of diseases is due to the presence of flavonoids and saponins, hence the use of *Hyptis spicigera* in the treatment of diarrhea, dysentery, colds and several other diseases by local herbalists or traditional healers is not surprising (Ladan *et al.*, 2014). This was evidenced by the use of other plants for the same purposes as described by Othira *et al.* (2009). Phenolic compounds like tannins present in plant cell are inhibitors of many proteolytic and hydrolytic enzymes used by plant pathogens (Aboaba and Efuwape, 2001; Mohanta *et al.*, 2007).

*Hyptis spicigera* extracts in ethylacetate and methanol resulted in high tannin content and it could probably be a source of phytochemicals for treatment of bacterial infection (Ladan *et al.*, 2014). Moreover, *Hyptis spicigera* is rich in saponin and it is traditional medicine lent credence to the medicinal potentials of the plant (De-Ruiz *et al.*, 2001; Elegani *et al.*, 2002). These plant (*Hyptis spicigera*) provides the natural anti-oxidant needed to enhance good living by scavenging free radicals that cause ill health in humans (Ladan *et al.*, 2014).

## 2.9 Insecticidal properties of *Hyptis spicigera* Lamiaceae

Several species from the *Labiatae* family have been tested for their insecticidal potency (Belmain *et al.*, 2001; Ogendo *et al.*, 2004) and are widely used insect pest control (Ke'ita *et al.*, 2000). In developing countries, Lamiaceae have traditionally been used for their insecticidal and repellent properties against several insects' species (Ngamo *et al.*, 2007). The insecticidal activity of *Hyptis* species using its volatile oils is also well documented Othira *et al.*, (2009); Sanon *et al.* (2006), Raja *et al.* (1990), Sharma (2002). *Hyptis spicigera*, has valid insecticidal properties (Facey *et al.*, 2005; Othira *et al.*, 2009). For example, dried whole plants are used for pest control while the oils, parts of the plants and extracts are found diffused application in ethno pharmacology in tropical countries (Falcao and Menezes, 2003).

The leaves are used in granaries to protect cowpea seeds against bruchids damages (Sanon *et al.*, 2006). It is also used as an effective insect repellent in traditional grain storage structures among the Acholi of Uganda (Wekesa *et al.*, 2011). Recent literature reports on essential oils of *Hyptis spicigera* and *Hyptis suaveolens* could be a valid option to chemical insecticides for the control of many stored-food pests (Ngamo *et al.*, 2007; Othira *et al.*, 2009; Sanon *et al.*, 2006; Noudjou *et al.*, 2007; Kéïta *et al.*, 2000; Raja *et al.*, 1990; Iloba and Ekraene, 2006; Kouninki *et al.*, 2007 and Kossou *et al.*, 2007). *Hyptis spicigera* and other species have been reported to exhibit nematicidal properties Jesse and Jada (2004).

## **2.10 The importance of food processing methods**

Food processing is important because of its effect on the availability of the nutrients and the degree at which nutrients are lost differs from one processing technique to the other.

### **2.10.1 Boiling**

Boiling is one of the domestic food processing techniques that involve using heat to prepare food for consumption. The common domestic cooking conditions include shallow frying which is at approximately 140–160<sup>0</sup> C, in soups and stews (which may include an initial frying period, followed by boiling at 100<sup>0</sup>C or at a higher temperature if the dish is cooked in the oven) Richard and Mariette (2015). Pressure boiling and steaming can also be used for this purpose. High pressure processing technology may provide high quality such as flavor, color and biological active components food products (Knorr, 1999).

### **2.10.2. Roasting**

Roasting is a type of heat treatment used to induce the development of the typical colour, taste and flavor. It also changes the chemical composition, modifies nutritional value and shelf life (Ozdemir and Devres, 2000). During roasting, pleasant aromas and flavours (nut-like or peanut butter-like) are transferred to the oil. Roasting is the key step for making condiment oil, because the colour, flavour, composition and quality of the oil are affected by the processing conditions used (Lee *et al.*, 2004).

Lipid oxidation strongly affects shelf life and sensory characteristics of oil seeds and depends on many factors such as the concentrations of unsaturated fatty acids, enzymatic activity, mineral composition and the presence of natural antioxidants (Ozdemir and Devres, 2000). Roasting is an important treatment for oily seeds prior to oil extraction and it causes some desirable or undesirable changes in physical, chemical and nutritional properties of the seeds (Açar et al., 2009). The process is carried out for promoting more flavor, desired color and texture changes that ultimately increases the overall palatability. The most important conditions of roasting process are the temperature and time (Kothe *et al.*, 2013).

### **2.10.3. Soaking**

Soaking is an integral part of a number of treatments, such as germination, cooking and fermentation. It consists of hydration of the seeds in water for a few hours. Several studies indicate that soaking can reduce the levels of minerals, phytic acid and proteolytic enzyme inhibitors which can be partly or totally solubilized and eliminated with the discarded soaking solution (Prodanov, *et al.*, 2004). Soaking could be one of the processes to remove soluble anti- nutritional factors, which can be eliminated with the discarded soaking solution. However, some metabolic reactions can take place during soaking which will affect some of the constituent compounds Akande *et al.* (2010).

### **2.10.4. Germination**

Germination involves sprouting of seeds at the beginning of the development of seeds into plants (Zhu et al., 2005). It is also a natural process that occurs during growth period

of seeds which meet their minimum condition for growth and development (Sangronis *et al.*, 2006). Germination does not require intensive energy output and also yields natural products. During seed germination, storage proteins are hydrolyzed, while amino acids are transported into the growing seedling axis (Akande *et al.*, 2010). Also reserve materials are degraded, commonly used for respiration and synthesis of new cells prior to developing embryo (Vidal-Valverde *et al.*, 2002).

### **2.11 Anti-nutrient factors and their effect in plant food**

Anti-nutrients are chemicals which are produced by plants for their own defense, among other biological functions and reduce the maximum utilization of nutrients especially proteins, vitamins, and minerals, thus preventing optimal exploitation of the nutrients present in a food hence decreasing the nutritive value (Dan *et al.*, 2014). Some of these plant chemicals have advantage to human and animal health if consumed at appropriate amounts (Ugwu *et al.*, 2006).

Anti-nutrients are compounds that act to reduce the nutrient utilization of ingested food (Osagie, 1998). Anti-nutritional factors are compounds that interfere with the digestion of proteins (e.g trypsin inhibitor), minerals (e.g phytates) and vitamins (e.g tannins) (Carolyn *et al.*, 2007). Most of the anti-nutritional factors become ineffective by simple processing measures such as heating, soaking, germination or autoclaving (Nowak and Haslberger, 2000).

### **2.11.1 Tannins**

Tannins are polyphenols and sometimes called plant polyphenols (Okuda and Ito, 2011). They are secondary compounds of various chemical structures widely occurring in plant kingdom (Francis *et al.*, 2001). It is an astringent, bitter plant polyphenol compound that either binds or precipitates proteins and many other organic compounds including amino acids and alkaloids (Tadele, 2015). The term tannin refers to the use of tannins in tanning animal hides into leather; however, the term is widely applied to any large polyphenolic compound containing sufficient hydroxyls and other suitable groups to form strong complexes with proteins and other macromolecules (Ashok *et al.*, 2012).

#### **2.11.1.1 Classification of tannins**

Tannins are another major group of polyphenols in our diets and usually subdivided into two groups named hydrolysable tannins and condensed tannins. The hydrolysable tannins are compounds containing a central core of glucose or another polyol esterified with gallic acid (Khanbabaee and van Ree, 2001). They are also called gallotannins, or with hexahydroxydiphenic acid, also called ellagitannins. The structure of these compounds has a many possibilities in forming oxidative linkage. Intermolecular oxidation reactions give rise to many oligomeric compounds having a molecular weight between 2,000 and 5,000 Daltons (Khanbabaee and van Ree, 2001). The condensed tannins are oligomers or polymers of flavan-3-ol linked through an interflavan carbon bond. They are called proanthocyanidins because they decompose to anthocyanidins

through acid-catalyzed oxidation reaction upon heating in acidic alcohol solutions (Khanbabaee, and van Ree, 2001).

#### **2.11.1.2 Effect of processing on the tannins**

Food processing methods including soaking (Vidal Valverde et al., 2002) germination, decortications, fermentations, cooking and addition of enzymes have been suggested to reduce the concentration of anti-nutritional factors. Tannins are heat stable and they decrease protein digestibility in animals and humans, probably by either making protein partially unavailable or inhibiting digestive enzymes and increasing fecal nitrogen Gemedede and Ratta (2014). The anti-nutritional effects of tannins include interference with the digestive processes either by binding enzymes or by binding to food components like proteins or minerals (Hagerman *et al.*, 1992). It also has the ability to complex with vitamin B<sub>12</sub> (Francis *et al.*, 2001). Tannins inhibits digestive enzymes and causes irritation of the gut (Hang and Preston, 2009; Hang and Binh, 2013). Soaking reduces tannins as a result of it leaching into soaking water (Oboh et al., 2010).

#### **2.12 Oxalate**

Oxalate is a salt formed from oxalic acid, for example, calcium oxalate, which has been found to be widely distributed in plants (Nachbar *et al.*, 2002). It is a naturally occurring substance found in plants and in the human body (Liebman and Wahsh, 2011).

### **2.12.1 Classification of oxalates in plant**

In chemical terms, oxalate belongs to a group of molecules called organic acids (Liebman and Wahsh, 2011). Some oxalate salts, such as sodium and potassium, are soluble, whereas calcium oxalate salts are basically insoluble. The insoluble calcium oxalate has the tendency to precipitate (or solidify) in the kidneys or in the urinary tract, thus forming sharp-edged calcium oxalate crystals when the levels are high enough (Apata and Badalona, 2012).

These crystals play a role to the formation of kidney stones in the urinary tract when the acid is excreted in the urine. Oxalate is an anti-nutrient which under normal conditions is confined to separate compartments. However, when it is processed or digested, it comes into contact with the nutrients in the gastrointestinal tract; when released, oxalic acid binds with nutrients, rendering them inaccessible to the body (Gemedede and Ratta, 2014). Strong bonds are formed between oxalic acid, and various other minerals including calcium, magnesium, sodium, and potassium resulting in the formation of oxalate salts (Gemedede and Ratta, 2014).

In plants with a cell sap of approximately pH 2, such as some species of *Oxalis* and *rumex* oxalate exists as the acid oxalate ( $\text{HC}_2\text{O}_4$ ), primarily as acid potassium oxalate. Plants with a cell sap of approximately pH 6, such as some plants of goosefoot family it exists as oxalate ( $\text{C}_2\text{O}_4$ )<sup>2-</sup> ion usually as soluble sodium oxalate and insoluble calcium and magnesium oxalates. Calcium oxalate is insoluble at a neutral or alkaline pH, but freely dissolves in acid (Gemedede and Ratta, 2014).

### **2.12.2 Effect of processing on oxalates**

The importance of the anti-nutritive activity of oxalic acid has been recognized for over fifty years. Oxalic acid forms water soluble salts with  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{NH}_4^+$  ions, it also binds with  $\text{Ca}^{2+}$ ,  $\text{Fe}^{2+}$ , and  $\text{Mg}^{2+}$  rendering these minerals unavailable to animals. However,  $\text{Zn}^{2+}$  appears to be relatively unaffected (Noonan and Savage, 1999). Oxalates have been reported to form complexes with mineral, particularly calcium thereby making them unavailable to the body (Agwunobi *et al.*, 2002; Ndimantang *et al.*, 2006; Okereke, 2012). Oxalate interferes with calcium absorption in the digestive tract and also limits nitrogen retention (Hang and Preston, 2009; Hang and Binh, 2013).

### **2.13 Phytate**

Phytate (is also known as Inositol hexakisphosphate (InsP6) and it is the salt form of phytic acid, it is found in plants, animals and soil (Mueller, 2001). It is primarily present as a salt of the mono- and divalent cations  $\text{K}^+$ ,  $\text{Mg}^{2+}$ , and  $\text{Ca}^{2+}$  and accumulates in the seeds during the ripening period. Phytate is regarded as the primary storage form of both phosphate and inositol in plant seeds and grains. In addition, phytate has been suggested to serve as a store of cations, of high energy phosphoryl groups, and, by chelating free iron, as a potent natural anti-oxidant (Mueller, 2001). Among all the anti-nutritional components, phytic acid is one of prime concern for human nutrition and health management (Kumar *et al.*, 2010).

### **2.13.1 Classification of Phytases**

Phytases have been classified as 3-phytases (EC 3.1.3.8), and 6-phytases (EC 3.1.3.26) based on the position of first phosphate hydrolyzed. The 3-phytases initiate dephosphorylation of phytic acid at the 3 position of phytic acid and 6-phytases at position 6. The 3-phytases are the largest group of phytases which are generally found in bacteria and fungi. The 6-phytases act basically on the carbon atom next to C5 of the inositol ring. Plant phytases act preferentially at the C6 carbon and are 6-phytase. Phytases can be categorized into acid phytases and alkaline phytases on the basis of pH optimum (Milko et al., 2008). On the basis of catalytic property phytases have also been classified as Histidine acid phosphatase (HAP),  $\beta$ -Propeller phytase (BPP), cysteine phosphatase (CP) and purple acid phosphatase (PAP) (Vats and Banerjee., 2004; Mullaney and Ullah., 2003; Singh et al., 2011).

### **2.13.2 The chemistry of phytate**

The chemical description of phytic acid is myoinositol (1, 2, 3, 4, 5, 6) - hexakisphosphoric acid (Oatway et al., 2001). Phytate is ubiquitous among plant seeds and grains, comprising 0.5 to 5 percent (w/w) (Loewus, 2002). Phytate works in a broad pH-region as a highly negatively charged ion, and therefore its presence in the diet has a negative impact on the bioavailability of divalent, and trivalent mineral ions such as  $Zn^{2+}$ ,  $Fe^{2+/3+}$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Mn^{2+}$  and  $Cu^{2+}$  (Mueller, 2001). The unique structure of phytic acid offers it the ability to strongly chelate with cations such as calcium, magnesium,

zinc, copper, iron, and potassium to form insoluble salts. Therefore, it adversely affects the absorption and digestion of these minerals, as seen in animals (Oatway et al., 2001).

### **2.13.3 Effect of processing on phytate**

Phytic acid (PA) can be found in many cereals, legumes, oilseeds and other plants (Fardet, 2010). It is considered as an anti-nutrient because of its potential to reduce mineral absorption by binding of mineral divalent and trivalent ions such as calcium, magnesium, zinc, copper, iron and potassium into phytic acid-mineral complexes called phytates (Kumar et al., 2010; Tavajjoh et al., 2011). Various food processing and preparation techniques, such as decortications, soaking, cooking, germination and fermentation, are the major efforts made to reduce the amounts of phytate in foods (Kumar et al., 2010). Phytic acid binds to minerals and makes them unavailable due to its chelating property. It has been reported that phytic acid inhibits absorption of iron, zinc calcium, magnesium and manganese (Phillippy, 2006).

High levels consumption of phytate-containing foods will result in mineral deficiency and it will depend on what else is being consumed (Mueller, 2001). Phytate chelates minerals in the body. Thus, makes certain important minerals such as zinc, iron and to a lesser extent mineral such as calcium and magnesium biologically unavailable to the body (Mullaney and Ullah, 2012). It can depress absorption of nutrients due to the damaged pyloric caeca region of the intestine (Francis *et al.*, 2001).

