DETERMINATION OF PHYTOCHEMICAL COMPOSITIONS, NUTRITIONAL CONTENTS AND ANTIOXIDANT PROPERTIES OF *Ocimum basilicum* L. AND *Ocimum gratissimum* L. Leaves

BY

EZEKIEL MALGWI ANJILI

DEPARTMENT OF CHEMISTRY,

FACULTY OF PHYSICAL SCIENCES,

AHMADU BELLO UNIVERSITY,

ZARIA, NIGERIA

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Ezekiel Malgwi, ANJILI

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DEPARTMENT OF CHEMISTRY,
FACULTY OF PHYSICAL SCIENCES,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

SEPTEMBER, 2021

DECLARATION

I declare that the work in this dissertation entitled **"Determination of Phytochemical compositions, nutritional contents and antioxidant properties of *Ocimum basilicum L.* and *Ocimum gratissimum L.* Leaves",** has been carried out by me in the Department of Chemistry, under the supervision of Prof. A.A. Nuhu and Dr. S. Uba. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree or diploma at this or any other institution.

ANJILI Malgwi Ezekiel _____ _____

Signature Date

CERTIFICATION

This dissertation titled **"DETERMINATION OF PHYTOCHEMICAL COMPOSITIONS, NUTRITIONAL CONTENTS AND ANTIOXIDANT PROPERTIES OF *OCIMUM BASILICUM L.* AND *OCIMUM GRATISSIMUM L.* LEAVES''** by Ezekiel Malgwi ANJILI meets the regulations governing the award of the degree of Master of Science (M.Sc.) in Analytical Chemistry of the Ahmadu Bello University, and is approved for its contributions to knowledge and literary presentations.

Prof. A.A. Nuhu _____ _____
Chairman, Supervisory Committee, Signature Date

Dr. S. Uba _____ _____
Member, Supervisory Committee, Signature Date

Prof. G. I. Ndukwe _____ _____
The Head of Department Signature Date

Prof. S.A. Abdullahi _____ _____
Dean, School of Postgraduate Studies, Signature Date

DEDICATION

I am dedicating this research work to God Almighty first, my family, my friends and to the Department of Chemistry Ahmadu Bello University, Zaria, Kaduna state.

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I wish to thank God Almighty for the grace and opportunity given to me during the process and accomplishment of this research work. He has been faithful to me.

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ABSTRACT

Scent leaves is one of the important nutritious and medicinal plants used in Nigeria and other parts of the world. The aim of this work was to determine the phytochemical composition, nutritional contents and antioxidant properties of *O. basilicum* and *O. gratissimum* leaves. Elemental and proximate analyses were used for nutritional contents. N-hexane and methanol solvents were used to extract leaves of *O. basilicum* and *O. gratissimum* for phytochemical screening (for carbohydrate, cardiac glycoside, tannins, terpenoid and flavonoid) and antioxidant properties (for DPPH scavenging and total phenol contentassay) at concentration of 50µg/cm^3, 100µg/cm^3, 150µg/cm^3, 200µg/cm^3 and 250µg/cm^3. The elemental analysis for leaves showed that Ca (33024.60mg/kg), Cu (28.50mg/kg), K (2350.00mg/kg), Zn (98.80mg/kg) and S (5091.33mg/kg), were highest in leaves found in Awgu town, while Fe (2940.00mg/kg) and P (4123.24mg/kg) were highest in leaves found in Nsukka town.Mn (101.60mg/kg) in leaves found at G. R. A Damboa Road and N (34.05 mg/kg) in leaves found at 707Housing Estate were highest in *O. basilicum* leaves found at Maiduguri. Cobalt was not detected in both *O. basilicum* and *O. gratissimum* leaves. The soils where both *O. basilicum* and *O. gratissimum* were planted were analysed and showed that Ca (7911.20mg/kg) and K (490.00 mg/kg) in 707Housing Estate samples were highest and Co (2.40mg/kg), Cu (18.30mg/kg), Fe (35480.20mg/kg), Mn (338.60mg/kg), Zn (211.20mg/kg) and P (1012.72 mg/kg) in Nsukka town samples were highest; S (1988.23mg/kg) and N (2.27 mg/kg) in Awgu town samples were highest. The n-hexane extracts of both leaves of *O. basilicum* and *O. gratissimum* showed presence of all the phytochemical compositions except tannins while the methanol extracts of both leaves of *O. basilicum* and *O. gratissimum* showed presence of all the phytochemicalsanalysed. Both the leaves and soil were not toxic or contaminated compared with WHO/FAO standard for vegetable and soil and NAFDAC standard for food in

the elemental concentration except with Fe. The proximate analysis showed that highest Carbohydrate content (31.58%) and ash content (15.33%) werefound at 707Housing Estate;highest moisture content (26.66%), crude fiber (32.0%), and crude fat (18.66%) were found at G.R.A Damboa road while highest protein content (7.61%), was found in*O. gratissimum* leaves at Nsukka town. The pH of *O. basilicum* leaves was slightly acid while that of *O. gratissimum* leaves was slightly basic. The antioxidant properties analysis showed that the methanol extract of *O. basilicum* leaves has maximum reducing effect of 98.56% in DPPH assay at 250 µg/$cm^3$and n-hexane extract of*O. basilicum* leaves has maximum value of 102.00 mgGAC/g in total phenol content assay at 50µg/cm^3. From the analysis the n-hexane and methanol extracts of both leaves of *O. basilicum* and *O. gratissimum* revealed good antioxidant effects. There results indicate the potential effectiveness of both leaves in health and medicinal applications. Statistically both leaves showed no differences in all the content checked in respect to the antioxidant properties and nutritional contents.

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LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviation	Translation
ANOVA	Analysis of Variance
AAS	Atomic Absorption Spectroscopy
DMSO	Dimethyl Sulfoxide
FAO	Food and Agricultural Organization
G.R.A	Government Residential Area
NAFDAC	National Agency for Food and Drug Administration and control
MESS	Multi-Element Standard Solution
SPSS	Statistical Package for Social Sciences
WHO	World Health Organization
Max.	Maximum
Conc.	Concentration
DPPH	2,2-diphenyl-1-picrylhydrazyl Assay
Symbols	**Full Meaning**
+	Present
-	Absent

CHAPTER ONE

1.0INTRODUCTION

1.1 Background of the Study

Ocimum plants originated from the tropical regions of Asia, Africa, and Central and South America (Bailey, 1924; Staff, 1976; Darrah, 1980). Generally, these *Ocimum* plants have square stems, fragrant opposite leaves, and whorled flowers on spiked inflorescences (Darrah, 1980). Herbs are generally used to add a distinctive aroma and flavour to food and for medicinal purposes (Gad *et al.,* 2013). Scent leaves is one of the important nutritious and medicinal plants commonly used in Nigeria and other parts of the world. In the northern part of Nigeria, the leaves is called "Daidoya" by the Hausas while in the southern part of Nigeria it is called "Nchonwn" by the Igbo (Effrain *et al*., 2000). Scent leaves contain nutrients such as protein, carbohydrate, fat, vitamin, and mineral elements which are very useful to the human body (Katarzyna, 2010). Scent leaves has been known in Nigerian folk medicine in the management of different diseases and symptoms such as diarrhoea, malaria, headache, fever, infectionsand pneumonia.(Omale*et al.,* 2008).

Ocimum basilicum is found in North-Eastern Nigeria andis recognized by its strong scent and short leaves. It contains useful elements and is highly nutritious and is used as seasoning in cooking. It also consists of phytochemical contents and is highly medicinal (Katarzyna, 2010). It has been reported to be hepato-protective, immunomodulatory, anti-hyperglycermic, anti-toxic, anti- inflammatory, and anti-fungal (Ahmed*et al.,* 2015). The leaves are used for the treatment of diseases and symptoms such as malaria, fever, headache, ulcer, cold, etc. The plant is also used as insect repellant and controls some reptiles (Omale *et al.,* 2008).

Ocimum gratissimum is recognized by its broad leaves and has less aroma than the *O. basilicum*. It is a perennial plant which is widely distributed in the tropics of Africa and Asia (Ladipo *et al.,* 2011). It is found in the South-Eastern part of Nigeria andis also very nutritious and used as seasoning in cooking. It consists of different composition of elements. It is used through West Africa as anti-malarial and anti-convulsant and for the management of stomach pain, and catarrh. Oil from the leaves have been found to possess antiseptic, anti-bacterial and anti-fungal activities (Idris *et al.,* 2011). The plant is traditionally used in the management of different conditions like diarrhoea, headache, fever, skin disease, and pneumonia (Njoku *et al.,* 2011). The present research was focused on the type of scent leaves (*Ocimum* leaves) found in the North-Eastern and South-Eastern regions of Nigeria.

1.2 Statement of Research Problem

Scent leaves are important food substances which have nutritional and medicinal values. Determination of the qualities of the leaves in terms of their phytochemical composition, nutritional and antioxidant properties is important in order to know the different constituents which might affect human health either positively or negatively. The wide consumption of scent leaves by Nigerians necessitates the determination of the quality of the two species of the scent leaves (in Maiduguri and Enugu) with respect to their nutritional values and elemental composition. The quality of the soil on which the plants are grown affects the quality of these leaves.

1.3 Justification of the Research

The plants are abundantly found and consumed by people in the region of study (North-Eastern and South-Eastern Nigeria) with respect to their species. The plants are known for

their nutritional values and their antioxidant properties which would be determined and compared with respect to both species of the scent leaves. This would give a picture of the phytochemical composition, nutritional contents and antioxidant properties. Determination of the elemental composition of the leaves would provide a basis for assessing the health implication of their consumption.

1.4 Aim

The aim of this study was to determine the phytochemical composition, nutritional contents and antioxidant properties of *Ocimum* plant from the North-Eastern (Maiduguri) and South-Eastern (Enugu), Nigeria.

1.5 Objectives;

The specific objectives of this work were to:

i. determinethe phytochemical composition of scent leaves found in the North-Eastern and South-Eastern Nigeria.
ii. determinenutritional content of the scent leaves by proximate analysis.
iii. determinethe elemental composition of the soil and leaves samples from the two sampling locations.
iv. determinethe antioxidant properties of the scent leaves.

1.6 Scope and Limitations of the Research

The scope of this study was to investigate the phytochemical composition, nutritional contents, and antioxidant properties of the scent leaves and the elemental composition of the plants and soil on which the plants were grown. The major limitation of this research was the cost of analysis due to large sample size. Thus the sampling point was restricted to

two states, one state from each of the two geo-political zones selected for the study (in the North-Eastern and South-Eastern parts of Nigeria).

CHAPTER TWO

2.0LITRETURE OF REVIEW

2.1 Review of Recent Works on *Ocimum basilicum* and *Ocimum gratissimum*

Cobalt showed a positive effect on nutritional, chemical, and endogenous hormone content of *O. basilicum* plant (Gad *et al.,* 2013). *O. gratissimum* revealed higher phenolic content and reducing power when compared with *Vernonia amygdalina* leaves (Oriakhi *et al.,* 2014). *O. gratissimum* showed high phytochemical content such as saponin, phenol, flavonoid etc. than Uziza leaves (*Piper guineense*) (Nwankwo *et al.,* 2014). The ethanol and ethyl acetate extracts of *O. gratissimum* were able to protect the brain of rat from Fe^{2+} induced lipid peroxidation (Oluwafemi *et al.,* 2014). *O. basilicum* showed higher mineral composition of P, Ca, K, Na and Zn than others compared within the assessment of nutritional, phytochemical composition and likely medicinal benefit of *Vernonia amygdalina* and *Talinum triangulare* leaves (Agunbiade *et al.,* 2015). *O. gratissimum* is a good source of protein and fibre and contains phytochemicals in the leaves (Talabi and Makanjuola, 2017). *O. gratissimum* showed good constituents of phytochemicals in the leaves (Jumare, 2018). *O. gratissimum* leaves showed higher content of moisture, crude fibre and carbohydrate when compared with *Vernonia amygdalina* leaves (Gideon *et al.,* 2019). *O. basilicum* showed the presence of flavonoids, terpenoids, Tannins, glycosides and carbohydrate in phytochemical screening and good inhibition Capacity (IC_{50}) in DPPH and Hydrogen peroxide assay in leave extracts among various indigenous vegetables (Ajiboye *et al.,* 2021). *O. basilicum* revealed good antioxidant activity in total phenolic and DPPH assay in extracts of ethanol, methanol and water (Teofilovic *et al.,* 2021).

2.2Phytochemical Compositions

Phytochemicals are bioactive non-nutrient plant compounds obtainable in fruits, vegetables, grains, and other food plants that have been linked to reducing the risk of major chronic diseases. Large percentage still remains unknown and need to be identified before their health benefits are fully understood (Matos *et al.,* 2015). Phytochemicals are classified into two (2) namely, primary and secondary. Primary constituents include chlorophyll, carbohydrate, fats, amino acids and proteins, while the secondary constituents include terpenoid and alkaloid which are active constituents with antibacterial, anti-inflammation and antifungal properties (wadood *et al.,* 2013).

Phytochemicals such as alkaloids, reducing sugars, flavonoids, terpenoids and phlobatannins are significant and have economic value in the research institutes and pharmaceuticals companies in the manufacturing of new drugs for the treatment of various diseases (Wadood *et al.,* 2013). Flavonoids possess anti-allergic, antioxidant and anti-inflammatory activities (Devika and Koilpillai, 2014). Tannins indulge in the management of glycogen level in blood, stimulating the receptor cells to utilize carbohydrate. Flavonoids are known to amend cardiac function, reduce anginas and lower cholesterol levels. Terpenoids promote antioxidant properties and co-act with most regulatory amino acid. Cardiac glycosides are used in the regimen of congestive heart failure and cardiac arrhythmias as they escalate contractile force of the heart muscles (Nyamai *et al.,* 2016).