Phytates form insoluble complexes with zinc, iron, magnesium and calcium at physiological pH (Shah *et al.*, 2011). Phytates impair the utilization of protein and some

minerals resulting in poor performance (Hang and Preston, 2009; Hang and Binh, 2013). In cooking, phytic acid combines with the calcium and magnesium in the seed to form insoluble calcium and magnesium phytates (Khatab and Arntfield, 2009). Phytic acid serves as an important reserve of phosphate generated by the action of phytase during seed germination for the developing seedling.

## **2.14 Knowledge and research gap**

### **2.14.1 Determination of the nutrient content**

Given the fact that extensive studies have been conducted on the nutritional content of other oil seeds, however, there is very limited information on the nutritional content of *Hyptis spicigera* Lamiaceae seeds. Few authors carried out studies on the nutritional content of *Hyptis spicigera* Lamiaceae seeds. For example, Grindley, 1950 researched into the nutritional composition of *Hyptis spicigera* Lamiaceae seeds in Sudan and Acipa et al. (2013) did similar studies in Uganda.

### **2.14.2 Determination of anti-nutrient content of *Hyptis spicigera* Lamiaceae seeds.**

Data on the determination of anti-nutrient content of *Hyptis spicigera* Lamiaceae is not available. However; other oil seeds (sesame) were studied and well documented in literature.

**2.14.3 Evaluation of the effect of processing on the nutrient content of *Hyptis spicigera* Lamiaceae seeds.**

No single study was found on the subject. However, similar studies were carried out on other oil seeds (sesame) and were well documented elsewhere.

**2.14.4 Evaluation of the effect of processing on the anti-nutrient content of *Hyptis spicigera* Lamiaceae seeds.**

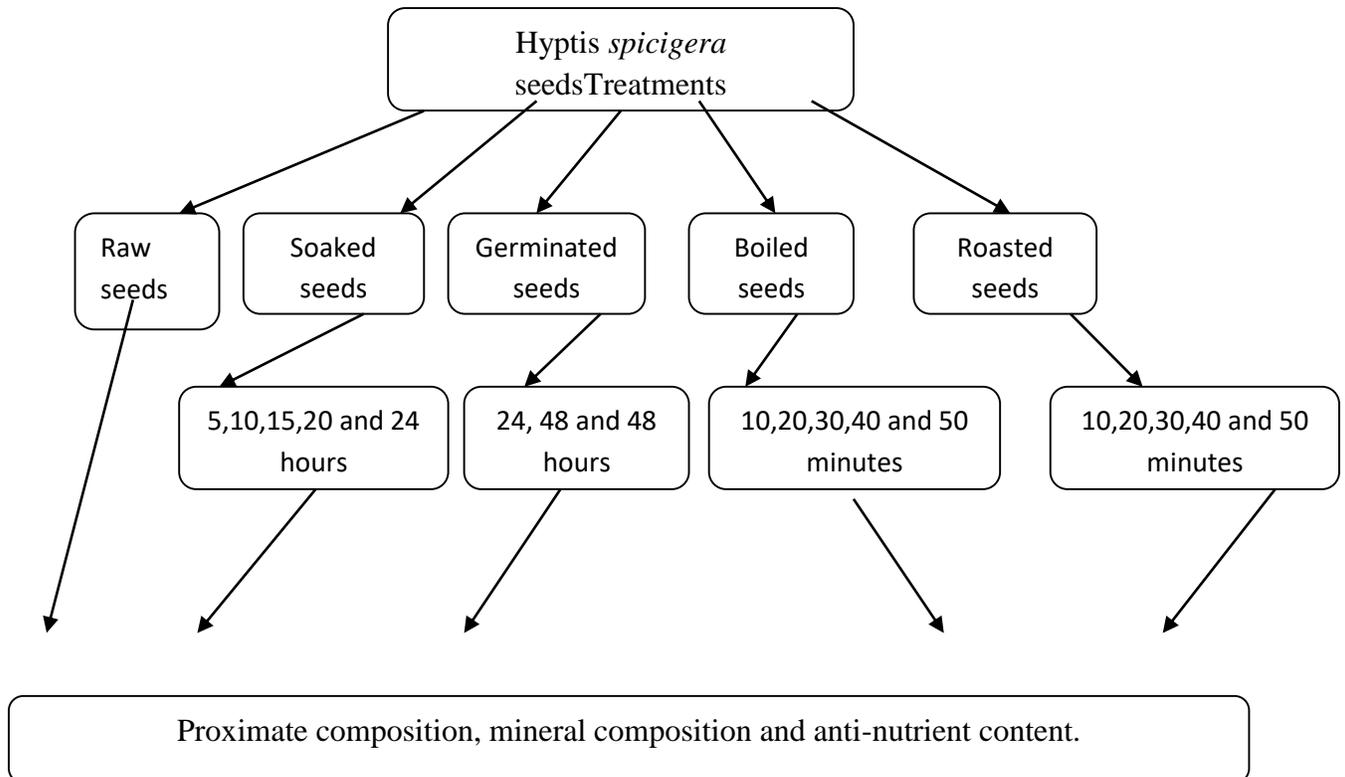
No research was carried on the effect of processing on the anti-nutrient content of *Hyptis spicigera* Lamiaceae seeds. Most of the studies carried out on *Hyptis spicigera* Lamiaceae seeds were on the chemical properties of *Hyptis spicigera* Lamiaceae seeds extract as well as its medicinal and insecticide properties.

## CHAPTER THREE

### MATERIALS AND METHODS

#### 3.1 Research Design

A completely randomized design was used in this study, whereby different processing techniques were the blocks while the nutrients (proximate analysis) and anti-nutrients (phytates, tannins and oxalates), were the assessments carried out. The seeds were randomly selected prior to processing and during analysis. The processing techniques used were: (Soaking, germination, boiling and roasting). Analysis was done to the unprocessed and processed samples for nutrient and anti-nutrient levels. Also the experiments were done in triplicates.



**Figure 3. 1: Flow chart of the treatments carried out**

## **3.2 Collection and preparation of the raw material**

### **3.2.1 Raw material collection**

Ten kilograms of *Hyptis spicigera* seeds were bought from local farmers in Yei, Morobo County in South Sudan. The seeds were packed in plastic bag, and then placed in closed box and transported to Jomo Kenyatta University of agriculture and technology laboratory. Then the seeds were kept in a cold room at 10°C until analysis was done.

### **3.2.2 Raw material preparation**

The seeds were manually cleaned and all the broken ones including the unwanted components such as debris of leaves, stones and seeds of other plants were removed before final processing. They were divided into five portions, each portion contained (500g) of the seeds: one portion was kept as whole seed sample, second portion was soaked in distilled water, and the third portion was soaked and germinated, fourth was boiled and fifth was roasted.

## **3.3 Sample preparation and treatments**

The raw seeds were subjected to processing methods such as: Soaking, soaking and germination, boiling and roasting. Each of these processing methods served as experimental treatment groups to be compared with control (raw grains). The different processing methods are described below:

### 3.3.1 Raw seed (Seeds not treated)

Five hundred grams (500g) of *Hyptis spicigera* seeds were cleaned, removed extraneous materials, then milled in to a fine flour to pass through a 0.3 mm sieve using a Kenwood grinding mill and stored in plastic bags under refrigeration at 10 °C until used and served as a control.



**Figure 3.2: Cleaned (raw *Hyptis spicigera* Lamiaceae seeds).**

### 3.3.2 Soaking treatment

Five hundred grams (500g) of *Hyptis spicigera* seeds were placed in a plastic bowl and then soaked in distilled water of seed to water ratio 2:1 (w/v) for 5, 10, 15, 20 and 24 hours respectively. This was carried out at room temperature. The water left after soaking was discarded. The soaked seeds were dried in an incubator at 50°C until constant weight was obtained, then the dried samples were milled in to a fine flour to pass through a 0.3 mm sieve using a Kenwood grinding mill and stored in plastic bags

under refrigeration at 10°C until used (Kanensi et al., 2011, Makinde & Akinoso, 2013).

The sample is shown in Fig 3.3.



**Figure 3.3: Soaked (*Hyptis spicigera* Lamiaceae seeds).**

### **3.3.3 Germination treatment**

The seeds were weighed in to clean gauze. Then, the seeds were steeped in distilled water at a seed to water ratio of 2:1(w/v) for 5 hours, 10 hours, 15 hours, 20 hours and 24 hours at room temperature. After steeping for the respective time periods, they were germinated for 24, 48 and 72 hours in the dark at room temperature respectively (Figure 3.4). The germinated seeds and rootlets all were dried in an incubator at 50°C until constant weight was obtained, then the dried sample were milled in to a fine flour to pass through a 0.3 mm sieve using a Kenwood grinding mill and stored in plastic bags under refrigeration at 10 °C until used (Kanensi et al., 2011, Makinde & Akinoso (2013).



**Figure 3.4 Germinated (*Hyptis spicigera* Lamiaceae seeds).**

### **3.3.4 Boiling treatment**

Five hundred grams of the seed were divided into five categories, the first category was boiled in distilled water (100°C), with the seed to water ratio of 2:1(w/v) for 10 minutes, and the second was boiled (100°C) for 20 minutes and the third category was boiled (100°C) for 30 minutes, the fourth was boiled for 40 minutes and the fifth was boiled for 50 minutes. After boiling, the water was drained off and boiled seeds were dried in an incubator at 50°C until constant weight was obtained, then the dried sample were milled into a fine flour to pass through a 0.3 mm sieve using a Kenwood grinding mill and stored in plastic bags under refrigeration at 10°C until used (Embaby, 2010). The sample is shown in Fig 3.5



**Figure 3.5 Boiled (*Hyptis spicigera* Lamiaceae seeds).**

### **3.3.5 Roasting treatment**

The seeds were roasted in an oven at 100°C for 10, 20, 30, 40 and 50 minutes according to the method described by Mariod et al., 2012. The roasted seeds were removed from the oven to cool and then milled in to a fine flour to pass through a 0.3 mm sieve using a Kenwood grinding mill and stored in plastic bags under refrigeration at 10 °C until used.

The samples is shown in Fig 3.6



**Figure 3.6. Roasted (*Hyptis spicigera* Lamiaceae seeds).**

### **3.4 Nutritional composition of *Hyptis spicigera* seed flour on dry weight basis.**

#### **3.4.1 Proximate analysis**

Moisture content, crude fibre, crude fat, crude protein and total ash of the raw, soaked, roasted, germinated and boiled seeds were determined using method No. 950.46 (AOAC, 1995).

##### **3.4.1.1 Determination of moisture content**

The moisture was determined according to AOAC method 925.10-32.10.03 (AOAC, 1995). About five grams of fresh sample was weighed and placed in a clean dry moisture dish and the weight of the sample and dish taken. These were placed in a moisture oven and the temperatures adjusted to 105<sup>0</sup>C. The samples were dried for 3

hours and cooled in a desiccator and weighed till a constant weight is retained. The amount of moisture in the samples was calculated using the formula:

$$\% \text{ Moisture} = \frac{(\text{Weight of sample before drying} - \text{Weight of the sample after drying})}{\text{Weight of sample before drying}} \times 100$$

#### **3.4.1.2 Determination of crude ash content**

The ash content was determined by incinerating in a muffle furnace (AOAC, 1995) method 923.03-32.1.05. About five grams of fresh sample was weighed into a clean and weighed crucible, and charred by heating in a fume hood until smoking ceased. The charred samples were then transferred to a muffle furnace and temperature increased gradually to 550°C. The samples were then allowed to ash to completion of whitish colour. Temperature was reduced, samples removed and cooled in a desiccator before weighing. The amount of ash was calculated using the formula:

$$\% \text{ Ash} = \frac{\text{Weight of crucible with ash} - \text{Weight of empty crucible}}{\text{Sample weight}} \times 100$$

#### **3.4.1.3 Determination of crude fat content**

The crude fat was determined using the soxhlet method 920.85.32.1.13. (AOAC, 1995). An intermittent extraction of oil with excess of oil with fresh condensed organic solvent was used. Approximately five grams of sample was weighed into extraction thimbles and initial weight of extraction flasks taken. Fat extraction was done using petroleum spirit in soxhlet apparatus for eight hours. The extraction solvent was rotary evaporated and the extracts were dried in a hot oven for 15 minutes at 70°C before the final weight of the flasks with extracted oil taken. Calculation was done using the formula:

$$\% \text{ Crude fat} = \frac{(\text{Weight of fat extracted}) \times 100}{\text{Weight of sample}}$$

#### 3.4.1.4 Determination of protein

The protein content was determined using the semi-micro kjedahl method (AOAC, 1995), specification 950.46 method 20.87-37.1.22. Approximately 2 g of sample was weighed into a digestion flask together with a combined catalyst of 5 g potassium sulphate and 0.5 g of copper sulphate and 15 mL of sulphuric acid. The mixture was heated in a fume hood for two hours until the digest color turned blue. This signified the end of the digestion process. The digest was cooled, transferred to 100 mL volumetric flask and topped up to the mark with deionized water. A blank digestion with the catalyst was also made. Exactly 10 mL of diluted digest was transferred into the distilling flask and washed with distilled water. 15 mL of 40% NaOH was added and this also washed with distilled water. Distillation was done to a volume of about 60 mL distillate. The distillate was titrated using 0.02 N HCL to an orange color of the mixed indicator, which signified the end point. The nitrogen in the sample was calculated as:

$$\% \text{ Nitrogen} = \frac{V_1 - V_2 \times N \times F \div (V \times 100)}{\text{Sample weight}}$$

Where:  $V_1$  is the titre of sample in ml

$V_2$  is the titre for the blank in ml

N=normality of standard HCL (0.02)

f = factor of standard HCL solution

V= volume of diluted digest taken for distillation (10 mL)

S = weight of sample taken for distillation (1 g).

The protein content was then calculated as:

% Protein = Nitrogen  $\times$  Protein factor (6.25)

#### **3.4.1.5 Determination of crude fibre**

The crude fibre was determined according to (AOAC., 1995, Method 920.86-32.1.15). Approximately two grams of the sample was weighed into a 500 ml conical flask. About 200ml of boiling 1.25% H<sub>2</sub>SO<sub>4</sub> was added and boiling done for 30 minutes under reflux condenser. Filtration was done under slight vacuum with Pyrex glass filter (crucible type) and the residue washed to completely remove the acid with boiling water. Approximately 200ml of boiling 1.25% NaOH was added to the washed residue and boiling done under reflux for another 30 minutes. Filtration was done using the same glass filter previously used with the acid. The residue was rinsed with boiling water followed by 1% HCl and again washed with boiling water to rinse the acid from the residue. The residue was washed twice with alcohol and thrice with ether. It was then dried in a hot-air oven at 105<sup>0</sup>C in a porcelain dish to a constant weight. Incineration was then done in a muffle furnace at 550<sup>0</sup>C for three hours and then the dish was cooled in a dessicator. The final weight taken and calculation was done using the formul

$$\text{crude fibre(\%)} = \left( \frac{w1 - w2}{w} \right)$$

W1-Weight of acid and alkali digested sample

W2-Weight of incinerated sample after acid and alkali digestion

W-Weight of sample taken

#### **3.4.1.6 Determination of total carbohydrate content**

The contents of total carbohydrates were calculated by subtracting the sum of moisture, protein, fat and ash from 100 (AOAC, 1995).

% Total carbohydrate = 100- (% Moisture + % Fat+ % Ash+ % Crude Protein).