2.3 Nutritional contents

Nutrients are the basic substances that provide nourishment for the maintenance and sustenance of life and growth. Nutrients are basically classified into two; these are macronutrients and micronutrients. Macronutrients are required in larger quantity; they are substances which supply bulk energy for the metabolic system to function; while micronutrients are required in very small quantity, and supply the main co-factors for metabolic processes(Clementson, 2014). Protein, fats and carbohydrate are examples of macronutrients while zinc, sodium, magnesium, manganese are micronutrients (Shovon *et al.,* 2013; Sodamade *et al.,* 2013; Paul *et al.,* 2014).High fibre content can decrease high cholesterol levels in the body; Lipid is a very good source of energy which aids in conveying fat soluble vitamins, treats and shields internal tissues. Macronutrients such as carbohydrate, protein and fats are the active constituents for building and regulating the body system (Akpabio and Ikpe,2013; Clementson, 2014; Paul *et al.,* 2014).The nutritional contents found in plant such as the essential nutrients play a vital role on the health of humans or animal that feed on plants. They help in controlling metabolic functions in the body system as explained below;

(a). Iron

Ironis one of the essential elements for all living organisms which participate in various metabolic activities, such astransport of oxygen, deoxyribonucleic acid (DNA) synthesis, and electron transfer.Iron can form free radicals, which can lead to damaging of tissue. Disorders and problems of iron metabolism are among the most familiar diseases in humans that encompass a broad spectrum of diseases with different clinical manifestations, varying from anaemia to neurodegenerative diseases (Abbaspour *et al.,* 2014).

(b) Zinc

Zincis an essential mineral that people need to stay healthy; the paucity in humans is now known to be a significant malnutrition case world-wide. Zinc deficiency is the cause of declinedgrowth and causesimmune, epidermal, skeletal, gastrointestinal, reproductive system and central nervous problems (Roohani *et al.,* 2013).

(c) Copper

Copper is an essential trace element, which acts as the cofactor of many redox enzymes. There is no consensus regarding copper recommended intake for humans, as indicated by disparities between references issued by various national authorities. Lack of copper in the body causes impaired evolution of the cardiovascular system,immunologic abnormalities and neurologic and bone malformations. High intake of copper results in oxidative cell damage and death (Bost *et al.,* 2016; Ackerman and Chang, 2017).

(d) Potassium

Potassium is an essential mineral nutrient. Potassium is an important cation in intracellular fluid. It partakes in acid-base balance, osmotic pressure regulation, nerve impulses conduction, muscle contraction andfunction of cell membrane. The significance of potassium to human health has been well acknowledged and new researches continue to stress its positive effects. High dietary consumption of potassium has been seen to prevent people from a number of problems that affect the kidneys, bones and cardiovascular system (Anonymous, 2013).

(e) Manganese

Manganese is an essential elementfor human health; it is important for antioxidant system, metabolism, reproductivehormone function andimmunological system function. High consumption of Manganese may lead to a condition known as manganism, which causes dopaminergic neuronal death (a neurodegenerative disorder)and parkinsonian-like signs (Avila *et al.,* 2013).

(f) Calcium

Calciumis an abundant essential mineral in human body with over 99% deposit in bone. The three (3) major activities for calcium homeostasis such as intestinal absorption, bone re-sorption, and kidney reabsorption are managed by calcium sensing receptor (CaSR) through a series of complexmechanisms. Any problems in these processes can lead to diseases related to dysfunctional regulation of calcium(Zhou *et al.,* 2013; Pu *et al.,* 2016). Calcium in diets and supplements participates in calcium homeostasis. Low calcium consumption can also lead to bone diseases. High intake of calcium can decrease the rate of bone loss and fracture incidence and also the risk of breast cancer. High intake of calcium can increase the chance of various diseases statesuch as increase in the risks of kidney stone, stroke, critical gastrointestinal occurrence, and cardiovascular diseases (like myocardial infarction). The intake and supplementation of calcium contribute a lot to the health status of humans (Pu *et al.,* 2016).

(g) Cobalt

Cobalt is a necessary trace element in the regulation of human body, which is a key constituent of cobalamin (vitamin B12). It has been noted that cobalt insufficiency was related with the disruption in vitamin B12 synthesis. Surplus of cobalt can lead tohypothyroidism and anaemia, as well as extend the risk of evolutional deformity and failure in infants (Falah and Saja, 2017).

(h) Phosphorus

Phosphorus is an element present in every cell of the body, normally in the form of phosphate as seen in RNA, DNA and cell membranes. Over 85% of phosphorus (P) is stored in the teeth and bones which help in castingteeth and bones, as well as mending bones. Phosphorus promotes nerve function and good muscle, regulates heartbeat, supports developmentand mendstissues and cells; stores and uses energy from food and maintains a good kidney function. Excessive intake of phosphorus might lead to unfavorable effects on bone (weak bones), kidney (chronic kidney disease), and heart (cardiovascular disease) (Calvo and Uribarri, 2013a; Karp, 2013; Markus *et al.*, 2014;Gal and Dahl, 2017).

(i). Sulphur

Sulphur is an essential element for plants as well as humans. Sulphur is present in plants asmethionine, amino acids,thiamine, Monocysteine, biotin, vitamins, while in humans it is found asmethylsulfonylmethane, glutathione, precursor of alicin, and glucisinolates. Sulphur helps in enzyme reactions and protein synthesis.It eliminates and deactivates many kinds of toxins in the body and protects the structural function around body, cartilage and skin (Chistopher and Elson, 2017; Prasad, 2014; Emmanuel *et al* 2014).

(j) Nitrogen

Nitrogen is an important nutrient in all organisms because it is the major constituent element in all amino acids. The amino acids are the fundamental building blocks for all exiting proteins which help in regulating some functions within the human body. High amount of amino acid can cause hepatic encephalopathy, a disorder found in liver and hyperammonemia which can damage the brain (Mathews and Van Holde, 2013; Mullins, 2014).

2.4 Antioxidant Properties

Antioxidants are substances that inhibit oxidative stress in living organisms; they reduce oxidation of other molecules. This oxidative stress caused by free radicalscauses diseases and abnormalities in living organisms.However, some scientists now discredit the claim that all free radicals are naturally bad for health.Plant has a natural capacity to biosynthesize a range of non-enzymatic antioxidants able to enervate reactive oxygen species that cause oxidative harm. Also plants are used to discover potent antioxidants against reactive oxygen species in the human body (Kasote *et al.,* 2015). Radical scavenging antioxidants present an excellent function in the prevention and management of variousdiseases(Szymanska, *et al.,* 2016; Renata*et al.,* 2015). The capacity of inherent antioxidants found in foods, spices, fruits, beveragesand appurtenance has attracted much consideration from the public as well as scientists in anticipation of their function indisease prevention and retardation of ageing. Various systems had been evolved and applied to impose the ability to scavenge free radicals (Niki, 2016).

CHAPTER THREE

3.0MATERIALS AND METHODS

3.1 Materials

3.1.1 List of Apparatus and Equipment

i. Beaker
ii. Volumetric flask
iii. Conical flask
iv. Crucible
v. Whatman No. 1 filter paper
vi. Sohlet setup
vii. Test tubes
viii. Test tube holder
ix. Spatula
x. Micro pipette
xi. Burette
xii. Aluminum foil
xiii. Sample bottles

3.1.2 List of Reagents

i. Molisch reagent
ii. Barfored’s reagent

3.2 Methods

3.2.1 Cleaning of apparatus

All apparatus such as glassware, rubber, crucibles, mortar, etc. which were used in the analysis were cleaned using the following techniques; scraping away of any thick solid material from glassware, wiping offany grease from the glass with a solvent like acetone, soaking of the glassware in a warm cleaning solution of detergent and water, followed by washing and rinsing with tap water first, and then deionized water.

3.2.2 Collection of samples

Fresh samples *of Ocimum* plant species and soil were collected from Government Residential Area along Damboa road and 707 Housing Estate (Maiduguri town) in North-Eastern Nigeria (Figure 3.1) and Nsukka and Awgu towns (Enugu State) in South-Eastern part of Nigeria (Figure 3.2). Both leaves were examined and authenticated at the herbarium of the Department of Botany, Ahmadu Bello University, Zaria with the following *voucher* numbers; 1285 for *Ocimum gratissimum L.* and 044 for *Ocimum basilicum L.* The soil was collected at each sampling location using clean hand trowel by piercing the soilto 10 cm from the top soil in three places.

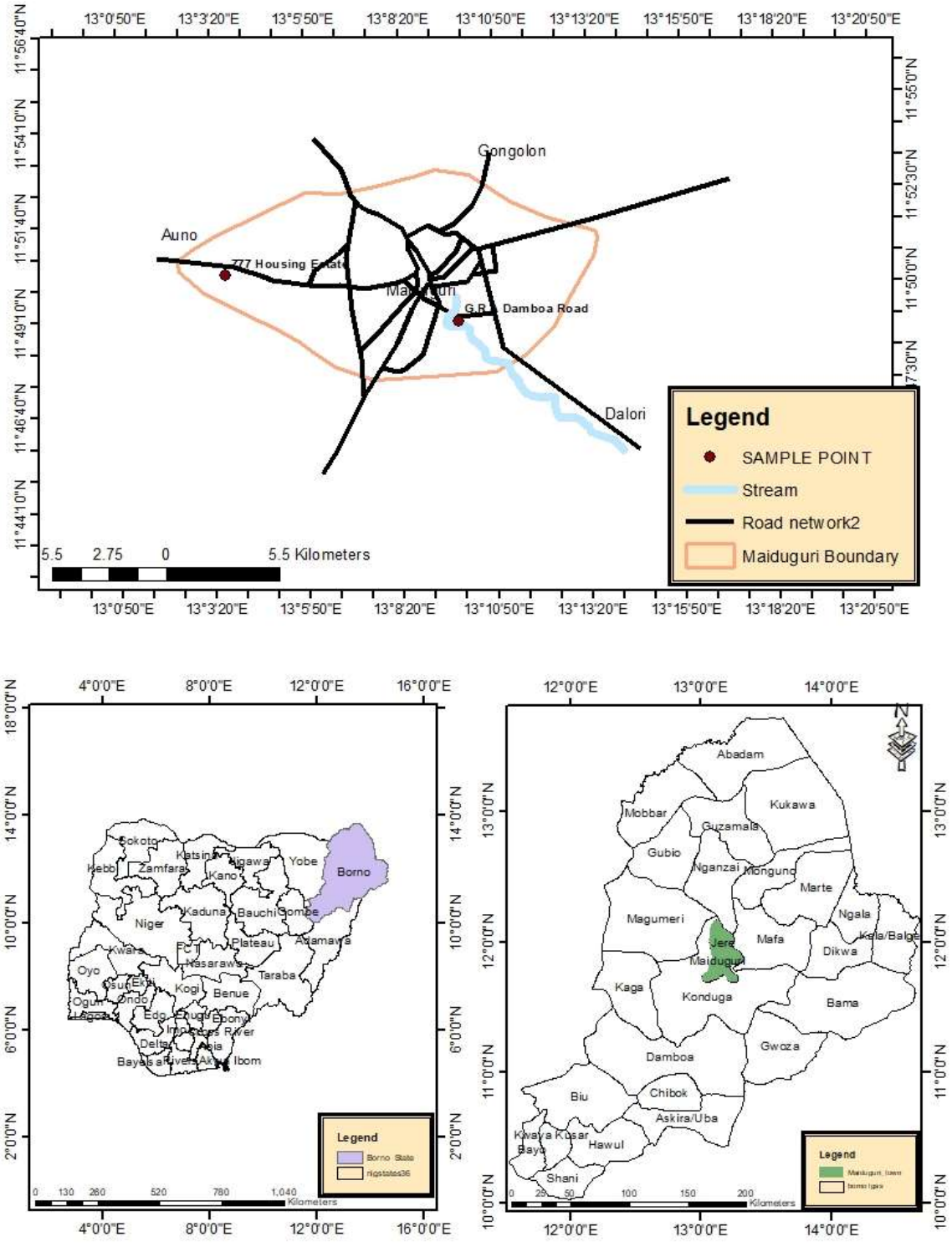


Figure 3.1: Map of Maiduguri, Borno State, Nigeria and the sampling locations

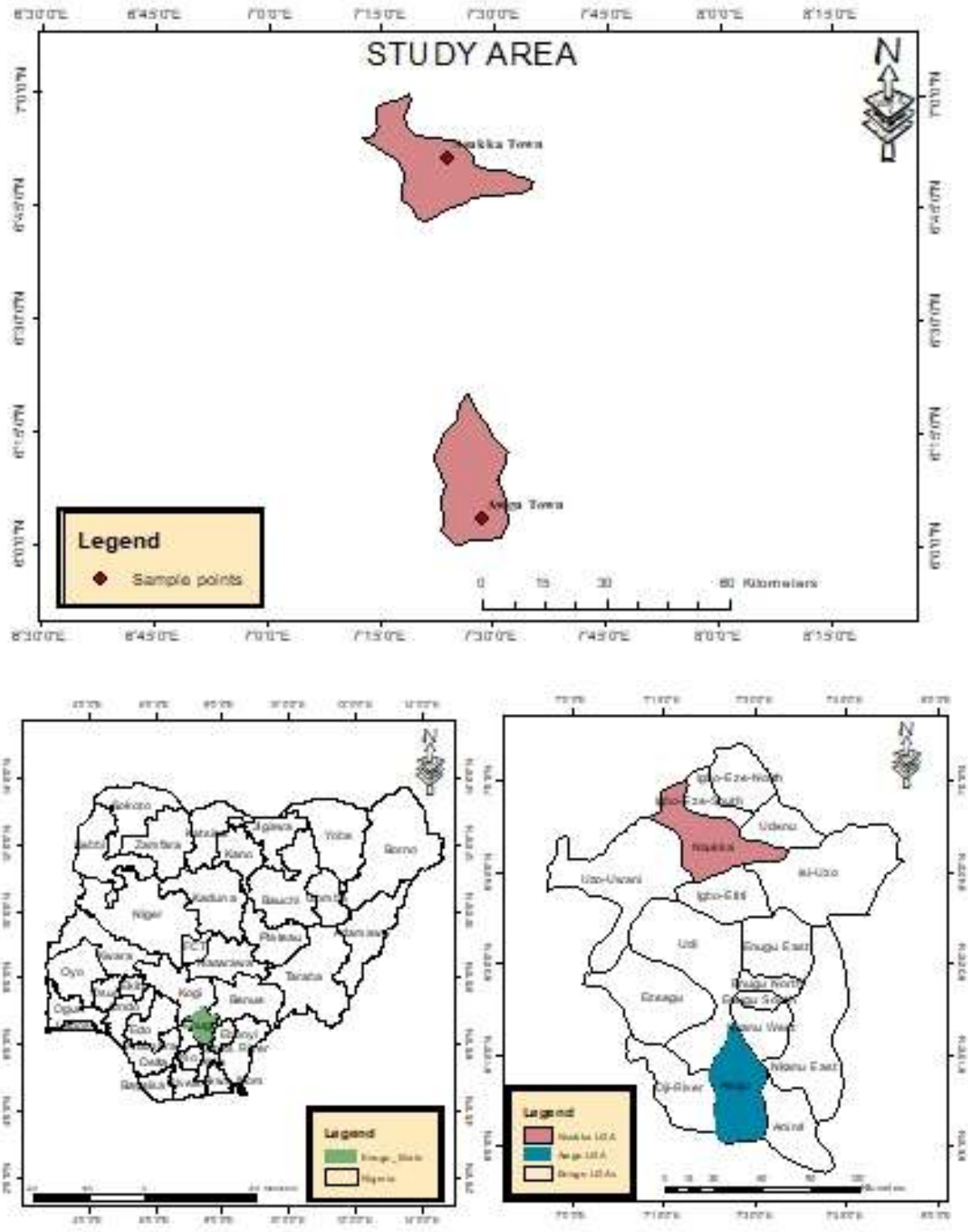


Figure 3.2: Map of Enugu, Enugu State, Nigeria and sampling locations

3.2.3 Sample pre-treatment

Fresh samples of scent leaves (*Ocimum gratissimum* and *Ocimum basilicum* leaves) were washed and dried at room temperature separately for 3 days. The two samples were then crushed in a mortar separately; the resulting powder was sieved with 0.02mm mesh size, weighed and stored in a low density polyethene bag. Likewise soil collected in three places at each sampling location was dried at room temperature for 3 days, then sieved and mixed in one place and stored in polythene bagsfor further analyses.

3.2.4 Sample extraction

The powdered samples of 250g were successively extracted by adding 2000 cm^3 of n-hexane and methanol as solvents in soxhlet apparatus for 48hr. The resulting extract was filtered through a Whatman filter paper No.1 and solvents were removed by using a rotator evaporator and subjected to freeze drying in a lyophilizer until dry extracts were obtained (Gulcin, 2005). The extracts were used for determination of phytochemical contents and antioxidant properties.

3.2.5 Phytochemical Analysis

Phytochemical composition test of n-hexane and methanol extracts of *Ocimum basilicum* and *Ocimum gratissimum* leaves was carried out to determine the presence of carbohydrate, tannins,cardiac glycoside, terpenoid, and flavonoid. Exactly 0.1 g of each of the extracts of *Ocimum basilicum* and *Ocimum gratissimum* plant was dissolved in 50 cm^3 of ethanol for phytochemical screening methods as follows;

a. Test for Carbohydrate

i. Molischs Test

Few drops of molisch's reagent were added to 2 cm^3 of the extract obtained, followed by the addition of concentrated sulfuric acid by the side of the test tube. The mixture was allowed to stand for 2 min and then diluted with 5 cm^3 of distilled water. Formation of a red or dull violet colour at the interphase of two layers indicated the presence of carbohydrate (Trease and Evans, 2002).

ii. Barfoed's Test (test for monosaccharides)

A solution of the extract (1 cm^3) obtained above was mixed with Barfoed's reagent in the test tube and then heated on a water bath for 2 min; red precipitate of cuprous oxide indicated the presence of monosaccharides like fructose and glucose (Trease and Evans, 2002).

iii. Test for soluble starch

An already prepared 2 cm^3 solution of the extract was boiled with 1 cm^3 of 5% potassium hydroxide (KOH), cooled and acidified with sulfuric acid (H_2SO_4). A yellow coloration indicated the presence of soluble starch (Voshnois, 1979).

b. Test for Tannins

Ten(10 cm^3) of distilled water was stirred with 0.1 gof each extractsand filtered. The filtrate was used for following test;

1. Ferric chloride Test

One(1%) of ferric chloride solution was added to 2 cm^3 of the filtrate. A blue-black, green or blue-green precipitate showed the presence of tannins (Trease and Evans 2002).

c. Test for cardiac glycoside

Two(2 cm^3) of prepared sample was dissolved in 2 cm^3 of chloroform. Sulfuric acid was carefully added to the mixture by the side of the test tube to form a lower layer.

Appearance of a reddish-brown colour or yellow at the interphase indicated the presence of steroid of ring (i.e. a glycone portion of cardiac glycoside) (Siver *et al.*, 1998).

d. Test for Terpenoids

Two(2 cm^3) of extract and 1 cm^3 of acetic anhydride were added in a test tube followed by the addition of conc. sulfuric acid. A colour change from pink to violet indicated the presence of terpenoids (Siver *et al.*, 1998).

e. Test for Flavonoids

i. Shinoda's Test

Two(2 cm^3) of the extract was warmed and filtered; three pieces of magnesium chips were added to the filtrate. A few drops of conc. hydrochloric acid were added and a change in colour from pink, orange to purple indicated the presence of flavonoids (Markham and David, 1988).

ii. Ferric chloride Test

Few amounts of the extract was boiled with distilled water and filtered. Few drops of ten(10%) ferric chloride solution were added to 2 cm^3 of the filtrate; a blue-green coloration indicated the presence of a phenolic hydroxyl group (Trease and Evans, 2002).

3.2.6 Proximate Analysis

Prepared samples were analysed for Carbohydrate, moisture, ash,crude fibre, pH, protein and crude fats contents using standard analytical methods:

a. Determination of carbohydrate content

Carbohydrate content was estimated by subtracting the percentage concentration obtained from fat, protein, moisture and ash content of the sample analyzed from 100% (A.O.A.C, 1990).

%Carbohydrate = [100 - (Moisture +ash +Protein + Fats)].

b. Determination of moisture content

One(1.5 g) of prepared sample was dried in oven at 105^{o}C for 24 hrs, and then cooled in desiccators. Further drying was done until constant weight of the content was obtained. The moisture content was calculated as percentage moisture according to the method described by Owoso and Ogunmoyela (2001) in equation 1.

$$\%\text{moisture}=\frac{(W1-W2)}{Wt}\text{x } 100 \quad \text{- - - - - - (1)}$$

W_1= initial weight of crucible + sample (g)

W_2= final weight of crucible + sample after heating and cooling (g)

Wt = weight of sample (initial) (g)

c. Determination of ash content

One(1.5 g) of the prepared sample was weighed and transferred into a crucible of known weight. This was then charred with Bunsen burner. The difference in weight was calculated according to the method described by Udo and Ogunwele (1986)in equation 2.

$$\%\text{Ash}=\frac{(W2-W1)}{Wt}\,x\ 100\text{- - - - - - - (2)}$$

Difference in weight of ash=W_2-W_1

W_1= weight of empty crucible (g)

W_2= weight of crucible and sample after heating and cooling (g)

Wt= weight of sample (g)

d. Determination of crude fibre

Exactly 1.5 g of prepared sample (W_0) was dissolved in 100 cm^3 distilled water in a crucible; exactly 20 cm^3 of 10% sulphuric acid was added and boiled gently for 30 mins. The sample was cooled and then filtered; the filtrate was subjected to treatment using 10% NaOH and filtered. The residue was passed through exactly 20 cm^3 of ethanol and petroleum ether and then dried at 105^oC. The dried residue was weighed (W_1) and ashed at 600^oC for 90 mins. This was then cooled and re-weighed (W_2) and the percentage of crude fibre was calculated (Owoso and Ogunmoyela, 2001)using equation 3.

$$\%\text{crude fibre} = \frac{(W1 - W2)}{W0} \times 100 \quad - \quad - \quad - \quad - \quad - \quad - \quad 3$$

e. Determination of pH

One(1.5 g) of the prepared sample was dissolved in 10 cm^3 of deionized water. The pH meter was inserted into the solution and the pH of the solution was directly read from the pH meter (Gul and Mahpara, 2009).

f. Determination of protein content

Percentage crude protein was determined using AOAC (1990) as;

$$\%\text{Crude protein} = \%N \times 6.25 \text{ (Protein factor)} \quad - \quad - \quad - \quad - \quad (4)$$

Percentage Nitrogen was determined in 3.2.6 (d).

g. Determination of fat content

Fats analysis was carried out using the soxhlet extraction method. One(1.5 g) of the prepared sample was mixed with about 100 cm^3 of n-hexane. The mixture was vigorously shaken with the separation flask knob open at interval to release the accumulated pressure. The fat extracted in the n-hexane was evaporated to dryness over a soxhlet extractor. The

fats extract was transferred into a glass dish with n-hexane washing and the n-hexane was evaporated on water bath; the dish was placed in the oven to dry at 105^{o}C for 30 mins. The glass dish was cooled in a desiccator and weighed (Gul and Mahpara, 2009). The percentage of fats content in the sample was calculated using equation 5;

$$\%\text{crude fats} = \frac{\text{Wt.of n-hexane extract}}{Wt.of sample} x\ 100 \quad - \quad - \quad - \quad - \quad - \quad (5)$$

3.2.7 Spiking experiment and quality control

One(1 g) of each soil and plants leaves samples from the sampling locations was digested separately; each sample was spiked with MESS (multi-elements standard solution) and digested in duplicate together with the Blank and was run on the AAS machine. The amount of metal present in the spiked and unspiked samples was determined from calibration curve (Uba *et al.,* 2013). Recovery was taken as a measure of accuracy while standard deviation was taken as a measure of precision.

a. Preparation of multi-element standard solution (MESS)

Fifty (50 mg/l) of the metals concentration of 0.057306 g of $MnCl_2$, 0.047660 g of KCl, 0.069180 g of $CaCl_2$, 0.072540 g of $FeCl_3$, 0.1009890 g of $CoCl_2.6H_2O$, 0.062838 g of $CuSO_4$, and 0.052108 g of $ZnCl_2$ were weighed and dissolved in a beaker and transferred into 500 cm^3 standard volumetric flask and made up to mark using deionized water. This gave multi-element standard solution (MESS) (Table 3.1) and this was used for spiking experiment.

Twenty(20 cm^3) of the multi-element standard solution (MESS) was drawn with a graduated pipette and used to spike 1.0 g of soil and leaves samples respectively. These were digested in duplicate together with the blank and then run on AAS for each of the

metal used in multi-element standard solution (Uba *et al.*, 2013). The concentration of metals in spiked and un-spiked sample was used to calculate the percentage recovery for each metal used in the multi-element standard solution.

$$\% \text{ Recovery of metals} = \frac{W2 - W1}{B} X\ 100 \quad - \quad - \quad - \quad - \quad - \quad (6)$$

Where W_2 = Concentration of metal in spiked sample

W_1 = Concentration of metal in un-spiked sample

B = Concentration of metal in MESS

Table 3.1: List of MESS salts, their Molar mass and their calculated weight in grams needed to prepare 50mg/l of the respective metals

S/N	Element	Salt of the element used	Molar Mass (g/mol)	Weight of salt calculated (g)
1	Manganese (Mn)	$MnCl_2$	125.84	0.057706
2	Potassium (K)	KCl	74.550	0.047660
3	Calcium (Ca)	$CaCl_2$	110.98	0.069180
4	Iron (Fe)	$FeCl_3$	162.20	0.072540
5	Cobalt (Co)	$CoCl_2.6H_2O$	237.93	0.100989
6	Copper (Cu)	$CuSO_4$	159.60	0.062838
7	Zinc (Zn)	$ZnCl_2$	136.31	0.052108

3.2.8 Sample digestion for determination of metals and non- metals

a. Digestion of plant and soil samples for metals determination

The prepared plant extractof each species was digested by weighing 1.0g into an acid washed porcelain crucible and then charred for few minute on a Bunsen burner. The crucible was removed from the burner and cooled. Two ratiosone(2:1) of 3cm^3 of 70% HCl and 3cm^3 of conc. HNO_3 were added to the sample inside the digesting tubes and heated for an hr. The mixture was cooled and filtered through a Whatman No.1 filter paper into a 100cm^3 volumetric flask and then made up to the mark with deionized water (Saeid, 2012). The elemental composition in the digested sample was determined using Atomic Absorption Spectrometry which was done in duplicate. The equation 7 was used to calculate the concentration in mg/kg of each metal.

$$\text{Concentration (mg/kg)} = \frac{Concentrationinppm \quad (\frac{mg}{dm^3})}{weig\,ht\ of\ Sample \quad (kg)} X\ vol.of\ filtrate\ (cm^3)\ X1000 \text{- (7)}$$

b. Digestion of plant and soil samples for determination of phosphorus (P)

Four(4cm^3) of conc. perchloric acid, 10cm^3 conc. HNO_3 and 2cm^3 conc. H_2SO_4 were added to 1.0 g of sample in a pyrex conical flask under fume curboard. The sample mixture was heated under hot plate at 70^{o}C for 30min until dense white fumes appeared which was then allowed to cool. It was heated again for 15min at 80^{o}C followed by cooling and addition of 50cm^3 of distilled water. This was boiled for 15min at the same temperature under hot plate, cooled and filtered with Whatman No. 1 filter paper. The filtrate was topped up with distilledwater to 100cm^3 in a volumetric flask.Phosphorus developer reagent(Ammonium molybdate + metabanadite + Nitric acid) was added to the solutionand

then stored in a sample bottle for elemental determination using spectrophotometer at 620 nm which was carried out in duplicate(AOAC, 1970). The equation 8 was used to calculate the concentration of phosphorus in mg/kg.