#### **3.5 Determination of total mineral content (Iron, zinc calcium and magnesium)**

The mineral analysis was determined according to AOAC (1995) method. Ash that was previously determined (refer to 3.4.1.2) was cooled. Then 15ml of 6N HCl was added to samples in crucibles before transferring to 100ml volumetric flasks. Distilled water was used to top up to the mark (100ml). An Atomic Absorption Spectrophotometer (AAS) was used to determine all the minerals (Model A-6200, Shimadzu, Corp., and Kyoto, Japan). Clean equipment's and ready standards purchased from the supplier were used. Standards curves for iron, zinc, calcium and magnesium are shown (refer to appendix 1)

### 3.6 Anti-nutrient content analysis

#### 3.6.1 Determination of tannin content of *Hyptis spicigera* seed flour on dry weight basis.

Tannin content was determined by the method in Kirk and Sawyer (1998). Approximately 5g of flour samples were weighed into a volumetric flask and 50 ml of distilled water was dispensed inside the volumetric flask, shaken for 30 minutes. The mixture was allowed to stand for about 30 minutes 28°C before it was filtered through Whatman No.42 filter paper. 2 ml of the extract and the standard tannin solution (tannic acid) 0, 0.1, 0.2, 0.3, 0.4 and 0.5mg/ml were dispersed into a 50ml flask. Similarly, 2 ml of distilled water was put in a separate volumetric flask as a blank to calibrate the instrument to zero. 2 ml of Folin Denis reagent was added to each of the flasks followed by 2.5ml of saturated Na<sub>2</sub>CO<sub>3</sub> solution. The content of each flask was made to 50ml with distilled water and allowed to incubate at 28°C for 90 minutes.

Their respective absorbance was measured in a UV-vis spectrophotometer (UV mini 1240 model, Shimadzu Corp., Kyoto, Japan) at 760nm.

$$\text{Mg}/100 = \left( \frac{Y/M}{SW} \times \frac{1000}{100} \right)$$

Y- absorbance.

M - gradient of the standard curve

SW- sample weight

### **3.6.2 Determination of oxalates in *Hyptis spicigera* seed flour**

Oxalates were analyzed by HPLC method (Libert, 1981) with modifications suggested by Yu *et al*, (2002). A 0.5 g fresh weight of sample was homogenized in 4 mL of 0.5N HCL. The homogenate was heated at 80<sup>0</sup> C for 10 minutes with intermittent shaking. To the homogenate, distilled water was added up to a volume of 25 mL. About 3 mL of the solution was withdrawn and centrifuged at 12000 rpm for 10 minutes. About 1 mL of supernatant was passed through a micro filter (0.45μ) before HPLC analysis.

Standards were prepared at varying concentrations for quantification. HPLC analysis was carried by using Shimadzu UV-VIS detector, Hypsil C18 column (5μ M, 4.6 mm \*250 mm) equipped waters 550 was used as the static phase and the mobile phase was a solution 0.01 N H<sub>2</sub>SO<sub>4</sub>. Flow rate was 0.6 mL min<sup>-1</sup>, pressure of 62 kg f and detection wave length of 221 nm.

$$\text{Mg}/100 = \left( \frac{Y/M}{SW} \times \frac{1000}{100} \right)$$

Y- absorbance

M - gradient of the standard curve

SW- sample weight

### **3.6.3 Determination of phytates in *Hyptis spicigera* seed flour**

Phytates were analyzed by HPLC analysis method of phytic acid according to Camire and Clydesdale (1982). Approximately 0.5 g of sample was extracted with 10 mL of 3%

H<sub>2</sub>SO<sub>4</sub>. The content was filtered and the filtrate transferred to a boiling water bath for 5 minutes followed by 3 mL of FeCl<sub>3</sub> solution (6 mg ferric iron per mL in 3% H<sub>2</sub>SO<sub>4</sub>) added contents were heated for 45 minutes to complete precipitation of the ferric phytate complex. This was centrifuged at 2500 revolutions per minute (rpm) for 10 minutes and the supernatant discarded. The precipitate was washed with 30 mL distilled water, centrifuged and the supernatant discarded. A 3 mL of 1.5 N NaOH was added to the residues and the volume brought to 30 mL with distilled water. The content was heated for 30 minutes in a boiling water bath to precipitate the ferric hydroxide. The cooled sample was centrifuged and the supernatant transferred into a 50 mL volumetric flask. The precipitate was rinsed with 10 mL distilled water, centrifuged and the supernatant added to the contents of the volumetric flask. This was micro filtered and kept waiting for HPLC analysis. HPLC analysis was done using Shimadzu Refractive Index Detector (RID- 6A). The mobile phase was 0.005 N sodium acetate in distilled water, at a flow rate of 0.5 µL/min.) The standard curve is shown in (appendix 4).

$$\text{Mg}/100 = \left( \frac{Y/M}{SW} \times \frac{1000}{100} \right)$$

Y- height of the peak

M - gradient of the standard curve

SW-sample weight

### **3.7 Data analysis**

The data obtained were subjected to Analysis of Variance (ANOVA) using Genstat 14<sup>th</sup> Edition). The mean values were displayed with standard deviation (SD). The mean comparisons for the treatments were made using Duncan's Multiple Range Test (DMRT) and significant difference was accepted at  $p \leq 0.05$ .

## CHAPTER FOUR

### RESULTS AND DISCUSSION

There is limited information on the nutritional and anti-nutrient content of *Hyptis spicigera* Lamiaceae seed. Therefore, based on this limitation the results and discussion will be in form of comparison with other edible oilseed plants.

#### **4.0 Nutritional composition of *Hyptis spicigera* Lamiaceae seed**

##### **4.1 The proximate composition of *Hyptis spicigera* Lamiaceae seed on dry weight basis.**

The seed had a high fat content (35.79%). The proximate composition of *Hyptis spicigera* Lamiaceae seed flour is shown below in Table 4.1. The ash content 2.97% for *Hyptis spicigera* Lamiaceae seed flour was lower than the 5-6% range for sesame seed reported by Lee et al. (2005) and Borchani et al. (2010). High level of ash makes the oilseed a good source of mineral nutrition to the consumer (Afolabi, 2008).

Crude protein content 16.83% for *Hyptis spicigera* Lamiaceae seed flour is higher than of 0.04% reported by Acipa et al. (2013). This deviation could be attributed to variety of the seed and type of soil. However, these results is comparable to raw beni-seed seeds (18.87%) reported by Borchani et al. (2010). Consumption of 100g of *Hyptis spicigera* seeds would provide 200% RDA protein for infants 6-12 months, 100% RDA for children 1-8 years, males 9-13 years and females 9-13 years (WHO, 1998). Proteins have an important function in nutrition and supply adequate amounts of required amino acids to the body (Pugalenthi et al., 2004).

The crude fibre content 19.10% for *Hyptis spicigera* Lamiaceae seed flour obtained is lower than 61.28% reported by Acipa et al. (2013) but higher than the findings (4% – 5%, 6.26%, 6.09% and 6.17%) for sesame seed flour reported by Lee et al. (2005); Adeniyani et al.(2013) and Zebib et al (2015) respectively. Consumption of 100g of *Hyptis spicigera* seeds would provide 100% RDA fibre for children 1-8 years, males 9-70 years, females 9-70 years, pregnant mothers 18-50 years and lactating (WHO, 1998).Crude fibre plays an important role in normal peristaltic movement of the intestinal tract hence diet containing low fibre could cause constipation and could lead to colon diseases, piles, cancer and appendicitis (Ibironke and Ademola, 2014).

Crude fat content 35.79% for the seed is within the range 18% and 37% reported by Onayade et al. (1990). It is also comparable to 38.54% for sesame seed reported by Mbaebie et al. (2010).Consumption of 100g of *Hyptis spicigera* seeds would provide 100% RDA fat for infants 0 -12 months (WHO, 1998).The result shows that *Hyptis spicigera* Lamiaceae seeds could be a good source of oil. Fat acts as flavour retainer and increases the palatability of food (Iwe and Egwuekwe, 2010).

Carbohydrate content 25.31% for the *Hyptis spicigera* Lamiaceae seed flour was similar to 23.21% reported for *Hyptis spicigera* seed grown in Sudan (Grindley, 1950).It is higher than 11.69% -11.39% ranges for sesame seeds reported by Zebib et al. (2015). The carbohydrate value of the flour shows that seed is not a rich source of energy when consumed alone.

**Table 4.1: Proximate composition of *Hyptis spicigera* Lamiaceae seed flour on dry weight basis.**

Parameter	Percentage (%)
Ash	2.97 ±0.13
Crude fibre	19.10 ±0.71
Crude Fat	35.79 ± 0.49
Crude Protein	16.83 ±0.20
Total Carbohydrate	25.31 ±0.78

Values are means of triplicate determinations ± S.D.

#### **4.2. The mineral content of *Hyptis spicigera* Lamiaceae seed flour.**

The minerals selected for this study are of public health concern. Magnesium is the most abundant mineral found in the seed (297.9 mg/100g). The mineral content of *Hyptis spicigera* Lamiaceae seed flour is shown in Table 4.2.

Magnesium content of *Hyptis spicigera* Lamiaceae seed is higher than 119.29 mg/100g value reported by Acipa et al. (2013) Magnesium is important in supporting system activity of hormones, manufacturing of energy, maintenance of reproductive system, immune system and regulation of an intimate rhythm and arterial pressure together with calcium (Eryomenko, 2010). Iron content 2.72 mg/100g of the seed was within the range 2.29% reported by Acipa et al. (2013). Consumption of 400g of *Hyptis spicigera* seeds

would provide 100% RDA calcium and magnesium for infants 0 -12 months and children 1 -8 years. According to Bello et al. (2008), calcium helps in regulation of muscle contractions, transmission of nerve impulses and bone formation. Consumption of 100g of *Hyptis spicigera* seeds would provide 100%RDA zinc for all groups; with the exception of iron which needs to be increased to 300g to meet the 100% RDA iron for all group age per day.

**Table 4.2: The mineral content of *Hyptis spicigera* Lamiaceae seed flour on dry weight basis.**

Parameters	mg/100g
Iron	2.72 ±0.24
Calcium	252.6 ± 0.38
Zinc	2.52 ± 0.08
Magnesium	297.9 ± 0.74

Values are means of triplicate determinations ± S.D.

#### **4.3 The anti-nutrient content of *Hyptis spicigera* Lamiaceae seed**

The seeds contained high levels of oxalates (434.8 mg/100g) as given in Table 4.3 below. Oxalates can bind with calcium and magnesium, and interfere with their metabolism, cause muscular weakness and paralysis (Soetan & Oyewole, 2009). The majority of urinary stones formed in humans are calcium oxalate stones and patients are advised to limit their intake of foods with a total intake of oxalate not exceeding 50–60 mg per day (Massey et al. 2001). Phytate content for the seed flour was within the range

(379- 494 mg/100g) reported for sesame seed by Thunyarat et al. (2012). The tannin content 332.0 mg/100g is within the range (85 - 660 mg/100g) reported for sesame seed by Deme et al. (2017). Tannins are one of the most important bioactive constituents of plants (Ajayi et al., 2011), but they are considered anti-nutrients as they bind nutrients including zinc and iron.

**Table 4.3: Anti- Nutrient composition of *Hyptis spicigera* Lamiaceae seed on dry weight basis.**

Parameter	mg/100g
Tannins	332.0 ± 12.98
Oxalates	434.8 ± 57.71
Phytates	390.4 ± 19.60

Values are means of triplicate determinations ± S.D.

#### **4.4 Effect of soaking on the nutrient composition of *Hyptis spicigera* Lamiaceae seed flour**

Nutrient composition of the seed had a slight increased with soaking time with exception of protein content which decreased to 11.39 % as shown in Table 4.4 below. The *Hyptis spicigera* Lamiaceae seed flour soaked for 5 hours had the highest fat content as compared to the raw seed flour.

Seeds soaked 24 hours produced a non- significant increase in ash content ( $p=0.280$ ), in relation to the raw seeds. Nsa et al. (2011) reported similar findings and attributed it to leaching of the mineral elements into the water during soaking. However, these results are within the range of 3.67% - 5.39% for sesame seeds as obtained by Ozcan and Akgul (1995). Ash represents the mineral matter left after food material is burnt in oxygen (Enwereuzoh et al., 2015).

**Table 4.4: Effect of soaking on the nutrient composition of (*Hyptis spicigera* Lamiaceae) seed flour on dry weight basis.**

Treatments	Ash%	Crude Fibre%	Crude Fat%	Crude Protein%	Total Carbohydrate%
Raw	2.97±0.13 <sup>b</sup>	19.10±0.71 <sup>a</sup>	35.79± 0.49 <sup>a</sup>	16.83±0.20 <sup>b</sup>	25.31±0.75 <sup>c</sup>
Soaking 5 hours	1.86±0.08 <sup>a</sup>	16.48±1.14 <sup>a</sup>	52.25±1.45 <sup>c</sup>	17.00± 0.72 <sup>b</sup>	12.41±1.71 <sup>a</sup>
Soaking 10 hours	2.70±0.06 <sup>b</sup>	17.87±2.30 <sup>a</sup>	48.04± 1.80 <sup>bc</sup>	16.16±0.69 <sup>b</sup>	15.23±3.89 <sup>ab</sup>
Soaking 15hours	2.71± 0.35 <sup>b</sup>	19.46±0.86 <sup>a</sup>	46.06±2.43 <sup>b</sup>	11.56±0.40 <sup>a</sup>	20.21±3.57 <sup>bc</sup>
Soaking 20 hours	3.08 ±0.26 <sup>b</sup>	24.23±1.07 <sup>a</sup>	35.52±0.19 <sup>a</sup>	11.45±0.59 <sup>a</sup>	25.72±1.37 <sup>c</sup>
Soaking 24 hours	3.10±0.08 <sup>b</sup>	26.22±1.44 <sup>b</sup>	34.83±0.59 <sup>a</sup>	11.39±0.08 <sup>a</sup>	24.46±1.17 <sup>c</sup>

Values are means of triplicate determinations ± S.D. Means in the same column followed by the same superscript are not significantly different at  $p<0.05$ . Mean comparison for the treatments were made using Duncan's Multiple Range Test.

Soaking had an effect on the crude fibre content were seeds soaked 24 hours (26.22 %) had high crude fibre content and low in seed soaked 5 hours (16.48%) compared to the raw seeds. Crude fiber was significantly increased ( $p=0.001$ ) in soaked compared to the raw. Consumption of 100g of *Hyptis spicigera* soaked seeds would provide RDA fibre

for children 1-8 years, males 9-70 years, females 9-70 years, pregnant mothers 18-50 years and lactating (WHO, 1998). According to Makinde et al. (2016), this increase could have been due to protein–fiber complexes formed after possible chemical modification induced by the soaking of the seeds. Crude fibre is an indication of the roughage/bulkiness of the sample and its presence in the diet serves in reducing constipation by increasing bowel movement (Abiodun and Adegbite, 2012).

Fat content of the soaked seeds was highest in seeds soaked for 5 hours (52.25%) and least in seeds soaked for 24 hours (34.83%). There was a significant increase ( $p=0.001$ ) in the fat content of the soaked seeds compared to the raw seed. Similar findings were also reported on sesame seeds by Kajihansa et al. (2014) and attributed the increase to high fat content or oil content of the seeds. Consumption of 100g of *Hyptis spicigera* soaked seeds would provide RDA fat for infants 0 -12 months (WHO, 1998).