Concentration of P in mg/kg = $\frac{Cu\ x\ Vs\ x\ Vd}{g\ \ x\ Vt}$ - - - - - (8)

C_u = Concentration of Phosphorus (mg/dm^3) obtained from calibration curve

Vs = Volume of sample extracted or digested (cm^3)

V_d = Volume of sample + developer reagent (Ammonium molybdate + metabanadite + Nitric acid) (cm^3)

g = Weight of sample(g)

V_t = Volume of sample without developer(cm^3)

c. Digestion of plantand soil samples for determination of sulphur (S)

One(1.0 g) of sample was weighed and mixed with 25cm^3 of KH_2PO_4 in to anErlenmeyer flask of 50cm^3. It was shaken for 30min on a mechanical shaker and centrifugedat 350 rpm for 5 min. The solution was then filtered through a Whatman No. 1 filter paper and sulphurdeveloper reagent(Gelatin + Barium chloride) was added to the filtrate and stored in a sample bottle for determination using spectrophotometer at 228 nmwhich was done in duplicate (AOAC, 1970). Equation 9 was used to calculate the concentration of sulphur in mg/kg.

Concentration of S inmg/kg = $\frac{Cu\ x\ Vs\ x\ Vd}{g\ \ x\ Vt}$ - - - - - (9)

C_u = Concentration of Sulphur (mg/dm^3) obtained from calibration curve

Vs = Volume of sample extracted or digested (cm^3)

V_t = volume of sample without developer(cm^3)

g = Weight of sample (g)

V_d = Volume of sample + developer reagent (Gelatin + Barium chloride) in cm^3

d. Digestion of plant and soil samples for determination of nitrogen (N)

One(1 g) of the plant sample wasweighed and added to exactly 2 cm^3 of distilled water in to a 50cm^3 digestion flasks to stand for 30min. One(1 g) of copper sulfate , 1g, Na_2SO_4(catalyst) and 30cm^3 of conc. H_2SO_4 were added respectively to the sample mixture in the flask. The sample mixture was heated on a digestion rack until frothing stops. After the frothing stopped the heat was increased to gently boil for 3hrs.The boiled sample was allowed to cool for 5 min; exactly 20cm^3 of deionized water was then added to the digested sample and filtered using Whatman paper No. 11,and then was add up to 100 cm^3 of the filtrate in the volumetric flask. A distillation apparatus was set by placinga 250 cm^3 receiving flask containing exactly 10cm^3 of boric acid indicator under the condenser of the distillation apparatus. The flask containingten (10cm^3) of the filtratewas attached to the steam jet of distillation apparatus and ten(10cm^3) of 40% NaOH solution was added through the funnel stopcock and distillation was commenced. The sample was distilled to fifty(50cm^3)and then stopped.Titration of the distilled sample was carried out to first pink color with 0.01 N of $H_2SO_4$and also with blank (using standard) in duplicate (AOAC, 1990). Equations 10 and 11 were used to solve for the percent nitrogen and mg/kg in the sample.

$$\%\text{Nitrogen}=\frac{(T-B)\times N\times 14\times VD\times 100}{s\times V} \quad \text{- - - - - - -(10)}$$

$$\text{N(mg/kg)}=\frac{\%Nitrogen\ \times 1000}{100} \quad \text{- - - - - - - (11)}$$

T = Sample titre volume (cm^3)

B = Blank titre volume (cm^3)

N = Normality of H_2SO_4

S = Sample weight in grams

V= Aliquot taken (cm^3)

VD= volume of dilution (cm^3)

3.2.9 Determination of Antioxidant Properties

The n-hexane and methanol extracts of *Ocimum basilicum* and *Ocimum gratissimum* leaves were used for antioxidant assays of Total phenolic content estimation assay and 2,2-diphenyl-1-picrylhydrazyl assay. Exactly 0.1 g of each of the extracts was dissolved in $2cm^3$ of ethanol, $2cm^3$ 20%DMSO; this was made to mark of 50 cm^3 with 50% methanol and storedin a sample bottle.Exactly 50 µg/cm^3, 100 µg/cm^3, 150 µg/cm^3, 200 µg/cm^3, and 250 µg/cm^3 of the prepared solution ofn-hexane and methanol extracts of *O. basilicum* and *O. gratissimum* leaves were used for all the antioxidant assays except total phenol content estimation assayfor which only 50 µg/cm^3 was used. Also Exactly 0.1 g of Ascorbic acid was dissolved in 50 cm^3 of 50% methanol solvent and exactly 50 µg/cm^3, 100 µg/cm^3, 150 µg/cm^3, 200 µg/cm^3, and 250 µg/cm^3were prepared respectively as reference standards used for allthe assays except Total phenol assay.

a. Total phenol content estimation assay

Exactly $1cm^3$ of each prepared sample of 50% methanol only was mixed with $1cm^3$ of folin-ciocalteu's phenol reagent. After 5min, exactly $10cm^3$ of 7% Na_2CO_3 solution was added to the mixture followed by the addition of exactly $13cm^3$ of deionized distilled water

and mixed thoroughly. The mixture was kept for 90min at 23^{o}C, after which the absorbance was read at 750nm using spectrophotometer. The phenolic content was determined from extrapolation of calibration curve which was made by preparing gallic acid solution. The estimation of phenolic compound was carried out in duplicate; and the total phenolic content was expressed as milligrams of gallic acid equivalent per g of dried sample (Saeed *et al*., 2012).

b. DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay

One(1mg) of 2, 2-diphenyl-1-picrylhydrazyl was dissolved in 25cm^3 of 50% methanol in a beaker to dissolve and was made up to 1000cm^3 using a volumetric flask and stored. Three(3cm^3) of aliquot of the solution of DPPH was mixed with 2 cm^3 of each of the fractions of the samples at different concentrations. The reaction mixture was shaken well and incubated in dark for 15min at room temperature. A blank solution was prepared also and the absorbance of both solutions prepared was taken at 517nm using a spectrophotometer and the assay was done in duplicate (Saeed *et al.*, 2012). The scavenging effect was calculated based on the percentage of DPPH radical scavenged using in equation 12.

$$\text{Scavenging effect (\%)} = \frac{(blank\ \ absorbance\ -sample\ \ absorbance\ )}{blank\ \ absorbance}\ x\ 100 \text{ -} \qquad \text{-} \qquad \text{-(12)}$$

3.2.10Statistical Treatment of Data

The datacollected was expressed as mean and standard deviation which was subjected to statistical analysis using Microsoft spreadsheet and statistical package for social science (SPSS) software for the analysis of variance (ANOVA).

CHAPTER FOUR

4.0RESULTS

The following results of phytochemical compositions, nutritional contents and antioxidant properties of the leaves *Ocimum basilicum* and *Ocimum gratissimum* and soil samples were analysed and the following data were obtained;

4.1 Phytochemical composition of crude extracts of n-hexane and methanol extracts of *Ocimum basilicum* and *Ocimum gratissimum* leaves

Phytochemical composition of extracts of n-hexane and methanol of *O. basilicum* and *O. gratissimum* leaves were obtained through screening the extracts of both leaves are presented in Table 4.1. The results shows that the presence of carbohydrate, cardiac glycoside, terpenoid and flavonoid content, also absence of tannins and monosaccharide in carbohydrate were obtained in n-hexane extracts of both *O. basilicum* and *O. gratissimum* leaves. The methanol extracts of *O. basilicum* and *O. gratissimum* leaves showed the presence of carbohydrate, tannins, cardiac glycoside, terpenoid and flavonoid; also monosaccharide (Barfoeds test for carbohydrate) in both extracts of the leaves.

Table 4.1: Phytochemical screening of n-hexane and methanol extracts of Ocimum basilicum and Ocimum gratissimum leaves.

Phytochemicals			*Ocimum basilicum*		*Ocimum gratissimum*	
			n-hexane Extract	Methanol Extract	n-hexane Extract	Methanol Extract
Carbohydrate	I.	Molishs test	-	+	-	+
	II.	Barfoeds test	_	_	_	_
	III.	Starch test	-	+	-	+
Tannins			_	+	_	+
Cardiac glycoside			+	+	+	+
Terpenoid			+	+	+	+
Flavonoid	I.	Shinoda's test	+	+	+	+
	II.	Ferric Chloride test	+	+	+	+

4.2 Proximate contents of plant leaves of *Ocimum basilicum* and *Ocimumgratissimum*

The Mean percentage of proximate contents of plant leaves sample of *Ocimum basilicum* and *Ocimumgratissimum* found in 707Housing Estate Maiduguri, G.R.A Damboa Maiduguri, Nsukka town Enugu and Awgu town Enugu town for the following contents of Carbohydrate, moisture, ash, crude fibre, pH value, protein and fats were obtained and presented in Table A3-1 in Appendix III and Figure 4.1 to 4.7. The result shows that 707Housing Estate has maximum percentage of carbohydrate and ash content of 31.58 ± 0.69% and 15.33 ± 2.82% respectively in *O. basilicum* leaves illustrated in Figure 4.1 and 4.3, G.R.A Damboa road has maximum percentage of moisture, crude fibre and crude fats content of 26.66 ± 1.88, 32.00% ± 0.00% and 18.66 ± 1.88% respectively in *O. basilicum* leaves illustrated in Figure 4.2, 4.4 and 4.7, Nsukka town has maximum percentage of protein content of 7.61 ± 0.09% in *O. gratissimum* leaves illustrated in Figure 4.6. 707Housing Estate and G.R.A Damdoa road has a pH of 6.90 ± 0.09 and 6.93 ± 0.06 respectively in *O. basilicum* leaves which are slightly acidic and Nsukka town and Awgu town has a pH of 7.48 ± 0.11 and 7.54 ± 0.08 respectively in *O. gratissimum* leaves which are slightly basic as illustrated in Figure 4.5.

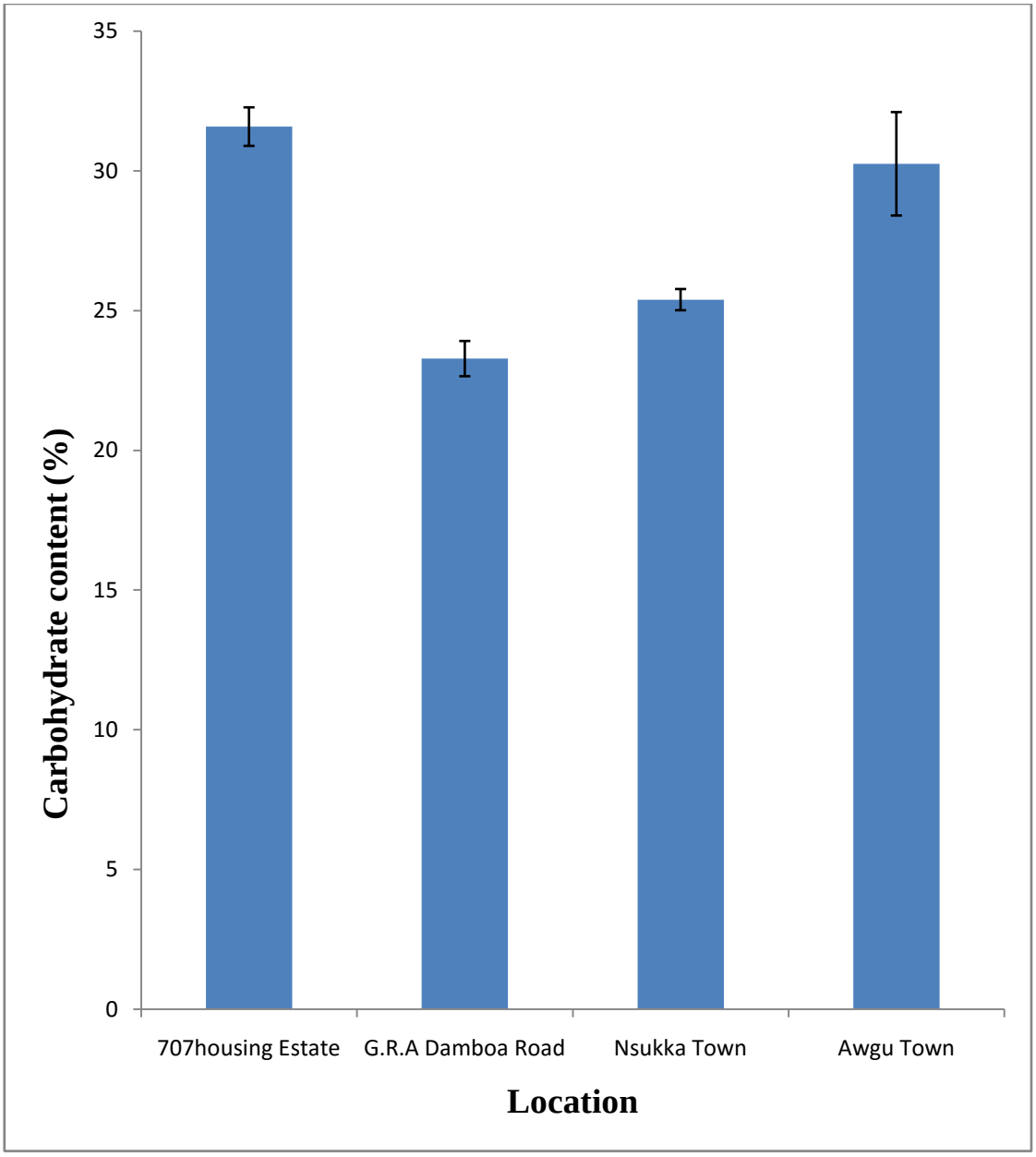


Figure 4.1: Percentage of carbohydrate in leaves of *Ocimum basilicum* obtained from 707housing Estate and G.R.A Damboa road and leaves of *Ocimum gratissimum* obtained from Nsukka town and Awgu town.