There was significant reduction ( $p=0.001$ ) in the protein content of the soaked seeds with respect to soaking period. Seeds soaked for 5 hours had the highest protein content (17 %), while seeds soaked 24 hours had the least protein value compared to the raw seed. Nsa et al. (2011) observed similar decrease, as soaking duration increased and attributed to some solubilization and leaching out of nitrogenous substances into the water. Acipa et al. (2013) reported a low value (0.04%) for *Hyptis spicigera* seed. Soaking the seeds for a short period of time would provide RDA protein for infants 6-12 months, children 1-8 years, males 9-13 years and females 9-13 years (WHO, 1998).

Seeds soaked for 20 hours had the highest carbohydrate content and seeds soaked for 5 hours had the least value compared to the raw seed. Soaking significantly decreased ( $p=0.008$ ) on the carbohydrate content of the soaked compared to the raw seed flour. The significant loss of carbohydrate noted in soaked *Hyptis spicigera* Lamiaceae seed might be as a result of solubility of carbohydrate in water. Carbohydrate provides energy to the cells in the body, particularly the brain, which is the only carbohydrate – dependent organ in the body (Enwereuzoh et al., 2015).

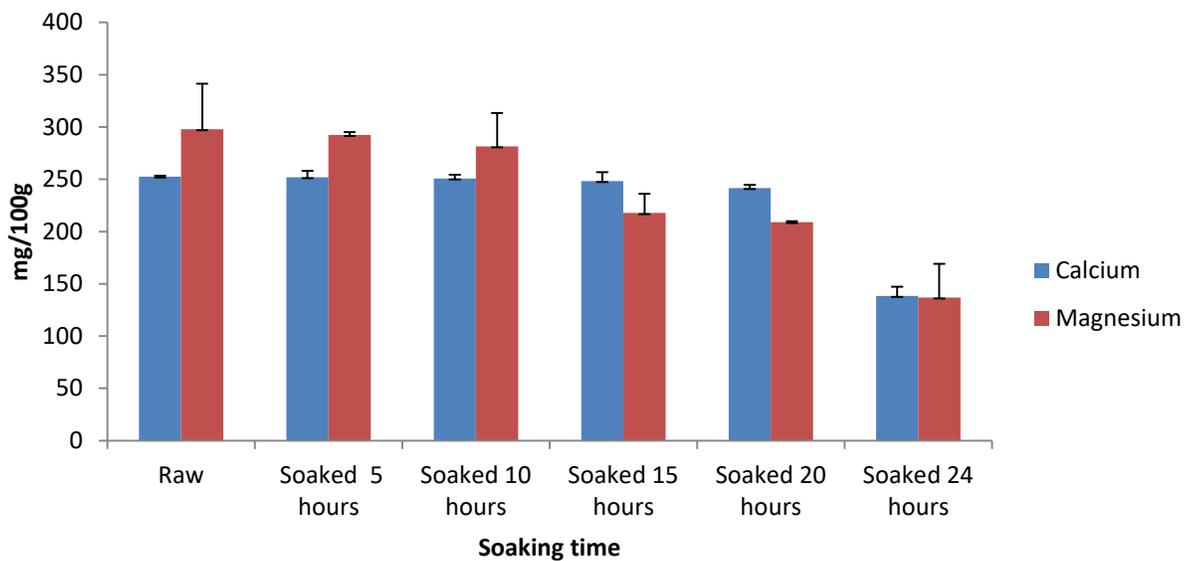
#### **4.5 Effect of soaking on the zinc and iron content of *Hyptis spicigera* Lamiaceae seed flour on dry weight basis.**

Iron level (2.72 mg/100g) was high in the raw seeds compared to the treated. Soaking had a non-significant reduction on both zinc content of the seed ( $p=0.210$ ) and iron ( $p=0.882$ ) compared to the raw seed. The reduction of minerals by soaking might be due to the loss of water soluble minerals by the steeping medium and the rinsing process (Bau et al., 1999).

#### **4.6 Effect of soaking on the calcium and magnesium content of *Hyptis spicigera* Lamiaceae seed flour.**

The effect of soaking on magnesium and calcium is demonstrated in figure 4.1 as below. Magnesium had the highest mineral content (297.9 mg/100g) in raw seed compared to the treated. There was a decrease in magnesium and iron content of the seed with increased soaking time compared the raw seed. The calcium content had decreased but not significantly ( $p=0.062$ ) with increase in soaking time. Meanwhile magnesium content had a significant decreased ( $p=0.001$ ) with increase in soaking time compared to

the raw seed. The calcium content of the raw *Hyptis spicigera* seed (252.6 mg/100g) obtained from the current study is within the range for other oilseeds like Flaxseed 255mg/100g (Rohini *et al.*, 2015). Calcium is important, because it is the major component of the bone and assists in teeth development (Okaka and Okaka., 2001). The reduction of minerals by soaking might be due to the loss of water soluble minerals by the steeping medium and the rinsing process (Bau *et al.*, 1999).



**Figure 4.1 Effect of soaking on the calcium and magnesium content of (*Hyptis spicigera* Lamiaceae) seed flour on dry weight basis.**

**4.7 Effect of soaking and germination on the nutrient composition of *Hyptis spicigera* Lamiaceae seed flour on dry weight basis.**

Soaking and germinating *Hyptis spicigera* seed improved the nutrient content of seed. Seeds soaked 20 hours and germinated 24 hours had the highest crude fat as shown below in Table 4.5.

The ash content increased significantly ( $p=0.43$ ) in seeds soaked 10 hours and germinated 48 hours, and reduced in soaked 24 hours and germinated 72hrs compared to the raw seed. The results agree with the findings by Rekha *et al.* (2007), who reported that the ash content rose to 3.23% in cowpea germinated for 72 h. According to Fouad *et al.* (2015) this could be due to increase in phytase enzyme activity during germination, were the enzyme hydrolyzes the bond between the protein-enzyme minerals become free.

It was observed that the protein content increased in seeds soaked 10 hours and germinated 48 hours, and decreased in seeds soaked 10 hours and germinated 72 hours compared to the raw seed. There was a significant increase on protein ( $p=0.05$ ) for all the treatments. This result agrees with earlier reports of increased protein content during germination of various cereals, legumes and oilseeds (Inyang and Zakari, 2008; Onwuka, *et al.*, 2008). According to Olagunju and Ifesan (2012) this increase could be attributed to net synthesis of enzymic protein (proteases) by germinating seeds.

The fat content decreased significantly ( $p=0.021$ ) as germination progressed with the highest value in seeds soaked 20 hours and germinated 24 hours. However, the decrease was noted in seeds soaked 20 hours and germinated 72 hours compared to the raw seed. Hahm *et al.* (2008) reported similar reduction on sesame seed after germination and attributed that to the use of starch as a source of energy to start germination (Mubarak, 2005).

**Table 4.5: Effect of soaking and germination on the nutrient composition of (*Hyptis spicigera* Lamiaceae) seed flour on dry weight basis.**

Treatment	Ash%	Crude Fibre%	Crude Fat%	Crude Protein%	Total Carbohydrate%
Raw	2.97±0.13 <sup>b</sup>	19.10 <sup>abc</sup> ±0.71	35.79 <sup>a</sup> ±0.49	16.83 <sup>cd</sup> ±0.20	25.31±0.75 <sup>abcde</sup>
Soaked5hours,germinated 24 hours	3.19±0.03 <sup>b</sup>	21.58±1.26 <sup>abc</sup>	43.16±4.28 <sup>cdefg</sup>	12.86±0.27 <sup>bcd</sup>	19.19±3.99 <sup>abcd</sup>
Soaked5hours,germinated 48 hours	3.06±0.03 <sup>b</sup>	20.54±1.24 <sup>abc</sup>	42.70±0.74 <sup>cdef</sup>	13.35±0.59 <sup>bcd</sup>	20.32±2.26 <sup>abcd</sup>
Soaked5hours,germinated 72 hours	3.02±0.02 <sup>b</sup>	23.11±2.06 <sup>c</sup>	47.24±6.33 <sup>defg</sup>	11.35±5.59 <sup>bc</sup>	15.25±10.6 <sup>a</sup>
Soaked10hours,germinated24hours	2.77±0.23 <sup>b</sup>	17.43±1.59 <sup>a</sup>	38.84 ±0.18 <sup>abc</sup>	13.35±0.28 <sup>abcd</sup>	27.59±3.36 <sup>f</sup>
Soaked10hours,germinated48hours	3.26±0.02 <sup>b</sup>	21.74±1.41 <sup>abc</sup>	39.50±2.00 <sup>abc</sup>	17.96 ±1.36 <sup>d</sup>	17.52±0.22 <sup>abc</sup>
Soaked10hours,germinated72hours	3.30±0.17 <sup>b</sup>	20.48±1.53 <sup>abc</sup>	35.75±1.72 <sup>ab</sup>	9.88 ±0.45 <sup>b</sup>	30.56±3.34 <sup>def</sup>
Soaked15hours,germinated24hours	3.19±0.05 <sup>b</sup>	17.87±0.28 <sup>a</sup>	40.37± 0.67 <sup>abc</sup>	12.22 ±0.47 <sup>bc</sup>	26.33±1.23 <sup>abcde</sup>
Soaked15hours,germinated48hours	2.35±0.85 <sup>b</sup>	19.35±0.44 <sup>abc</sup>	42.42±1.65 <sup>bcd</sup>	12.97 ±0.26 <sup>bcd</sup>	22.88±0.70 <sup>abcd</sup>
Soaked15hours,germinated72hours	2.47±0.31 <sup>b</sup>	22.82±0.73 <sup>bc</sup>	42.61±1.59 <sup>cde</sup>	14.94 ±0.70 <sup>bcd</sup>	17.13±1.67 <sup>ab</sup>
Soaked20hours,germinated24hours	2.45±0.16 <sup>b</sup>	18.53±0.52 <sup>abc</sup>	48.96±1.96 <sup>eg</sup>	10.98 ±0.35 <sup>bc</sup>	19.05±2.09 <sup>abcd</sup>
Soaked20hours,germinated48hours	2.77±0.05 <sup>b</sup>	18.17±2.45 <sup>ab</sup>	34.90±0.42 <sup>a</sup>	14.37 ±0.44 <sup>bcd</sup>	29.78±3.09 <sup>cde</sup>
Soaked20hours,germinated72hours	2.49±0.33 <sup>b</sup>	17.86 ±2.93 <sup>a</sup>	33.69±0.10 <sup>a</sup>	10.72 ±0.05 <sup>b</sup>	35.22±2.74 <sup>ef</sup>
Soaked24hours,germinated24hours	3.22±0.11 <sup>b</sup>	19.72±0.09 <sup>abc</sup>	37.40±2.69 <sup>abc</sup>	12.57 ±0.45 <sup>bc</sup>	27.07 ±2.48 <sup>bcde</sup>
Soaked24hours,germinated48hours	2.17±0.72 <sup>b</sup>	21.34±1.12 <sup>abc</sup>	40.31±0.74 <sup>abc</sup>	14.12 ±0.41 <sup>bcd</sup>	22.04±1.06 <sup>abcd</sup>
Soaked24hours,germinated72hours	1.04±0.29 <sup>a</sup>	17.44±0.48 <sup>a</sup>	38.02±2.05 <sup>abc</sup>	16.02 ±1.84 <sup>cd</sup>	27.46± 3.49 <sup>bcde</sup>

Values are means of triplicate determinations ± S.D. Means in the same column followed by the same superscript are not significantly different at p<0.05. Mean comparison for the treatments were made using Duncan's Multiple Range Test.

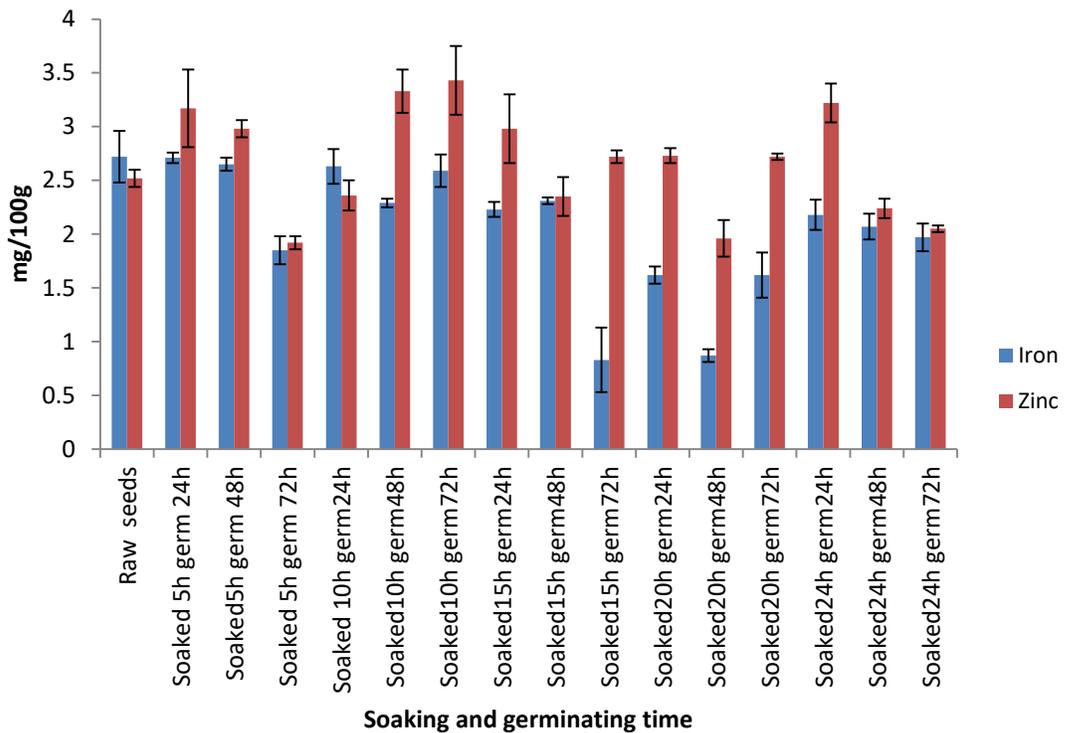
The crude fiber content was not significantly different ( $p=0.08$ ) for soaked and germinated seed compared to the raw seeds as shown in Table 4.5. Seeds soaked 15 hours and germinated 72 hours had the highest content crude fibre while the lowest in soaked 10 hours and germinated 24 hours compared to the raw seed. The values obtained in these results are higher than 9.24% – 9.42% reported for soaked and germinated sesame by Kajihansa *et al.* (2014). Crude fiber in diet is known to enhance digestibility, slowing down the release of glucose into blood stream, aiding bowel movement, and prevention of bowel cancers (Arinola & Adesina, 2014).

The seeds soaked for 10 hours and germinated 72 hours had the highest carbohydrate content and the lowest in seeds soaked 5 hours and germinated 72 hours compared to the raw seed. There was a significant increase ( $p=0.04$ ) on the carbohydrate content of the soaked and germinated seed and compared to the raw seed flour. This increase was explained in Eastmond and Graham (2001) as an extensive conversion of storage lipids to soluble carbohydrate after the onset of germination.

#### **4.8 Effect of soaking and germination on the zinc and iron content of *Hyptis spicigera* Lamiaceae seed flour**

The iron and zinc content for the soaked and germinated seeds decreased with increased soaking time (Figure 4.2). Seeds soaked 10 hours and germinated 72 hours had the highest zinc content and the lowest level in seeds soaked 5 hour and germinated 72 hours. There was no significant difference in zinc ( $p=0.143$ ) and iron ( $p=0.599$ ) content for the soaked and germinated seed compared to the raw seeds.

Pandey and Awasthi (2015); Duhan et al. (2002) reported on decreased iron content in germinated fenugreek seed flour and attributed that to leaching of iron into soaking medium. Dietary iron is known for its role in psychomotor development, maintenance of physical activity and resistance to infection (Black, 2003).

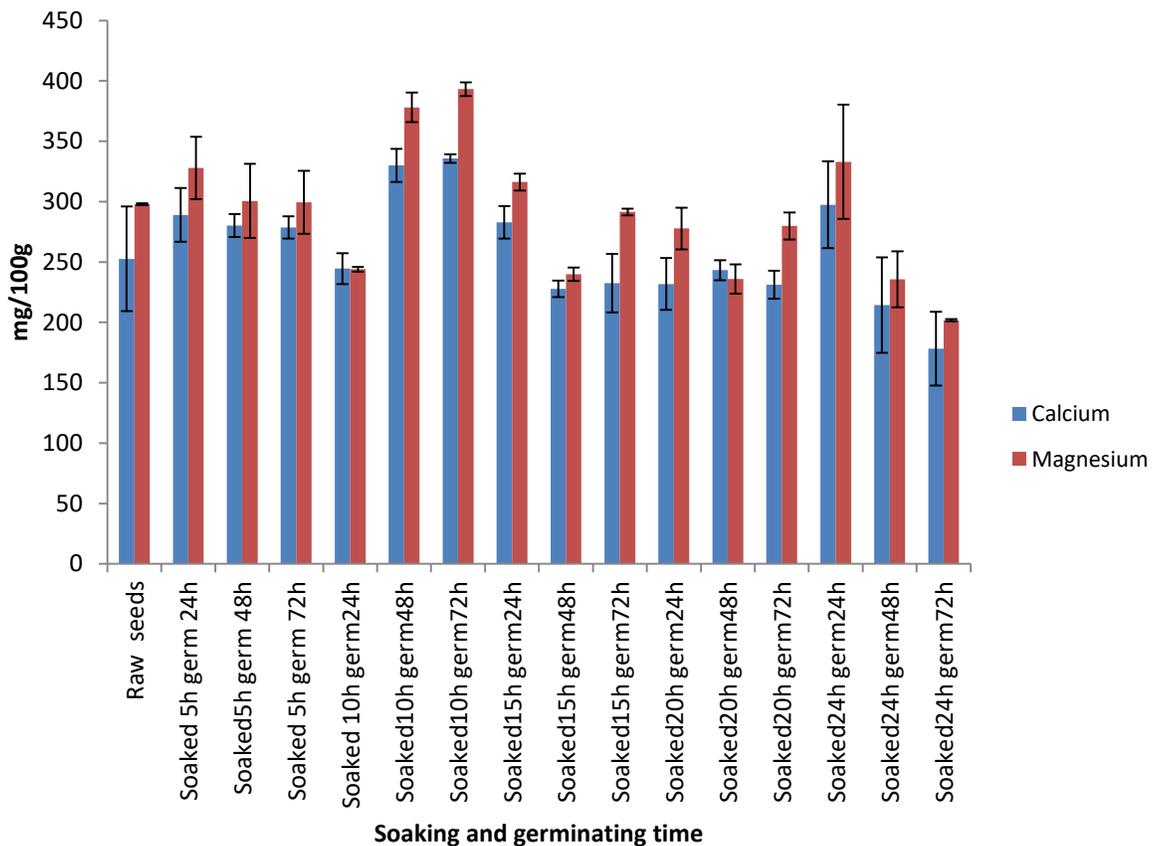


**Figure 4.2 Effect of soaking and germination on the zinc and iron content of (*Hyptis spicigera* Lamiaceae) seed flour on dry weight basis. h: hours.germ: germination.**

#### **4.9 Effect of soaking and germination on the calcium and magnesium content of *Hyptis spicigera* Lamiaceae seed flour.**

The magnesium content (393.1 mg/100g) for soaked and germinated seeds of *Hyptis spicigera* had the highest value (Figure 4.3). Calcium had the highest values in seeds soaked 10 hours; germinated 72 hours and least in seeds soaked 24 hours; germinated 72

hours compared to the raw seed. A significant increase ( $p=0.001$ ) was noted for calcium and magnesium compared to the raw seed. Similar results were reported by Hahm *et al.* (2009) who found that calcium content in sesame seeds increased after germination. According to El- Mahdy and El- Sebaiy (1982) the increase in calcium might be due to decrease in phytates, tannins and other anti-nutritional factors that bind the minerals.



**Figure 4.3 Effect of soaking and germination on the calcium and magnesium content of (*Hyptis spicigera* Lamiaceae) seed flour on dry weight basis. h:hours.germ: germination.**

#### **4.10 Effect of boiling on the nutrient composition of (*Hyptis spicigera* Lamiaceae) seed flour**

Boiling reduced the protein content of the flour when compared to the raw seed as represent below in Table 4.6

The result shows that the ash content for boiled *Hyptis spicigera* seed) is significantly low ( $p=0.001$ ) in seeds boiled for 5 minutes and seeds boiled for 50 minutes compared to the raw seeds ( Table 4.6 ). The observed reduction in the ash contents with time of boiling may be due to leaching of minerals into the water used for the wet-heat processing. The values are within the range (2.26-4.94%) results for raw and boiled beniseed reported by Adeniyani et al., 2013. Ash is used as a tool to measure the mineral content in any sample (Enwereuzoh et al., 2015).

There was a reduction in fibre content ( $p=0.001$ ) corresponding to an increase in boiling time as shown in Table 4.6. The highest value was recorded in seeds boiled for 10 minutes and least in seeds boiled for 50 minutes compared to the raw seed. According to Nsa *et al.* (2011) the decrease could be attributed to softening and subsequent loss of the seeds hard coat in the course of boiling and decanting of water. Adeniyani et al. (2013) reported high values (4.45-6.17-4.94%) for raw and boiled beniseed compared to the current results.

The comparison of raw and boiled *Hyptis spicigera* seed flour revealed that boiling significantly increased the crude fat content ( $p=0.001$ ) as shown in Table 4.6. The result shows that the highest value of boiled *Hyptis spicigera* seed flour was found in seeds

boiled 10 minutes and the lowest in seeds boiled 50 minutes compared to the raw seed. Adeniyani et al. (2013) reported on similar findings and attributed to the disruption of the cell structures and membrane partitions of the seeds by heat during boiling causing the fat to melt and be easily released from the seeds. The fat content is lower than (49.23-56.7%) results for raw and boiled beniseed reported by (Adeniyani et al., 2013).

**Table 4.6: Effect of boiling on the nutrient composition of (*Hyptis spicigera* Lamiaceae) seed flour on dry weight basis.**

Treatment	Ash%	Crude Fibre%	Crude Fat%	Crude Protein%	Total Carbohydrate%
Raw	2.97±0.14 <sup>b</sup>	19.10±0.71 <sup>c</sup>	35.79±0.49 <sup>a</sup>	16.83±0.20 <sup>b</sup>	25.31±0.75 <sup>a</sup>
Boiled 10 minutes	2.88±0.14 <sup>b</sup>	18.65±0.08 <sup>c</sup>	42.58±1.29 <sup>d</sup>	13.92±0.80 <sup>a</sup>	21.94±0.85 <sup>a</sup>
Boiled 20 minutes	2.84±0.25 <sup>b</sup>	18.817±1.04 <sup>c</sup>	39.84±0.55 <sup>c</sup>	13.51±0.60 <sup>a</sup>	25.61±1.24 <sup>ab</sup>
Boiled 30 minutes	2.79±0.14 <sup>b</sup>	17.55±1.33 <sup>c</sup>	39.30±0.53 <sup>bc</sup>	13.018±0.45 <sup>a</sup>	27.16±0.26 <sup>bc</sup>
Boiled 40 minutes	2.76±0.13 <sup>b</sup>	14.91±1.45 <sup>b</sup>	38.65±1.03 <sup>bc</sup>	13.10±0.28 <sup>a</sup>	30.55±2.50 <sup>c</sup>
Boiled 50 minutes	1.24±0.33 <sup>a</sup>	12.36±0.11 <sup>a</sup>	36.72±0.39 <sup>ab</sup>	12.74±0.57 <sup>a</sup>	36.92±0.39 <sup>d</sup>

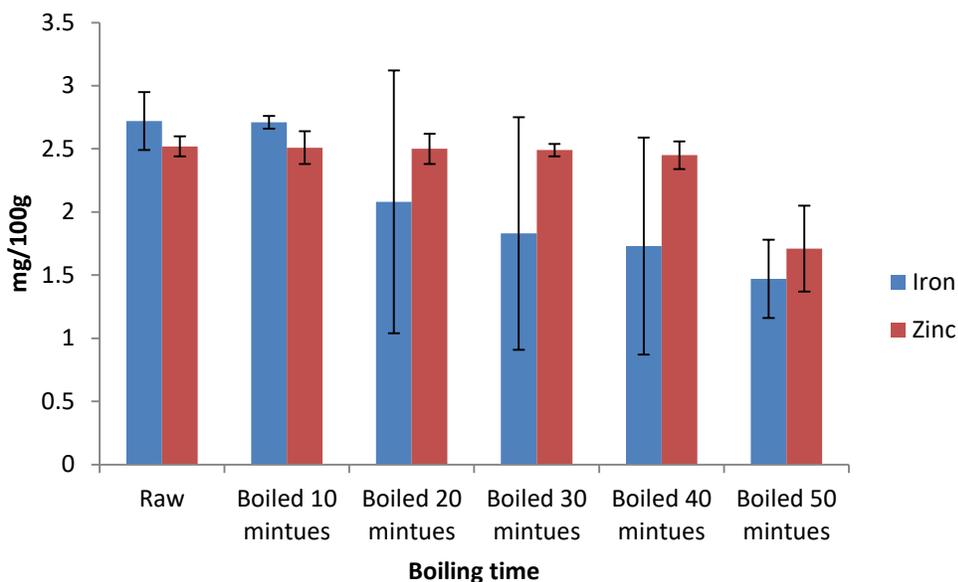
Values are means of triplicate determinations ± S.D. Means in the same column followed by the same superscript are not significantly different at p<0.05. Mean comparison for the treatments were made using Duncan's Multiple Range Test.

The highest protein content was found in seeds boiled 10 minutes and the least in seeds boiled 50 minutes compared to the raw seed. There was a significant reduction (p=0.007) on the protein content of the boiled and raw seed. Potter and Hotchkiss (2006) observed reduction in protein content in boiled beniseed with increase in boiling time and attributed to the denaturation of protein by heat. The values are within the range 14.1-18.87% reported for boiled beniseed (Adeniyani et al., 2013).

The carbohydrate content of the boiled *Hyptis spicigera* seed compared to the raw seeds had a significant increase ( $p=0.001$ ) with increased boiling time as shown in Table 4.6. Seeds boiled 50 minutes had the highest value and the least was in the boiled 10 minutes compared to the raw seed. The increase in the carbohydrate of *Hyptis spicigera* seed agreed with the report of Adeniyani et al. (2013) on boiled beniseed. Boiling cause the seed granules to break down, soften the cellulose and make the starch more available (Agiang *et al.*, 2010)

#### **4.11 Effect of boiling on the zinc and iron content of (*Hyptis spicigera* Lamiaceae) seed flour.**

The results of the zinc and iron analysis of boiled and raw *Hyptis spicigera* seed are presented in Figure 4.4. Boiling increased zinc and iron of the content compared to the raw seed. The highest value for zinc was found in seeds boiled for 40 minutes and the lowest was in seeds boiled for 30 minutes. A significant difference ( $p=0.024$ ) was noted for the zinc content of the boiled and raw seed flour. There was a non- significant decrease in iron content ( $p=0.720$ ) for the boiled seeds compared to the raw. Similar findings were also reported by Mariod et al., (2012) and attributed that to leaching of these minerals into the boiling water.



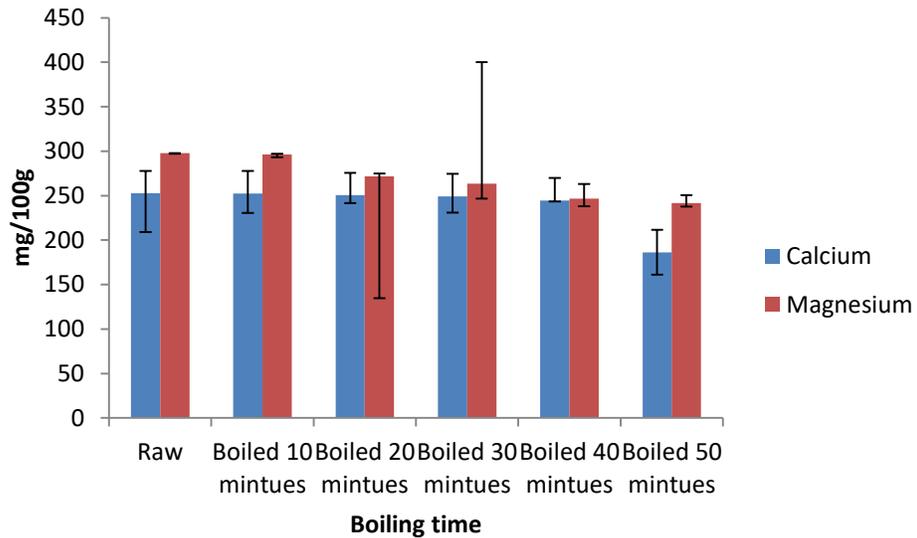
**Figure 4.4. Effect of boiling on the zinc and iron content of *Hyptis spicigera* Lamiaceae seed flour on dry weight basis.**

**4.12 Effect of boiling on the calcium and magnesium content of (*Hyptis spicigera* Lamiaceae) seed flour.**

Calcium and magnesium content of boiled and raw *Hyptis spicigera* seed are shown in Figure 4.5. Although magnesium had the highest value content (297.9mg/100g), boiling reduced the mineral content of the seed with increase in boiling time.

Seeds boiled 10 minutes had the highest calcium content and the least was in the seeds boiled 50 minutes. There was a non-significant decrease ( $p=0.366$ ) on the calcium content of the boiled and raw seed flour. Magnesium had the highest value in seeds boiled 10 minutes and the lowest in seeds boiled 50 minutes. There was no significant difference ( $p=0.965$ ) for magnesium content of the boiled and raw seed flour. According to Mariod et al. (2012) the reduction in mineral content could be attributed to leaching of these minerals into the boiling water. Calcium plays a role in supportive structures of the

body and its dietary deficiency, together with phosphorus and vitamin D, causes rickets in children, osteoporosis and osteomalacia in adults (Voet & Voet, 2004).



**Figure 4.5 Effect of boiling on the calcium and magnesium content of *Hyptis spicigera* Lamiaceae seed flour on dry weight basis.**

#### **4.13 Effect of roasting on the nutrient composition of (*Hyptis spicigera* Lamiaceae) seed flour.**

The roasted *Hyptis spicigera* seed had low fat and protein content compared to the raw seed flour and the reverse trend was found in the ash, carbohydrate and crude fibre content (Table 4.7).