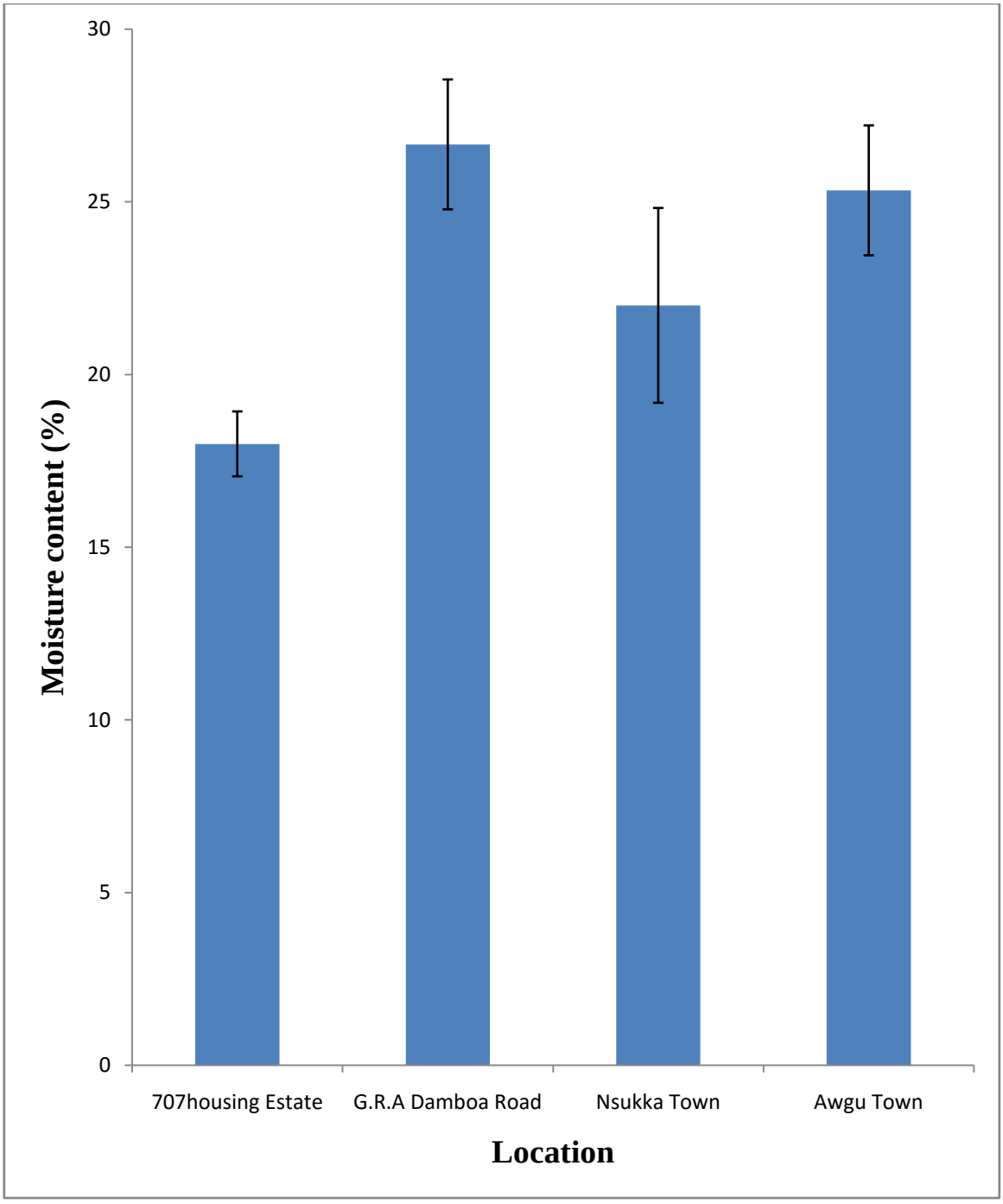


Figure 4.2: Percentage of moisture content in leaves of *Ocimum basilicum* obtained from 707Housing Estate and G.R.A Damboa road and leaves of *Ocimum gratissimum* obtained from Nsukka town and Awgu town.

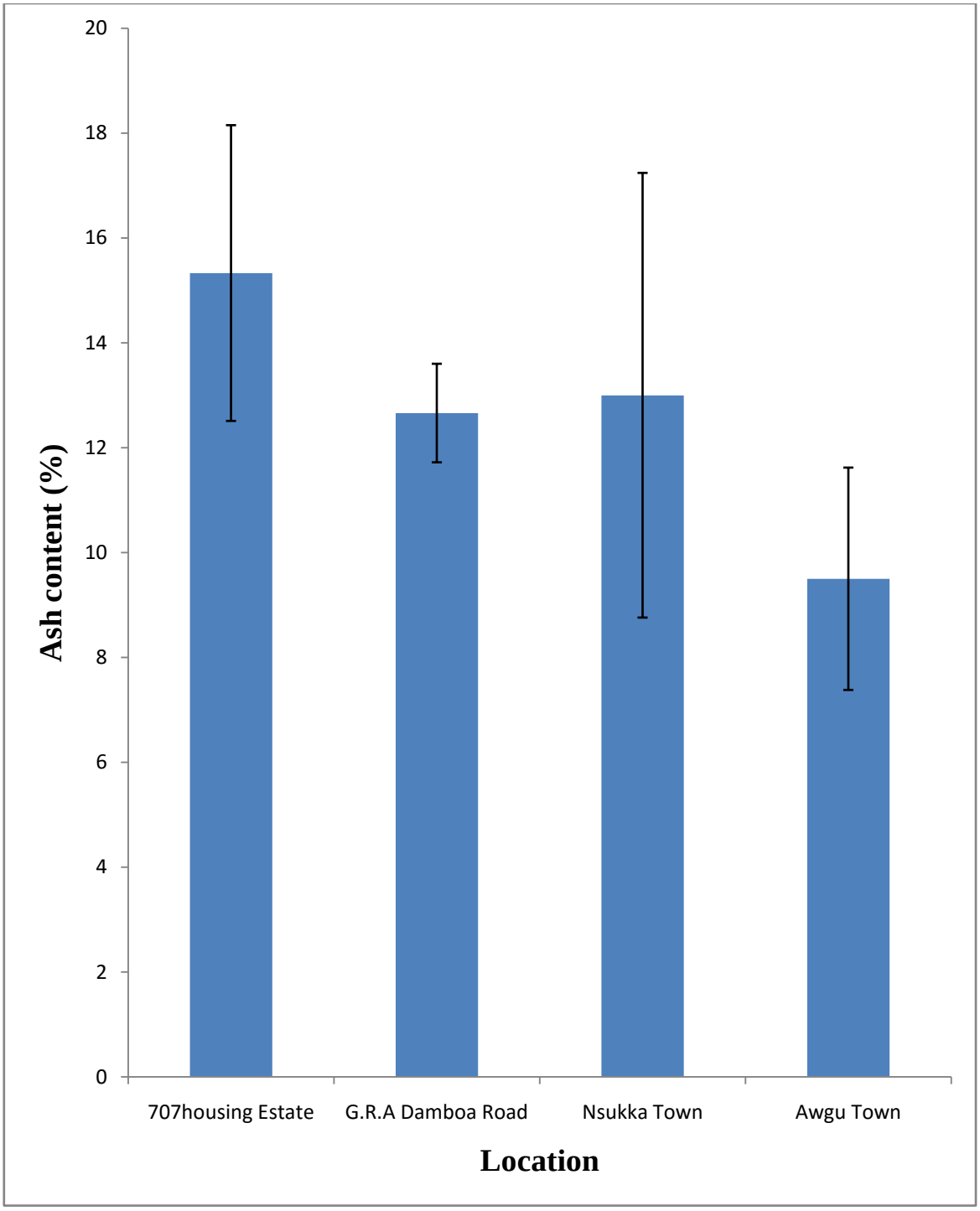


Figure 4.3: Percentage of ash content in leaves of *Ocimum basilicum* obtained from 707Housing Estate and G.R.A Damboa road and leaves of *Ocimum gratissimum* obtained from Nsukka town and Awgu town.

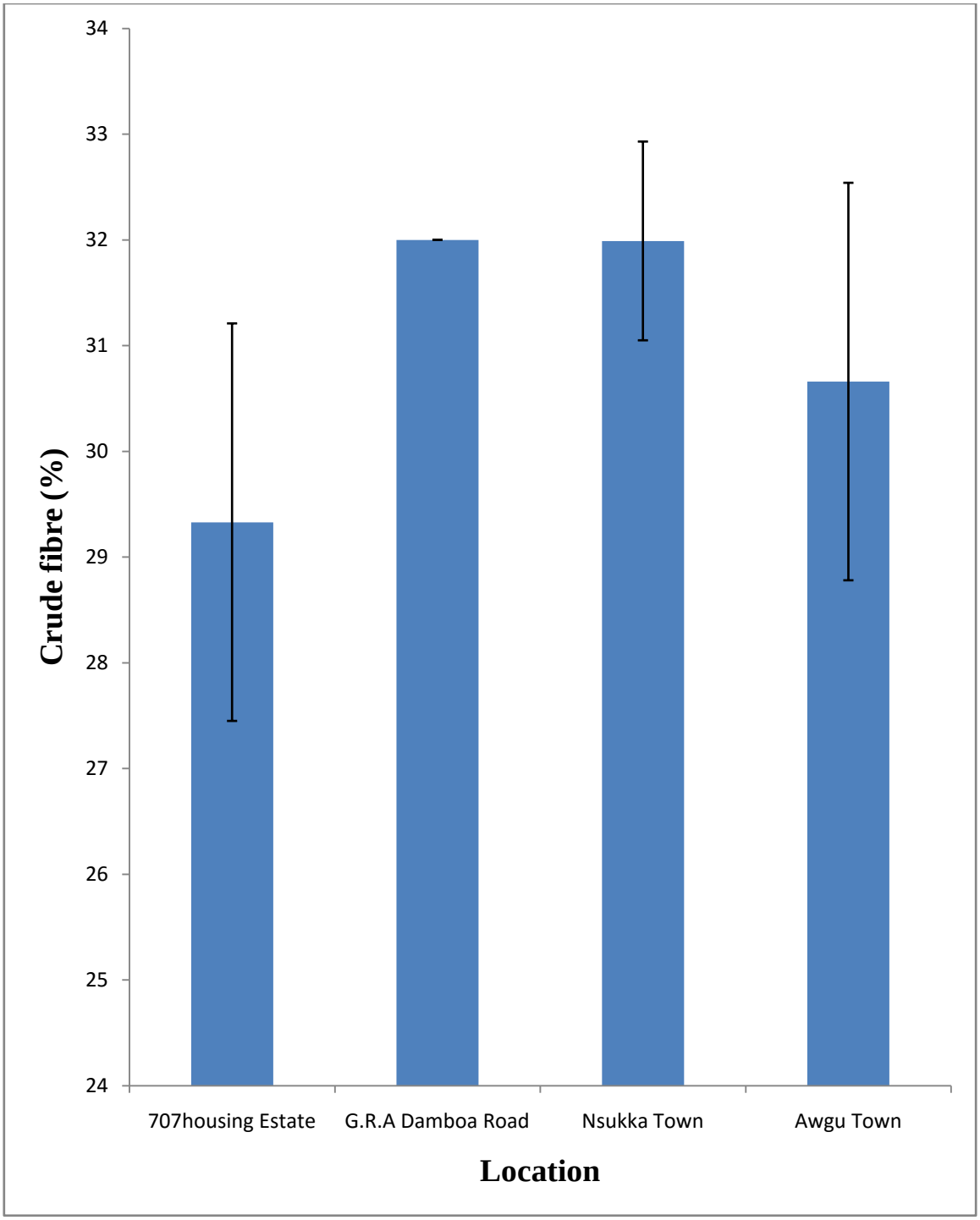


Figure 4.4: Percentage of crude fibre in leaves of *Ocimum basilicum* obtained from 707Housing Estate and G.R.A Damboa road and leaves of *Ocimum gratissimum* obtained from Nsukka town and Awgu town.