Ash content had the highest value in seeds roasted 10 minutes and the least in seeds roasted for 20 minutes compared to the raw seed. There was no significant difference ( $p=0.18$ ) on the ash content of the roasted and raw seed. The ash content of the roasted seed increased with increased roasting time. Makinde and Akinoso (2014) reported

similar findings and explained that the decrease in moisture content can be the reason for increased ash content of the seed.

There was no significant difference ( $P=0.08$ ) on the crude fibre content of the roasted and raw seed as represented in Table 4.7. The highest value 20.98% crude fibre was recorded in seeds roasted 10 minutes and the lowest was in seeds roasted for 30 minutes compared to the raw seed. The crude fibre content for the roasted seeds seemed to reduce with increased roasting time. Makinde and Akinoso (2014) reported similar findings in roasted sesame seeds and attributed the increase to the heat applied during roasting. The values are higher than (3.86% - 3.89%) reported by Makinde and Akinoso (2014). Crude fibre plays a physiological role in maintenance of internal distension for a normal peristaltic movement of the intestinal tract.

**Table 4.7: Effect of roasting on the nutrient composition of (*Hyptis spicigera* Lamiaceae) seed flour on dry weight basis.**

Treatment	Ash%	Crude Fibre%	Crude Fat%	Crude Protein%	Total Carbohydrate%
Raw	2.97±0.14 <sup>ab</sup>	19.10±0.71 <sup>ab</sup>	35.79±0.49 <sup>a</sup>	16.83±0.20 <sup>c</sup>	25.31±0.75 <sup>a</sup>
Roasted 10 minutes	4.84±1.74 <sup>b</sup>	20.98 ±0.19 <sup>b</sup>	35.95±1.78 <sup>a</sup>	12.94±0.19 <sup>b</sup>	25.27±2.82 <sup>a</sup>
Roasted 20 minutes	2.12±0.25 <sup>a</sup>	19.77 ±0.22 <sup>b</sup>	36.85±0.34 <sup>a</sup>	11.97± 0.28 <sup>b</sup>	29.27±0.24 <sup>b</sup>
Roasted 30 minutes	3.29±0.02 <sup>ab</sup>	15.42±2.28 <sup>a</sup>	46.04±2.25 <sup>c</sup>	10.59±0.61 <sup>a</sup>	24.63±3.19 <sup>a</sup>
Roasted 40 minutes	2.13±0.37 <sup>a</sup>	18.32±1.02 <sup>ab</sup>	41.05±0.17 <sup>b</sup>	12.15±0.29 <sup>b</sup>	26.33±1.14 <sup>a</sup>
Roasted 50 minutes	3.01±0.09 <sup>ab</sup>	17.85±1.24 <sup>ab</sup>	42.99±0.58 <sup>bc</sup>	11.78 ± 0.61 <sup>b</sup>	24.35±1.12 <sup>a</sup>

Values are means of triplicate determinations ± S.D. Means in the same column followed by the same superscript are not significantly different at p=0.05. Mean comparison for the treatments were made using Duncan's Multiple Range Test.

The fat content highest value was in seeds roasted 30 minutes and the least was found in seeds roasted 20 minutes compared to the raw seed as presented in Table 4.7. Makinde et al. (2014) reported an increase in oil content of sesame seed after roasting, but it was explained in Arinola and Adesina (2014) that application of dry heat has been known to facilitate release and extraction of oil in oilseed. The values are higher than the 3.86% - 3.89% range reported by Makinde and Akinoso (2014). Fat content decreased (p=0.001) on the roasted compared raw seed.

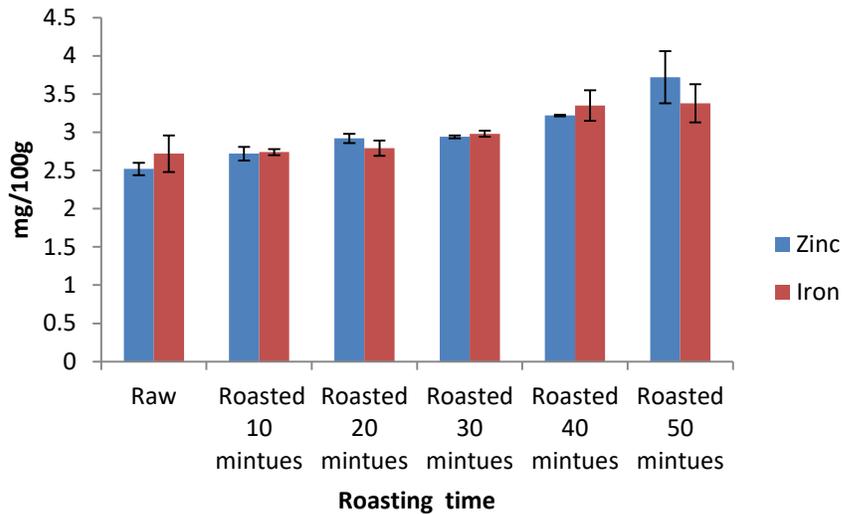
Protein had the highest value in seeds roasted 20 minutes and the least was in seeds roasted 30 minutes compared to the raw seed. There was a significant difference

( $p=0.001$ ) on the protein content of the roasted seed compared to the raw seed. Protein content reduced with increased roasting time. Hassan et al. (2016) reported on protein reduction in roasted pumpkin seeds and attributed that to protein denaturation. The values are higher than 3.86-3.89% range reported by Makinde and Akinoso (2014).

Carbohydrate content for the roasted seeds was high in seeds roasted 50 minutes and the lowest in roasted 30 minutes compared to the raw seed as presented in table 4.7. There was a significant increase ( $p=0.007$ ) on the carbohydrate content of the roasted compared to raw seed. According to Makinde et al. (2014), roasting cause sesame granules to break down, softens the cellulose, and makes the starch more available. The values are higher than that of 3.86% - 3.89% range reported by Makinde and Akinoso (2014).

#### **4.14 Effect of roasting on the zinc and iron content of *Hyptis spicigera* Lamiaceae seed flour.**

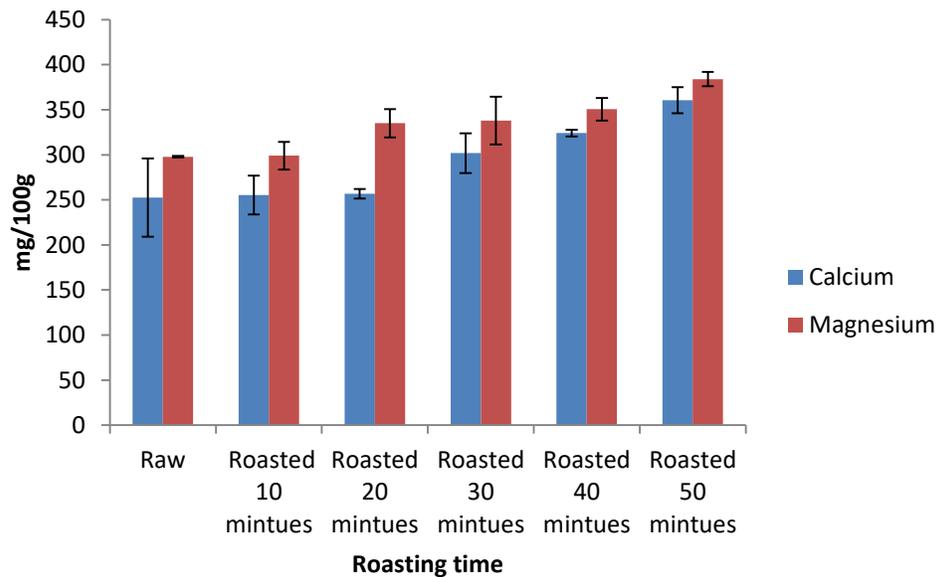
Roasting improved the iron content of the seed compared to the raw seed (Figure 4.6). The highest value the iron was found in seeds roasted 50 minutes and the least in seeds roasted 10 minutes. Roasting significantly increased the iron content of the seed ( $p=0.045$ ) as compared to the raw seed. Zinc content was high in seeds roasted for 50 minutes and the least in seeds roasted 10 minutes. There was a significant increase ( $p=0.032$ ) for zinc content of the roasted compared to the raw seed. The results are within range 0.3-8 mg/100g reported for sesame seed by Mohammed et al. (2011).



**Figure 4.6 Effect of roasting on the zinc and iron content of *Hyptis spicigera* Lamiaceae seed flour on dry weight basis.**

**4.15 Effect of roasting on the calcium and magnesium content of (*Hyptis spicigera* Lamiaceae) seed flour.**

Roasting increased the magnesium content of roasted seed compared to the raw *Hyptis spicigera* seed (Figure 4.7). Similar results were observed in calcium and magnesium levels in seeds roasted 20 minutes and roasted 10 minutes respectively. The heating method significantly increased the magnesium content of the seed ( $P=0.015$ ) compared to the raw seeds. Similar results were reported by Umoren et al. (2007); Obiajunwa et al. (2005), they explained that thermal processing improved the concentrations of some minerals. There was a significant difference ( $p=0.026$ ) for calcium content of the roasted and raw seed.



**Figure 4.7** Effect of roasting on the calcium and magnesium content of (*Hyptis spicigera* Lamiaceae) seed flour on dry weight basis.

#### **4.16 Effect of processing treatments on the anti-nutrient content of *Hyptis spicigera* Lamiaceae seed flour.**

##### **4.16.1 Effect of soaking on the anti-nutrient content of *Hyptis spicigera* Lamiaceae seed flour.**

Soaking treatment reduced the anti- nutritional factors in *Hyptis spicigera* seed flour as shown below in Table 4.8. The highest value for tannins, oxalates and phytates was in seeds soaked 5 hours and the least in seeds soaked for 24 hours compared to the raw seed.

Tannins and phytates had a significant decrease ( $p=0.001$ ) for the soaked and raw seed. Makinde and Akinoso (2013), reported similar reduction on anti-nutrient content of sesame seeds. According to Makinde and Akinoso (2013) and Aurelie et al. (2017), the

reduction is attributed to leaching out of oxalate and tannins as a result of soaking treatment. There was no significant difference ( $p=0.27$ ) for oxalate content of the soaked and raw seed.

**Table 4.8: Effect of soaking on the anti-nutrient composition of (*Hyptis spicigera* Lamiaceae) seed flour on dry weight basis.**

Treatment	Tannins (mg/100g)	Oxalates (mg/100g)	Phytates (mg/100g)
Raw	332.0 ± 12.98 <sup>c</sup>	434.8±57.71 <sup>a</sup>	390.4±19.60 <sup>c</sup>
Soaking 5 hours	90.46 ±23.99 <sup>b</sup>	378.0±174.2 <sup>a</sup>	199.4±33.44 <sup>b</sup>
Soaking 10 hours	48.96 ±19.36 <sup>ab</sup>	245.1±191.7 <sup>a</sup>	178.4±40.13 <sup>a</sup>
Soaking 15 hours	46.13± 24.08 <sup>ab</sup>	193.0±26.12 <sup>a</sup>	115.0±17.94 <sup>a</sup>
Soaking 20 hours	16.54± 16.54 <sup>a</sup>	191.4±22.07 <sup>a</sup>	107.0±16.16 <sup>a</sup>
Soaking 24 hours	11.29± 6.50 <sup>a</sup>	159.3±14.21 <sup>a</sup>	98.8±0.05 <sup>a</sup>

Values are means of triplicate determinations ± S.D. Means in the same column followed by the same superscript are not significantly different at  $p=0.05$ . Mean comparison for the treatments were made using Duncan's Multiple Range Test.

#### **4.16.2: Effect of soaking and germination on the anti-nutrient composition of (*Hyptis spicigera* Lamiaceae) seed flour.**

Soaking and germination process reduced the anti-nutrient content of *Hyptis spicigera* seed flour as shown below in Table 4.9. This reduction was observed with increased soaking and germinating time.

Seeds soaked 24 hours and germinated 72 had low anti-nutrient content compared to the other treatments. There was a significant decrease on all the anti-nutrient content of the seeds soaked and germinated ( $p=0.001$ ) compared to the raw seeds. Similiar reduction was reported on sesame seeds by Hahm et al. (2008). According to Mamudu et al. (2005), the reduction in phytates is attributed to sprouting process, in which phytic acid; a phosphate reserve degrades due to the action of phytase which is utilized by growing seedling. The reduction of phytates could enhance absorption of the minerals, as germination would reduce the immobilization effects of phytic acid and other anti-nutritional factors.

**Table 4.9: Effect of soaking and germination on the anti-nutrient composition of (*Hyptis spicigera* Lamiaceae) seed flour on dry weight basis.**

Treatment	Tannins (mg/100g)	Oxalates (mg/100g)	Phytate (mg/100g)
Raw seeds	332.0 ± 12.89 <sup>e</sup>	434.8 ± 57.71 <sup>e</sup>	390.4±19.60 <sup>c</sup>
Soaked 5hours germinated 24 hours	184.2 ± 30.97 <sup>bcd</sup>	244.6 ± 45.20 <sup>bcd</sup>	192.0±13.74 <sup>b</sup>
Soaked 5hours germinated 48 hours	126.2 ± 35.96 <sup>abc</sup>	243.3 ± 64.25 <sup>bcd</sup>	172.3±11.41 <sup>ab</sup>
Soaked 5hours germinated 72 hours	38.54 ± 32.52 <sup>a</sup>	208.3 ± 10.12 <sup>bc</sup>	156.0±23.04 <sup>ab</sup>
Soaked 10hours germinated 24hours	76.8±42.24 <sup>abc</sup>	293.2 ± 37.45 <sup>cd</sup>	153.5±23.08 <sup>ab</sup>
Soaked 10hours germinated 48 hours	57.2±28.63 <sup>ab</sup>	195.5 ± 18.95 <sup>bc</sup>	141.6±16.53 <sup>ab</sup>
Soaked 10hours germinated 72 hours	29.1±12.24 <sup>a</sup>	165.6 ± 31.33 <sup>b</sup>	139.4±22.40 <sup>ab</sup>
Soaked 15hours germinated 24 hours	289.9 ± 122.70 <sup>de</sup>	229.4 ± 12.16 <sup>bcd</sup>	137.9±58.03 <sup>ab</sup>
Soaked 15hours germinated 48 hours	102.7 ± 38.33 <sup>abc</sup>	215.2 ± 11.98 <sup>bc</sup>	135.9±31.38 <sup>ab</sup>
Soaked 15hours germinated 72 hours	55.5±30.52 <sup>ab</sup>	212.9 ± 20.47 <sup>bc</sup>	132.2±15.11 <sup>ab</sup>
Soaked 20hours germinated 24 hours	195.4±38.04 <sup>cd</sup>	338.9 ± 35.73 <sup>de</sup>	130.8± 20.78 <sup>ab</sup>
Soaked 20hours germinated 48 hours	83.5±40.61 <sup>abc</sup>	264.2 ± 43.33 <sup>bcd</sup>	128.8±12.22 <sup>ab</sup>
Soaked 20hours germinated 72 hours	31.0±10.41 <sup>a</sup>	248.1 ± 19.91 <sup>bcd</sup>	121.3±27.04 <sup>ab</sup>
Soaked 24hours germinated 24 hours	33.3±1.72 <sup>a</sup>	291.9 ±51.69 <sup>bcd</sup>	119.1±3.71 <sup>ab</sup>
Soaked 24hours germinated 48 hours	22.1 ±11.08 <sup>a</sup>	272.7 ±45.70 <sup>bcd</sup>	117.8±9.95 <sup>ab</sup>
Soaked 24hours germinated 72 hours	0.00±0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	100.9±12.35 <sup>a</sup>

Values are means of triplicate determinations ± S.D. Means in the same column followed by the same superscript are not significantly different at p=0.05. Mean comparison for the treatments were made using Duncan's Multiple Range Test.