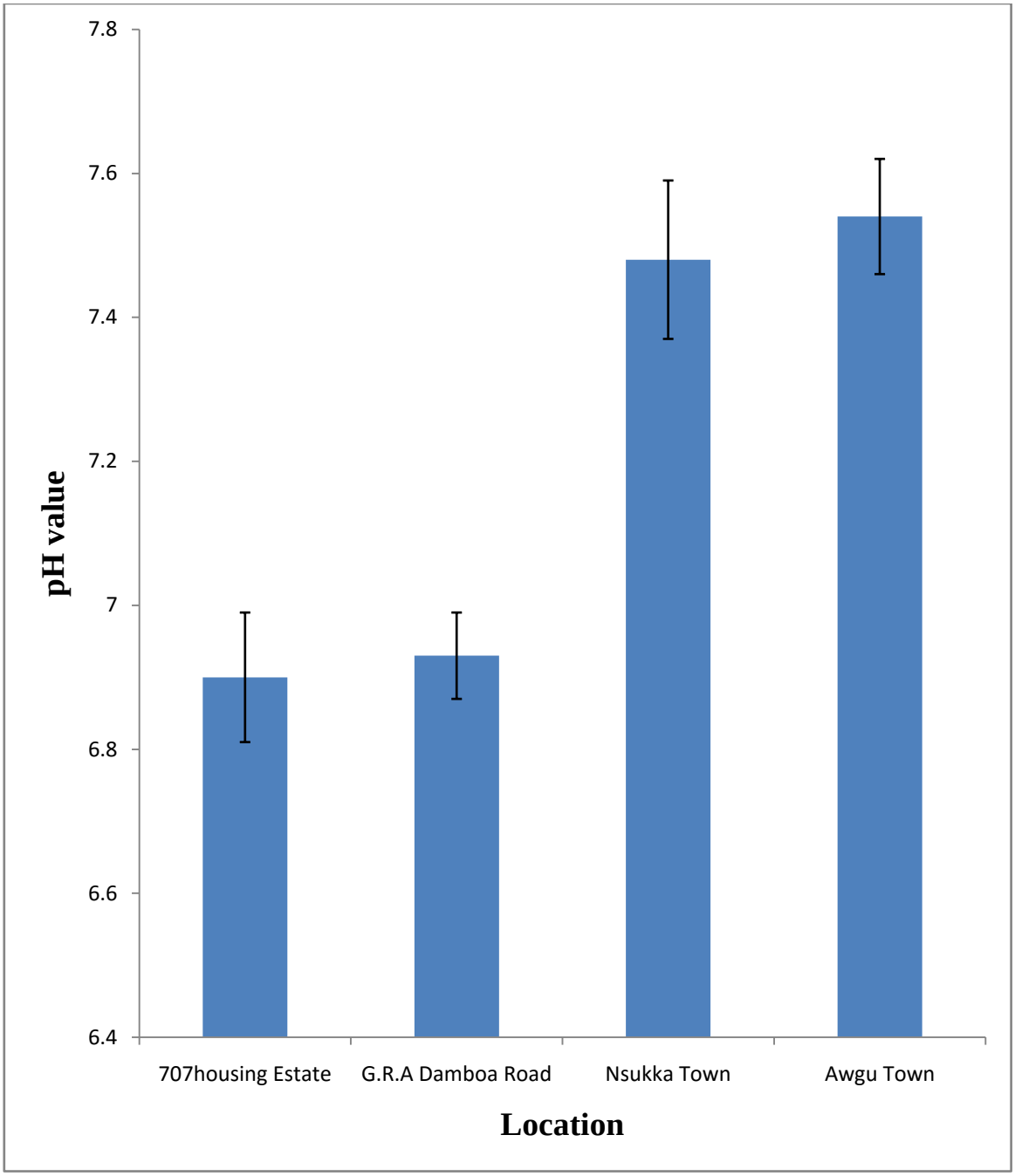


Figure 4.5: pH value in leaves of *Ocimum basilicum* obtained from 707Housing Estateand G.R.A Damboa road and leaves of *Ocimum gratissimum* obtained from Nsukkatown and Awgu town.

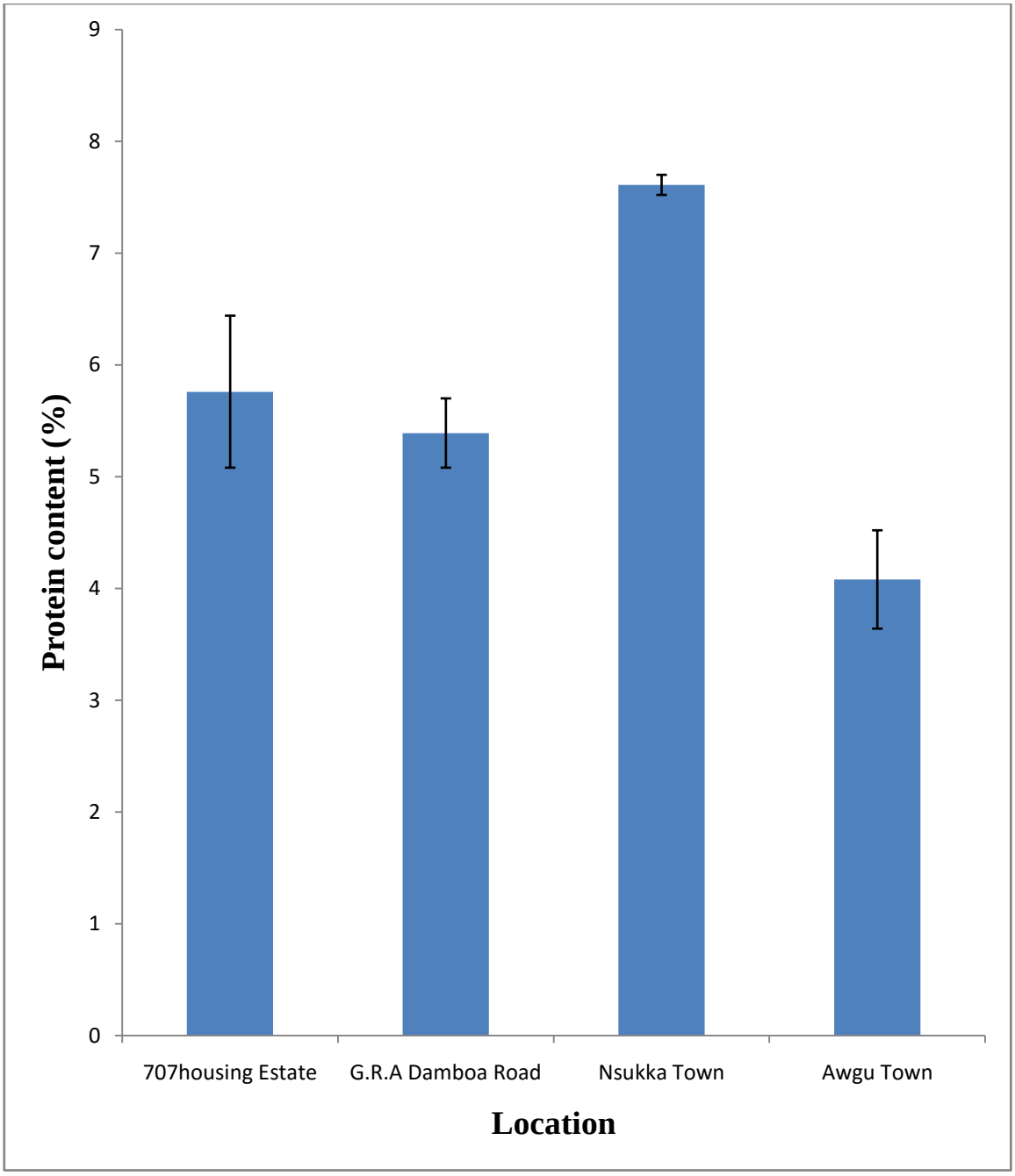


Figure 4.6: Percentage of protein content in leaves of *Ocimum basilicum* obtained from 707Housing Estate and G.R.A Damboa road and leaves of *Ocimum gratissimum* obtained from Nsukka town and Awgu town.

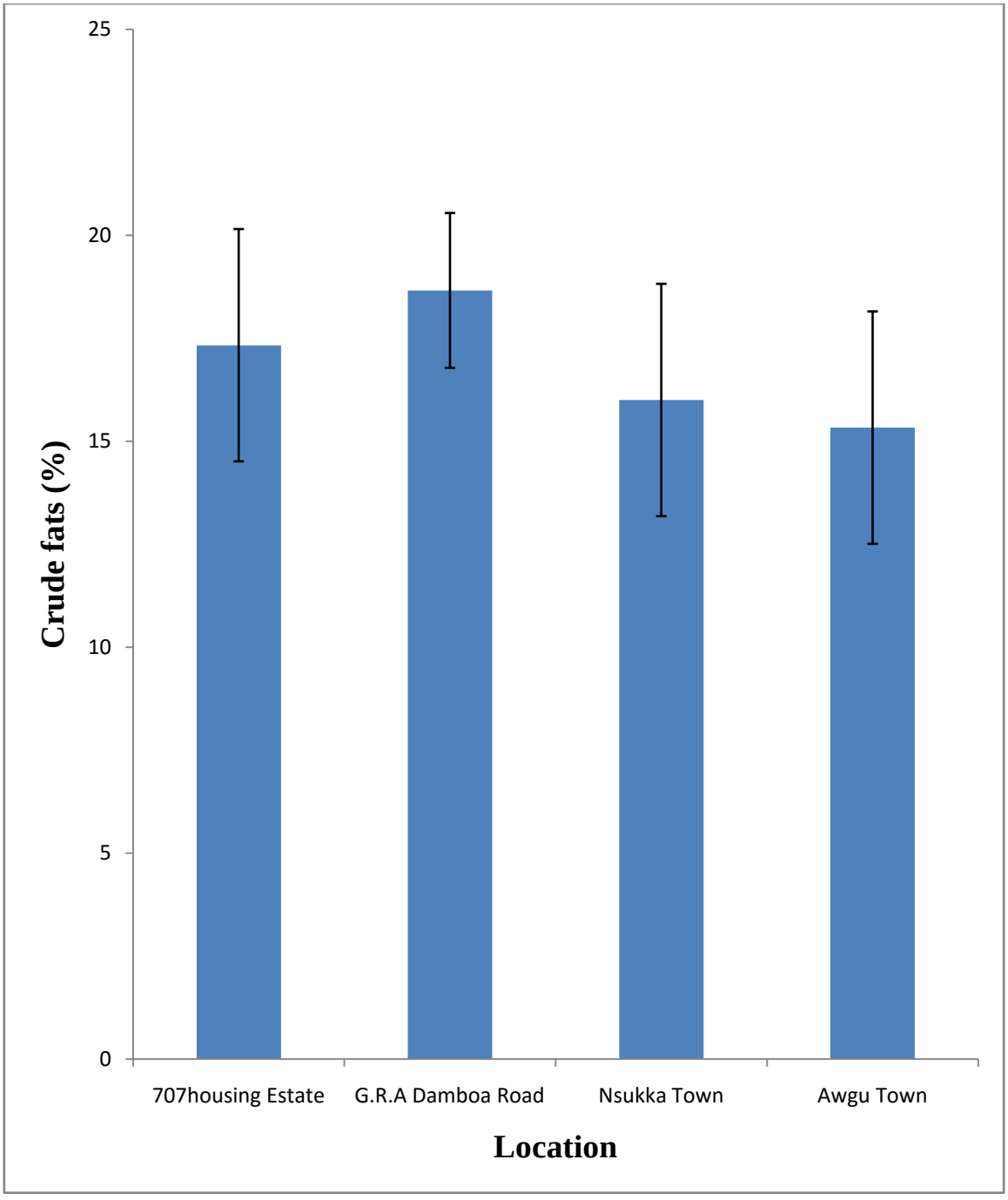


Figure 4.7: Percentage of crude fats in leaves of *Ocimum basilicum* obtained from 707Housing Estate and G.R.A Damboa road and leaves of *Ocimum gratissimum* obtained from Nsukka town and Awgu town.

4.3 Spiking analysis

Spiking analysis for MESS for both the plant and soil sample were determined and the data was calculated as illustrated in Appendix I. The percentage recovery for metals in plant leaves for Ca, Cu, Co, Fe, K, Mn, and Zn were found to be 125.1, 70.5, 61.8, 59.9, 90.3, 75.5 and 80.02% respectively, while the percentage recovery for metals in soil for Ca, Cu, Co, Fe, K, Mn and Zn were found to be 118.9, 63.5, 60.1, 88.0, 84.4, 78.7 and 55.6% revealed in Table 4.2. Thus from the %recovery of all the metals analysed with MESS in leaves and soil, therefore the results obtained by the use of AAS machine for the determination of metals in plant and soil for quality assurance was efficient.