#### **4.16.3: Effect of boiling on the anti-nutrient composition of *Hyptis spicigera* Lamiaceae seed flour on dry weight basis.**

The level of anti-nutritional factors (ANFs) as influenced by boiling treatment is shown below in Table 4.10. Boiling reduced all the anti-nutritional factors (ANFs) analyzed. This is in agreement with Medugu et al. (2012), who reported that boiling reduce the anti-nutritional factors and enhance the nutritional value of the plants. Tannin content was high in seeds boiled for 10 minutes and the least in seeds boiled 50 minutes compared to the raw seed. Tannin had decreased but not significantly ( $p=0.493$ ) with increase in boiling time. The reduction could be attributed to leaching of tannins to the boiling water. According to Arinola and Adesina (2014), tannins are polyphenols and all polyphenolic compounds are known to be water soluble in nature.

The oxalates content was high in the seeds boiled 10 minutes and the least in seeds boiled 50 minutes compared to the raw seed. Oxalates significantly decreased ( $p=0.001$ ) in the boiled seed and the raw seed. However, phytate content was high in seeds boiled for 10 hours and low in seeds boiled for 50 hours compared to the raw seed. A significant decrease ( $p=0.004$ ) was noted on the phytate content of the boiled and raw seeds. The reduction in phytate content may partly be due to leaching into the cooking medium, degradation by heat, and formation of insoluble complexes between phytate and other components such as protein and minerals (Siddhuraju and Becker, 2001). There was no significant difference ( $p=0.49$ ) on the tannin content of the boiled and raw seed.

**Table 4.10: Effect of boiling on the anti-nutrient composition of (*Hyptis spicigera* Lamiaceae) seed flour on dry weight basis.**

Treatment	Tannin (mg/100g)	Oxalate (mg/100g)	Phytate (mg/100g)
Raw seeds	332.0 ±12.9 <sup>a</sup>	434.8±57.71 <sup>b</sup>	390.4±19.60 <sup>b</sup>
Boiled 10 minutes	292.4±180.6 <sup>a</sup>	170.0±6.66 <sup>a</sup>	335.1±95.94 <sup>b</sup>
Boiled 20 minutes	228.6±76.48 <sup>a</sup>	158.7±2.55 <sup>a</sup>	183.8±24.07 <sup>a</sup>
Boiled 30 minutes	177.8±154.7 <sup>a</sup>	157.9±2.21 <sup>a</sup>	128.3±41.83 <sup>a</sup>
Boiled 40 minutes	127.4± 67.88 <sup>a</sup>	155.9±4.62 <sup>a</sup>	122.1±41.19 <sup>a</sup>
Boiled 50 minutes	45.8± 45.8 <sup>a</sup>	146.7±7.91 <sup>a</sup>	115.2±3.50 <sup>a</sup>

Values are means of triplicate determinations ± S.D. Means in the same column followed by the same superscript are not significantly different at p<0.05. Mean comparison for the treatments were made using Duncan's Multiple Range Test.

**4.16.4 Effect of roasting on the anti-nutrient composition of *Hyptis spicigera* Lamiaceae seed flour.**

Roasting treatment reduced tannins, oxalates and phytates content of the seed flour compared to the raw seed and increased the tannins content of seed flour as shown below in Table 4.11.

The highest values for tannin, oxalates and phytates was in seeds roasted 10 minutes and least in seeds roasted 50 minutes compared to the raw seed. There was a significant decrease ( $p=0.001$ ) on the oxalate and phytate content ( $p=0.001$ ) of the roasted and raw seed. Meanwhile, a non- significant decrease ( $p=0.19$ ) was recorded for tannin content of the roasted compared to the raw seed. The reduction in tannins contents during roasting treatments might be due to the loss of compounds while treating at a high temperature (Nithya *et al.*, 2007).

According to Embaby (2010) the reduction is attributed to the degradation or interaction with other components of seeds, such as proteins, to form insoluble complexes. Makinde and Akinoso (2014) reported similar findings on sesame seeds and explained that the only factor that could account for the lower concentrations of phytate and oxalate in roasted sesame could be attributed to the heat applied as these anti-nutrients are thermo labile in nature.

**Table 4.11: Effect of roasting on the anti-nutrient composition of (*Hyptis spicigera* Lamiaceae) seed flour on dry weight basis.**

Treatment	Tannins (mg/100g)	Oxalates (mg/100g)	Phytates (mg/100g)
Raw seeds	332.0±12.98 <sup>b</sup>	434.8±57.71 <sup>c</sup>	390.4±19.60 <sup>c</sup>
Roasted 10 minutes	26.92±15.19 <sup>a</sup>	178.3±25.91 <sup>ab</sup>	184.8±21.52 <sup>b</sup>
Roasted 20 minutes	25.26±12.63 <sup>a</sup>	211.7±14.92 <sup>b</sup>	124.7±41.95 <sup>ab</sup>
Roasted 30 minutes	24.55±0.35 <sup>a</sup>	195.5±5.89 <sup>ab</sup>	65.3±8.04 <sup>a</sup>
Roasted 40 minutes	17.41±2.28 <sup>a</sup>	124.4±1.60 <sup>ab</sup>	54.3±21.98 <sup>a</sup>
Roasted 50 minutes	15.40±9.85 <sup>a</sup>	110.5±11.18 <sup>a</sup>	48.4±6.37 <sup>a</sup>

Values are means of triplicate determinations ± S.D. Means in the same column followed by the same superscript are not significantly different at p<0.05. Mean comparison for the treatments were made using Duncan's Multiple Range Test.

## CHAPTER FIVE

### CONCLUSIONS AND RECOMMENDATIONS

#### 5.1 Conclusions

*Hyptis spicigera* Lamiaceae seed has a rich nutritional profile with relatively high fat content, carbohydrates, crude protein and minerals, such as magnesium and calcium, compared to seeds such as sesame. However, the crude fibre, ash and zinc content were relatively low compared to seeds like sesame. The anti-nutrient content was high especially oxalates, while phytates and tannins were within the range for sesame seeds.

The processing methods used namely soaking, germination, boiling and roasting had a varying effect on nutritional composition and anti-nutrient content of the seed. Germination and roasting improved the nutritional content of the seed (carbohydrates, fat and fibre) when compared to soaking and boiling treatments.

Processing by germination significantly reduced tannins and oxalates to very low levels. However, soaking, boiling and roasting had no significant effect on the tannins and oxalates.

#### 5.2 Recommendations

##### a) Recommendations for further research

1. Further studies are needed on the other varieties of *Hyptis spicigera* species in the whole of South Sudan and East Africa in general.

2. Further research to determine the content of vitamins, amino acid and fatty acid content in the raw and processed seed.
3. Further analysis to determine the functional properties of *Hyptis spicigera* seeds.

**b) Recommendations for practice**

1. From the results, this study recommends the use of soaking and germination as the most appropriate method for reduction of anti-nutrients and particularly tannins and oxalates but with further research on toxicity levels of cyanide and saponins.
2. Since roasting and germination retained most nutrient content of the seed, the method can be recommended in preparation of *Hyptis spicigera* seed flour and have them being utilized to fight against micronutrient deficiencies in the community.
3. This information provided could be included in the Nutritional Data Base in South Sudan's Ministry of Health. Hence, could be used in projects by the relevant governmental and non-governmental organizations concerned with the nutritional status of the South Sudanese population.

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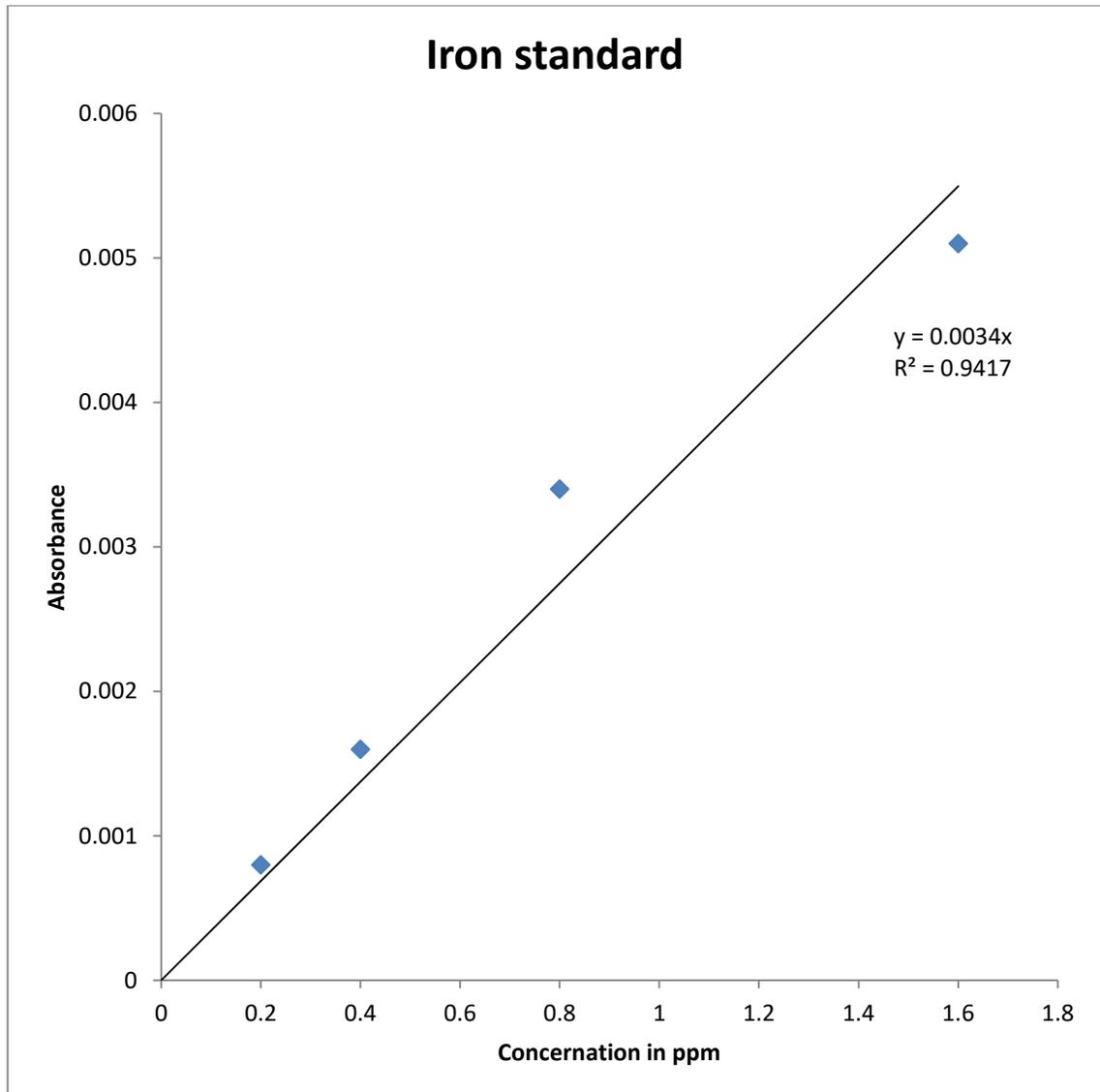
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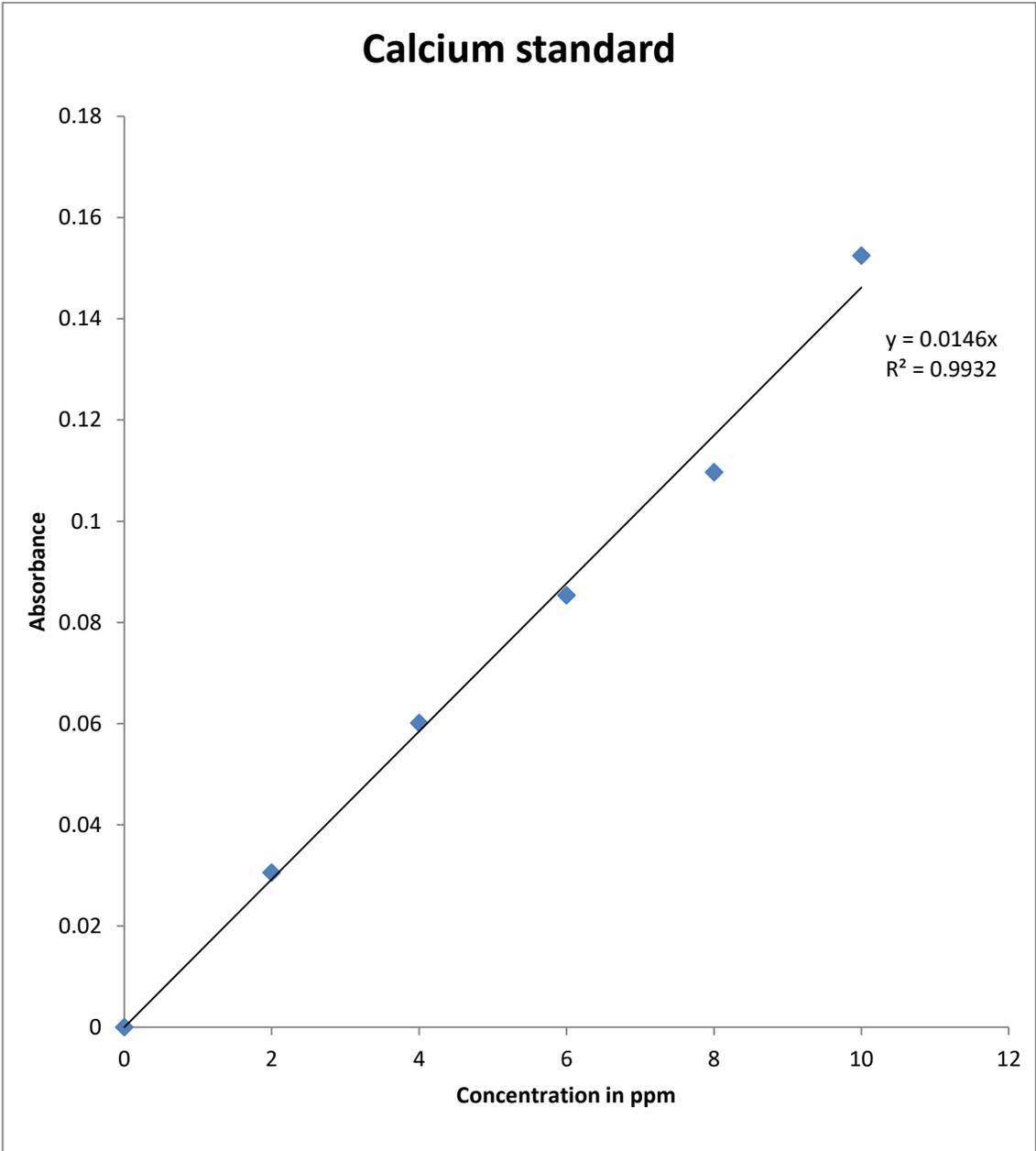
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## LIST OF APPENDICES