4.3.1 Concentration of elements in leaves of *Ocimum basilicum* and *Ocimum gratissimum*

The mean concentration of essential element of both plants leaves of *Ocimum basilicum* and *Ocimumgratissimum* found in 707housing estate Maiduguri, G.R.A Damboa Maiduguri, Nsukka town Enugu and Awgu town Enugu respectively was obtained and presented in Table 4.3 and 4.4 for the analysis of Ca, Cu, Co, Fe, Mn, K, Zn, P, S and N using Atomic absorption spectroscopy and other methods. The results for the metals analysed shows that Awgu town had the highest concentrations of Ca, Cu, K, and Zn of 33024.60 ± 477.19 mg/kg, 28.50 ± 0.31 mg/kg, 2350.00 ± 212.13 mg/kg, and 98.80 ± 1.10 mg/kg respectively in *O. gratissimum* leaves, Nsukka town had the highest concentration of Fe of 2940.00 ± 28.94 mg/kg in *O. gratissimum* leaves and G.R.A Damboa road had the highest concentration of Mn of 101.60 ± 0.41 mg/kg in *O. basilicum* leaves. Co showed low concentrations in both *O. gratissimum* and *O. basilicum* leaves. The concentrations of all the metals showed lower value than that of the WHO/FAO and NAFDAC except in Fe

as illustrated in Table 4.3. The results for the non-metals showed that Nsukka town had the highest concentration of Phosphorus of 1530.00 ± 72.14 mg/kg in *O. gratissimum* leaves, Awgu town had the highest concentration of suphur of 5091.33 ± 202.34 mg/kg in *O. gratissimum* leaves, and 707Housing Estate had the highest concentration of nitrogen of 34.05 ± 1.06 mg/kg in *O. basilicum* leaves as illustrated in Table 4.4.

Table 4.2: Percentage recovery of spiking analysis for plant leaves and soil

Metals	%recovery for plant leaves	%recovery for soil
Calcium R^2=0.999	125.1	118.9
Copper R^2=0.998	70.5	63.5
Cobalt R^2=1.0	61.8	60.1
Iron R^2=0.996	59.9	88.0
Manganese R^2=0.998	75.5	78.7
Potassium R^2=0.999	90.3	84.4
Zinc R^2=0.999	80.2	55.6

Table 4.3: Mean concentration ± STD of metals in *Ocimum basilicum* and *Ocimumgratissimum* plant leaves.

METAL	707Housing Estate Maiduguri *Ocimum basilicum*	G.R.A Damboa Road Maiduguri *Ocimum basilicum*	Nsukka town Enugu *Ocimum gratissimum*	Awgu town Enugu *Ocimum gratissimum*	FAO/ WHO STD In Vegt. Max. Permissible limit.	NAFDAC STD In Food Max. Permissible limit.
Ca (mg/kg) R^2=0.998	17089.90 ± 94.89	19160.00 ± 26.85	14096.00 ± 133.22	33024.60 ± 477.19	-	-
Cu (mg/kg) R^2=0.998	5.10 ± 0.09	12.30 ± 0.22	18.30 ± 1.84	28.50 ± 0.31	73.00	0-40.00
Co (mg/kg) R^2=1.000	ND	ND	ND	ND	50.00	0-3.50
Fe (mg/kg) R^2=0.996	526.00 ± 34.12	1797.30 ± 12.95	2940.00 ± 28.94	973.70 ± 12.52	425.00	10-40.70
K (mg/kg) R^2=0.999	1497.50 ± 85.20	1795.00 ± 43.13	1400.00 ± 113.13	2350.00 ± 212.13	-	-
Mn (mg/kg) R^2=0.998	54.00 ± 0.56	101.60 ± 0.41	84.80 ± 4.00	71.10 ± 0.60	500.00	-
Zn (mg/kg) R^2=0.999	37.30 ± 1.43	59.10 ± 1.52	88.20 ± 0.00	98.80 ± 1.10	100.00	0-50.00

ND = Not Detected

Table 4.4: Mean concentration ± STD of non-metals in *Ocimum basilicum* and*Ocimum gratissimum* plant leaves.

Non metals	707Housing Estate Maiduguri *Ocimum basilicum*	G.R.A Damboa Road Maiduguri *Ocimum basilicum*	Nsukka town Enugu *Ocimum gratissimum*	Awgu town Enugu *Ocimum gratissimum*
P (mg/kg) R^2=0.984	3580.66 ± 51.21	3219.02 ± 17.05	4123.24 ± 102.30	1530.49 ± 72.14
S (mg/kg) R^2=1.000	2351.00 ± 332.48	2057.15 ± 83.15	3467.72 ± 83.12	5091.33 ± 202.34
N(mg/kg)	34.05 ± 1.06	6.75 ± 0.35	29.10 ± 1.55	12.42 ± 0.24

4.3.2 Concentration of elements in soil samples

The mean concentrations of essential elements in soil sample found in 707Housing Estate Maiduguri, G.R.A Damboa Maiduguri, Nsukka town, and Awgu town for the analysis of Ca, Cu, Co, Fe, Mn, K, Zn, P, S and N determined using Atomic absorption spectroscopy and other methods were illustrated in Tables 4.5 and 4.6. The result for the metals in soil showed that 707Housing Estate had the highest concentrations of Ca and K of 7911.20 ± 24.91 mg/kg and 490.00 ± 29.69 mg/kg respectively in the soil, Nsukka town had the highest concentrations of Cu, Co, Fe, Mn and Zn of 18.30 ± 0.89 mg/kg, 2.40 ± 0.34 mg/kg, 3548.02 ± 481.33 mg/kg, 338.60 ± 1.16 mg/kg and 211.20 ± 12.20 mg/kg respectively in the soil showed in Table 4.5. The result for the non-metals in soil shows that Awgu town had the highest concentrations of sulphur and nitrogen of 1988.23 ± 75.87 mg/kg and 2.27±0.24 mg/kg respectively in the soil and Nsukka town had the highest concentration of phosphorus of 1012.72 ± 22.73 mg/kg in the soil showed in Table 4.6

Table 4.5: Mean concentration ± STD of metals in soil where *Ocimum basilicum*and*Ocimum gratissimum* were planted.

Metals	707Housing Estate Maiduguri Soil sample	G.R.A Damboa Road Maiduguri Soil sample	Nsukka town Enugu Soil sample	Awgu town Enugu Soil sample	FAO/WHO STD in soil Max. permissible limit
Ca(mg/kg) R^2=0.998	7911.20 ± 24.91	4568.60 ± 23.15	1503.50 ± 8.71	2779.00 ± 66.19	-
Cu (mg/kg) R^2=0.998	9.30 ± 0.94	7.00 ± 0.04	18.30 ± 0.89	34.00 ± 0.31	100.00
Co (mg/kg) R^2=1.0	ND	ND	2.40 ± 0.34	ND	50.00
Fe (mg/kg) R^2=0.996	2866.10 ± 197.58	5745.00 ± 64.47	35480.20 ± 481.33	22016.70 ± 108.71	5000.00
K (mg/kg) R^2=0.999	4900.00 ± 29.69	3407.50 ± 14.03	2370.00 ± 15.98	610.00 ± 18.38	-
Mn (mg/kg) R^2=0.998	100.00 ± 4.80	76.80 ± 2.09	338.60 ± 1.16	200.30 ± 1.21	2000.00
Zn (mg/kg) R^2=0.999	28.40 ± 0.84	69.10 ± 5.11	211.20 ± 1.22	37.70 ± 1.61	300.00

ND = Not Detected

Table 4.6: Mean concentration ± STD of non-metals in soil where *Ocimum basilicum* and *Ocimum gratissimum* were planted.

Non metals	707Housing Estate Maiduguri Soil sample	G.R.A Damboa Road Maiduguri Soil sample	Nsukka town Enugu Soil sample	Awgu town Enugu Soil sample
P (mg/kg) R^2=0.984	694.89 ± 5.18	643.00 ± 22.73	1012.72 ± 22.73	658.28 ± 33.24
S (mg/kg) R^2=1.000	301.71 ± 16.62	305.63 ± 11.08	1684.88 ± 55.41	1988.23 ± 75.87
N(mg/kg)	1.08 ± 0.04	1.04 ± 0.01	1.31 ± 0.12	2.27 ± 0.24

4.4 Antioxidant properties of extracts of n-hexane and methanol of *Ocimumbasilicum*and *Ocimum gratissimum* of plant leaves.

Antioxidant properties was determined using phenolic content assay and 2,2-diphenyl-1-picrylhyrazyl assay of both extracts of n-hexane and methanol of *O. basilicum* and *O. gratissimum* plant leaves were determined;

4.4.1 Total phenol content Assay

The Mean amount of total phenolic content for the extracts of n-hexane and methanol of both *Ocimum basilicum* and *Ocimum gratissimum* plant leaves at 50 µg/cm^{3}were determined as shown in Figure 4.8 and Table A4-1 in Appendix 4. The result shows that n-hexane and methanol extract of *O. basilicum* has high content of phenol of 102.00 ± 0.02 mgGAC/g and 99.00 ± 0.02 mgGAC/g respectively. Also *O. gratissimum* has phenol content of 11.75 ± 0.00 mgGAC/g and 10.25 ± 0.00 mgGAC/g in n-hexane and methanol extract respectively.

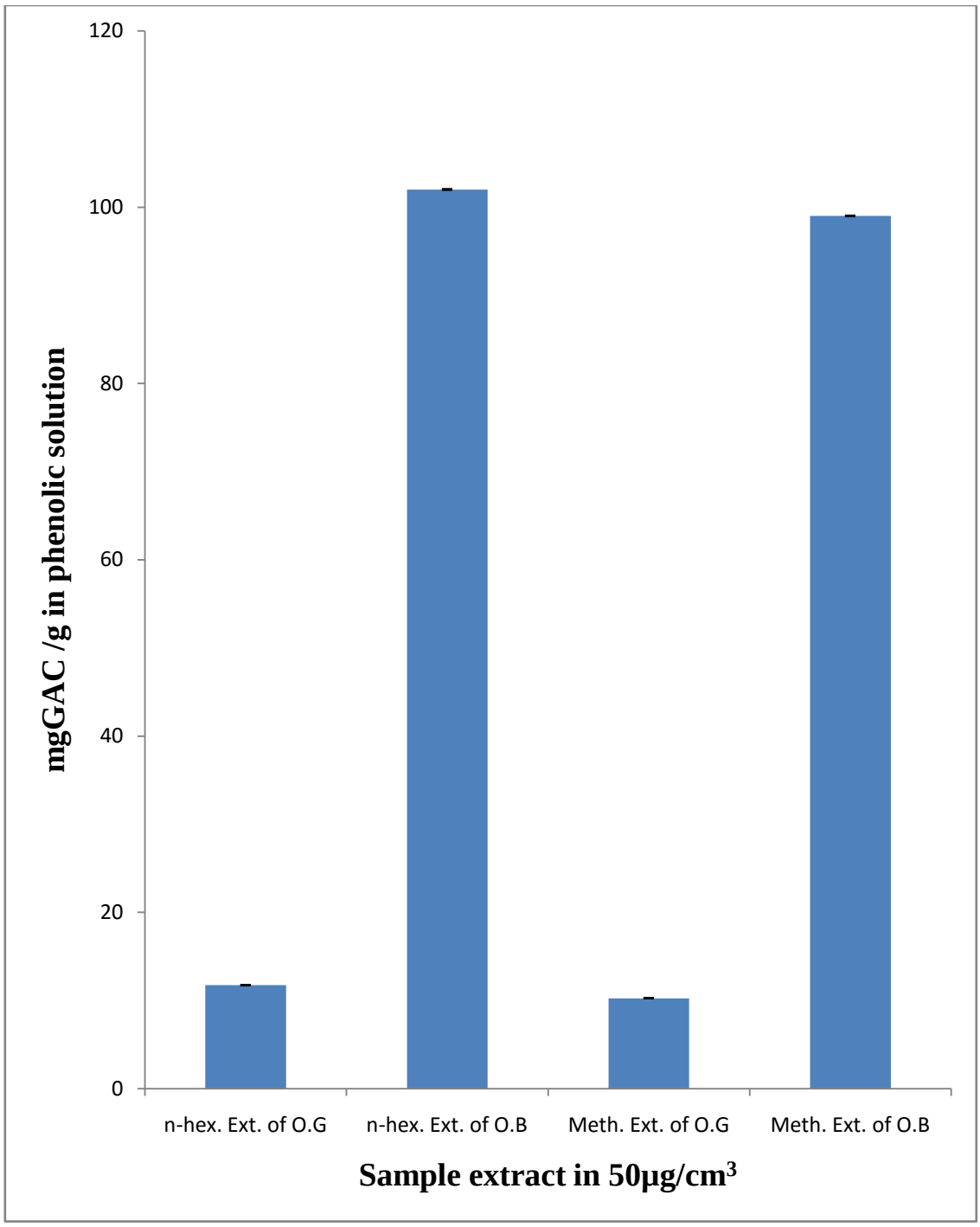


Figure 4.8: Mean concentration of phenolic content of n-hexane and methanol extracts in *O. basilicum* (O.B)and *O. gratissimum* (O.G) plant leaves.