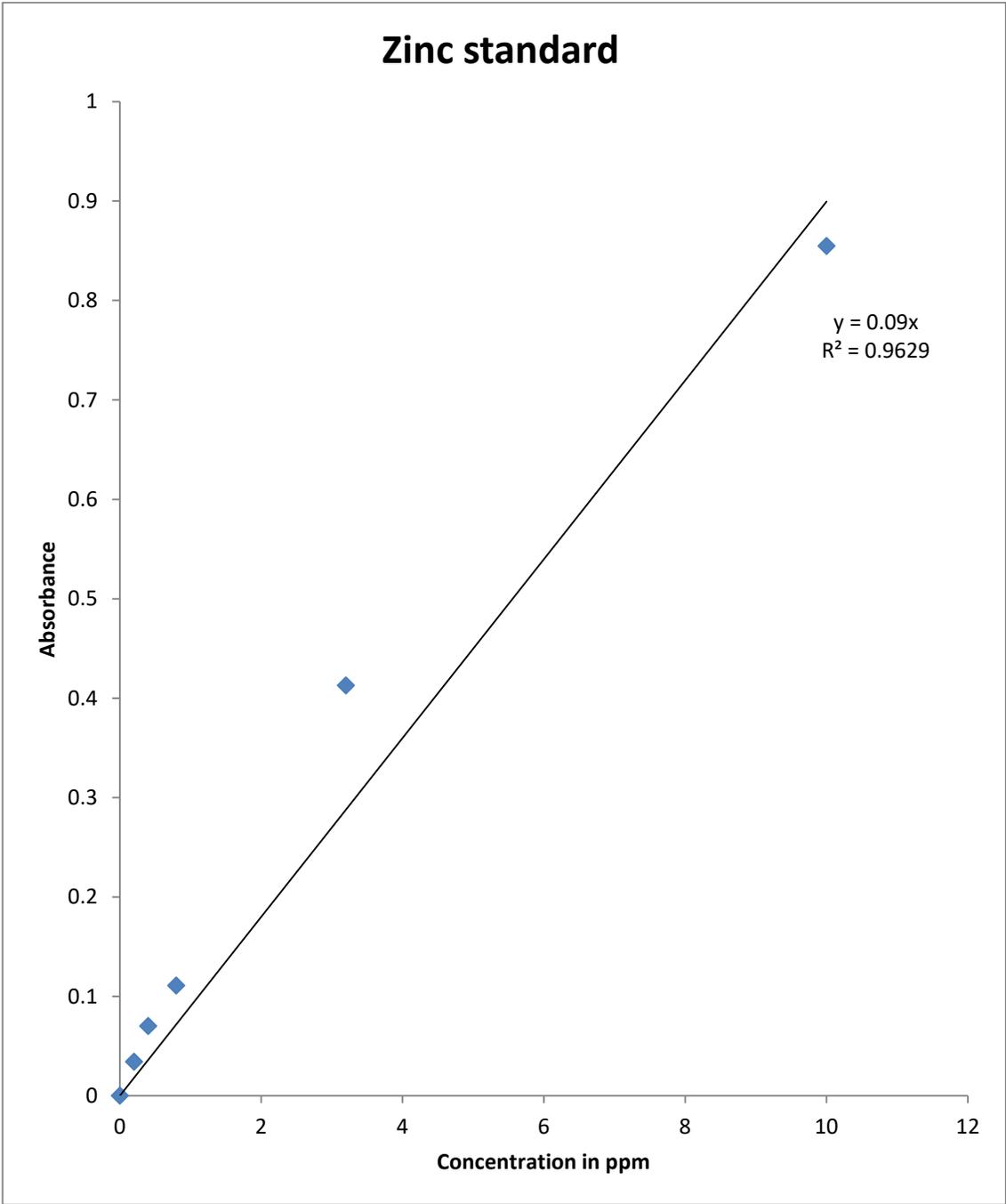
### Appendix 1: Standard curve for iron



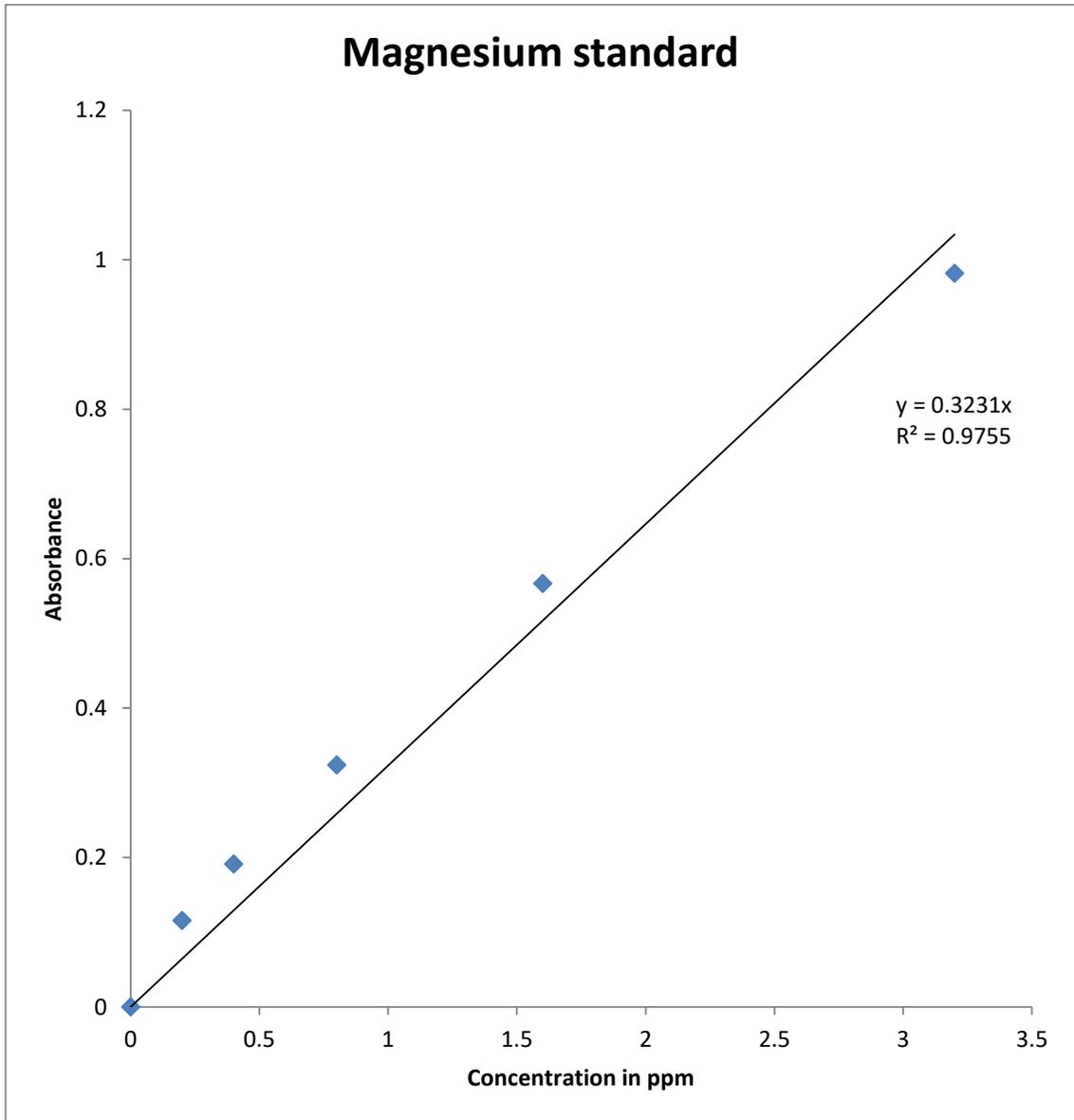
**Appendix 2: Standard curve for calcium**



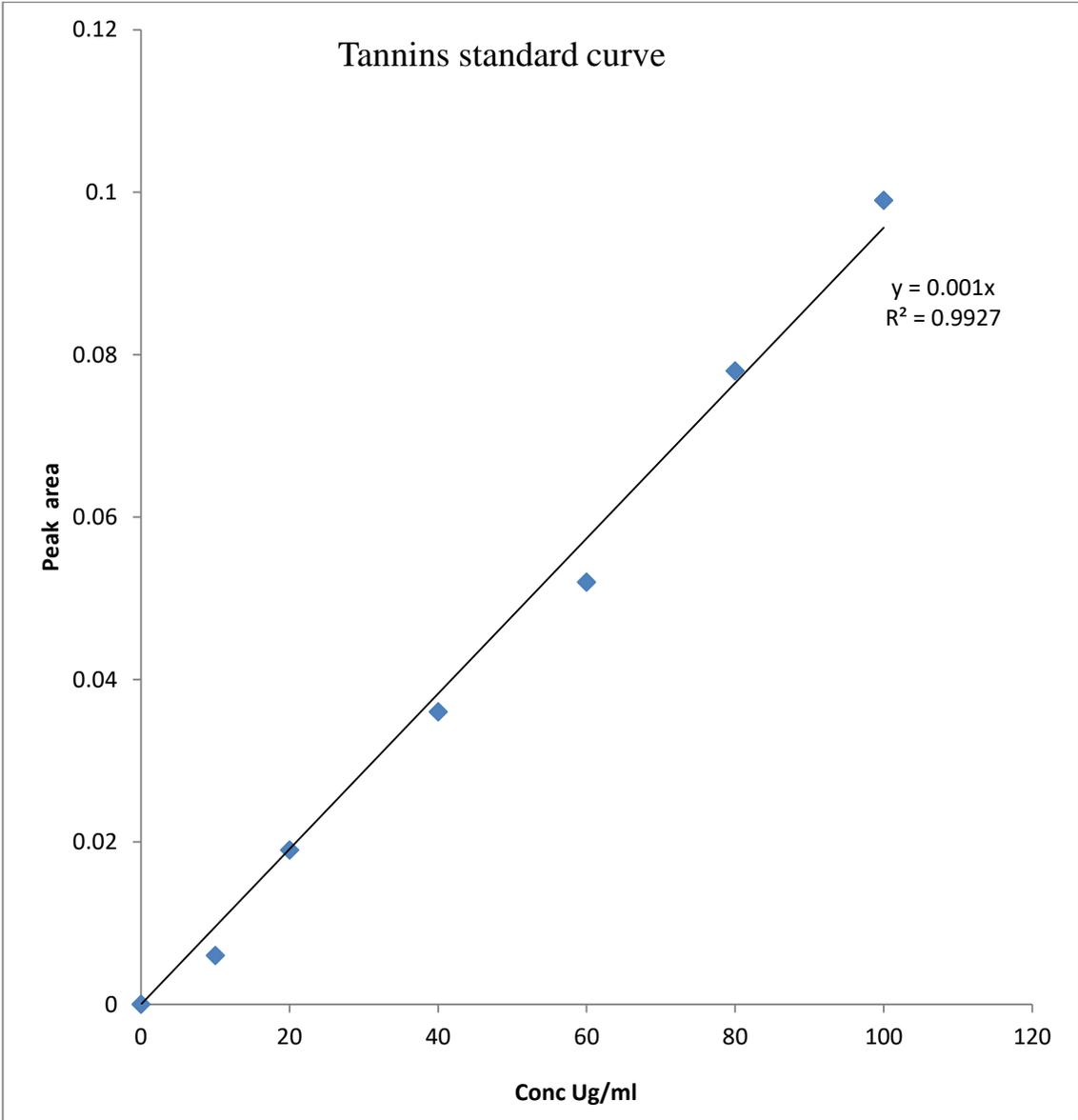
**Appendix 3: Standard curve for zinc**



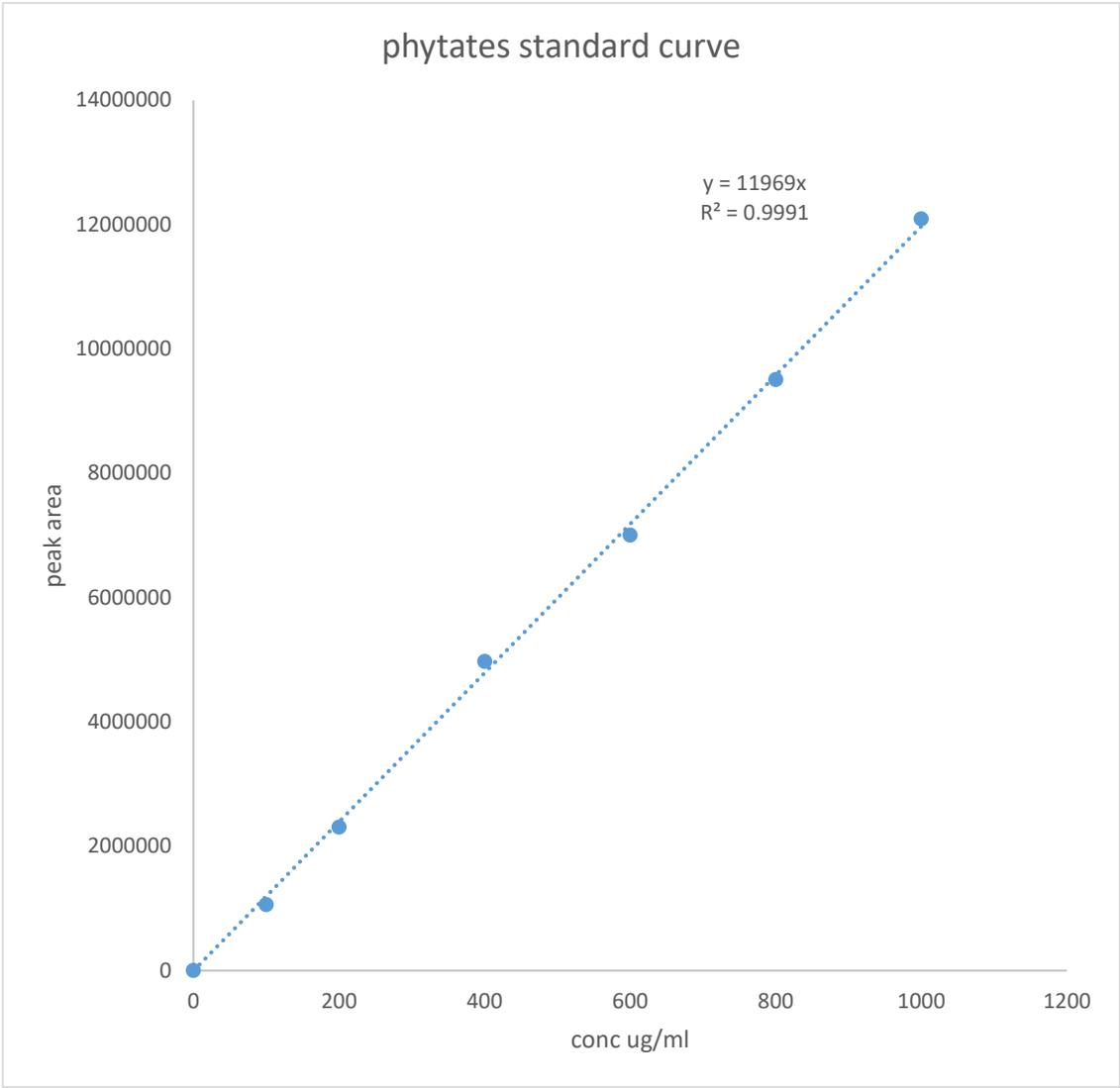
**Appendix 4: Standard curve for magnesium**



**Appendix 5: Standard curve for tannins**



**Appendix 6: Standard curve for phytates**



**Appendix 7: Standard curve for oxalate**