4.4.2 2-diphenyl-1-picrylhydrazyl (DPPH) assay

The antioxidant properties of *O. basilicum* and *O. gratissimum* leaves extracts was examined using 2,2-diphenyl-1-picrylhyrazyl assay,based on the ability of extracts to reduce the free radical in DPPH solution using ascorbic acid as the reference standard. The ability of n-hexane extracts of *O. basilicum*, *O. gratissimum* and ascorbic acid at various concentrations (50, 100, 150, 200 and 250 $\mu g/cm^3$) was determined and the value are shown in Figure 4.9 and Table A4-2 in Appendix 4. The maximum reducing property of both the n-hexane extract of *O. basilicum*, *O. gratissimum*, and ascorbic acid was obtained at 250 $\mu g/cm^3$ concentration was found to be 94.38 ± 0.53, 95.63 ± 0.18 and 99.56 ± 0.09 %, respectively. The reducing property of methanol extracts of *O. basilicum*, *O. gratissimum* and ascorbic acid at various concentrations (50, 100, 150, 200 and 250 $\mu g/cm^3$) was found to be 98.59 ± 0.09, 98.13 ± 0.18 and 99.56 ± 0.09 percent respectively at 250 $\mu g/cm^3$ shown in Figure 4.10 and Table A4-2 in Appendix IV.

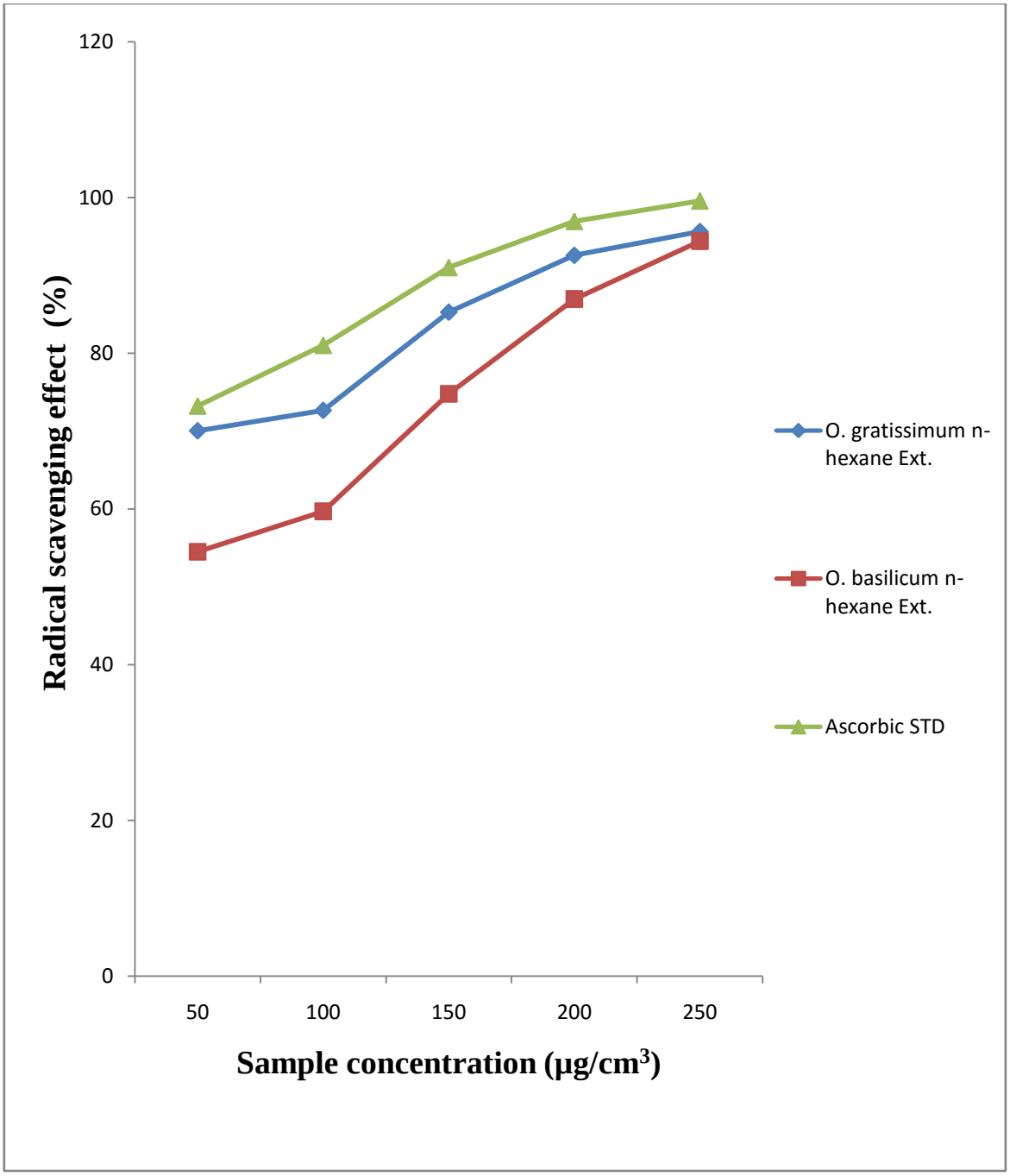


Figure 4.9: Mean percentage of radical scavenging effect of n-hexane extracts of*Ocimum basilicum* leaves, *Ocimum gratissimum* leavesand Ascorbic acid as reference standard in DPPH solution at various concentrations of extract.

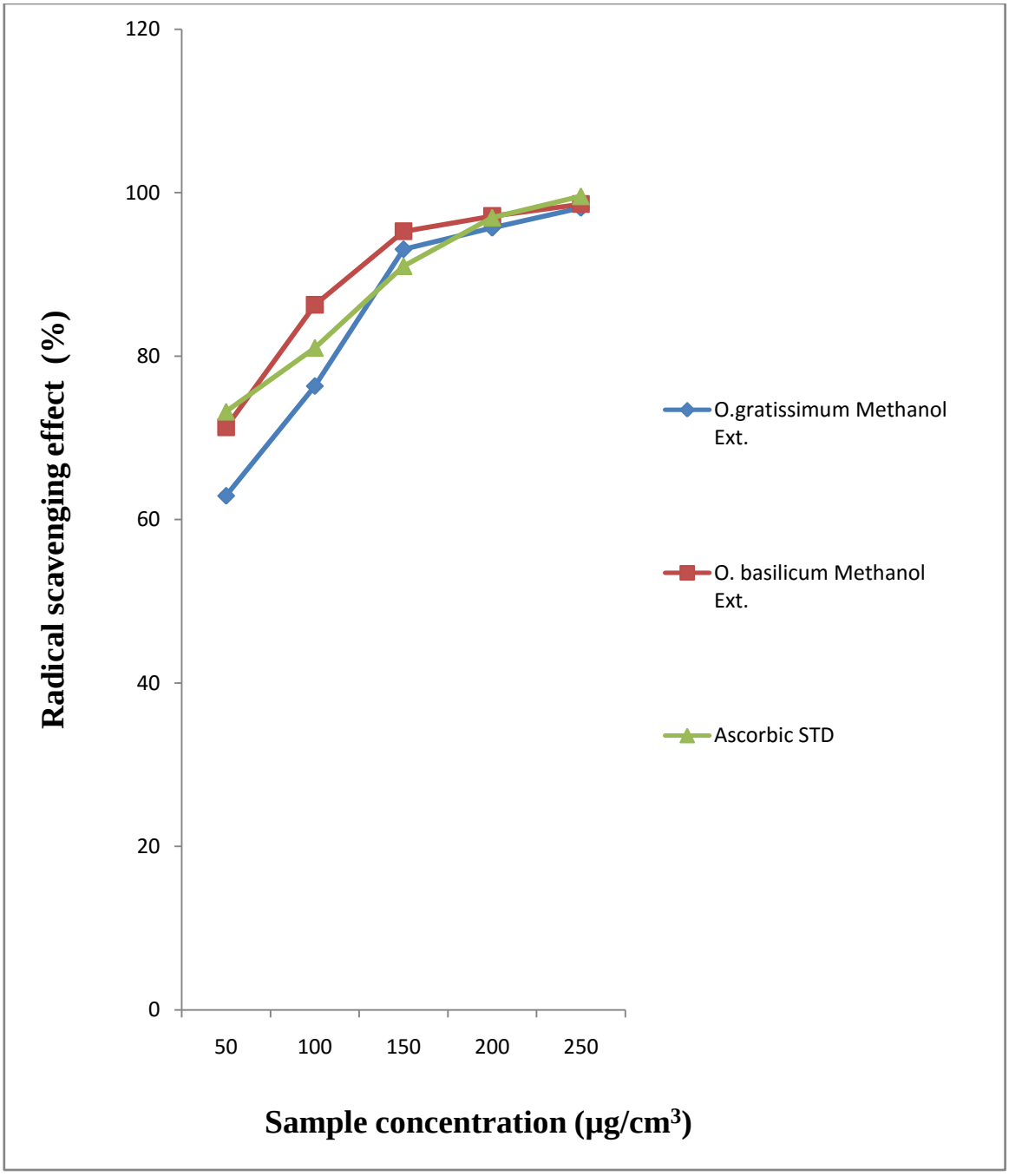


Figure 4.10: Mean percentage of radical scavenging effect of methanol extracts of*Ocimum basilicum* leaves, *Ocimum gratissimum* leavesand Ascorbic acid reference standard in DPPH solution at various concentrations of extract.

4.5 Statistical Analysis of data of Elemental analysis, proximate analysis and antioxidant properties of *Ocimum basilicum* and *Ocimum gratissimum* plant leaves with soil.

The statistical analysis of mean data of elemental composition, proximate contents and antioxidant properties of *Ocimum basilicum* and *Ocimum gratissimum* plant leaves and soil samples was done using special package for social science software (SPSS) version 16. A one way analysis of variance (ANOVA) was explored. The samples were divided into four (707Housing Estate for *O. basilicum* =1; G.R.A Damboa road for *O. basilicum* =2; Nsukka town for *O. gratissimum* =3 and Awgu town for *O. gratissimum* =4).

For mean concentration for elements of *Ocimum basilicum* and *Ocimum gratissimum* leaves in the four sampling sites, P=0.956 (p>0.05) as shown in Appendix 5 (Table A5-1).

For mean concentration of elements for soil in the four sampling sites,p=0.505 (p>0.05)as shown in Appendix 5 (Table A5-2).

Formean percentage proximate contents in*Ocimum basilicum* and *Ocimum gratissimum* leaves in the four sampling sites,p=1.00 (p>0.05)as shown in Appendix 5 (Table A5-3).

Also a one way ANOVA was exploredfor the impact of n-hexane and methanol leavesextracts of *Ocimum basilicum* and *Ocimum gratissimum* leaves on antioxidant properties using DPPHassay.

For the mean percentage inhibition of n-hexane and methanol leavesextracts of *Ocimum basilicum* and *Ocimum gratissimum* usingDPPH assay,p=0.409 (p>0.05)as shown in Appendix 5 (Table A5-4).

CHAPTER FIVE

5.0DISCUSSION

5.1 Elemental analysis for plant leaves and soil

The contamination of vegetables with minerals due to soil and atmospheric contamination poses a threat to its quality and safety. Dietary intake of elements also poses risk to animals and human health (Saeid*et al.*, 2012).

Calcium was obtained in all leaves samples, and Awgu town had the highestconcentration (33024.60mg/kg) in*O. gratissimum* leave followed by G.R.A Damboa road and 707 Housing Estate (19160.00 and 17089.90 mg/kg) in*O. basilicum*leaves respectively. Nsukka had the lowest concentration (14096.00mg/kg) of Ca in *O. gratissimum*leaves in Table 4.3.High concentration of Ca in *O. basilicum leaves* had been reported by Daniel *et al.*(2011). For *O. gratissimum* leaves,however, concentrations reported by Idris *et al.* (2011) and Asaolu *et al.*(2012) were very low compared to what we obtained.Calcium wasobtained in all the soil samples and 707Housing Estate had the highestconcentration(7911.20 mg/kg) followed byG.R.A Damboa Road and Awgu town(4568.60 and 2779.00mg/kg) respectively. Nsukka town had the lowest concentration (1503.50 mg/kg) as shown in Table 4.5. Both the *Ocimum* species had hidh content of Ca in the leaves was due to the high content of Ca in the soil.

Copper was obtained in leaves samples of both *O. basilicum* and *O. gratissimum* with Awgu town having the highest concentration (28.50 mg/kg) of Cu in *O.gratissimum* leaves followed by Nsukka town (18.30 mg/kg) in *O. gratissimum* leavesand G.R.A Damboa road (12.30 mg/kg)in *O.basilicum*leaves. 707Housing Estate had the

lowestconcentration(5.10mg/kg) of Cu in *O.basilicum* leaves. The WHO/FAO and NAFDAC standard showed that the leaves samples of both species of *Ocimum* were belowthe maximum concentration in vegetable shown in Table 4.3.Low concentration of Cu in *O. basilicum* leaves was reported by Agunbiade *et al.* (2015) and in*O. gratissimum* leaves as reported by Idris *et al.* (2011) and Asaolu *et al.* (2012). The results agreed withthe results obtained in the current study. In soil, Cu was found in all the samples of the soilswith Nsukka townhaving the highest concentration(18.30 mg/kg) followed by 707Housing Estate (9.30 mg/kg) andG.R.A Damboa road(7.00mg/kg)in soil. Awgu town had the lowest concentration (3.40 mg/kg) in soil samples. The WHO/FAO standard show that all the soil sampleshad concentrations of Cu that were below the maximum concentration in soilas shown in Table 4.5.

Cobalt was not detected in any of the leaves samples. In soil samples,however,only Nsukka samples confirmed Co at a concentration of 2.40 mg/kg. The WHO/FAO standard shows that the soil was not contaminated withCo as illustrated in Table 4.5.

Iron content was obtained in the leaves sample of *O. basilicum* and *O. gratissimum* of which Nsukka town had the highest concentration of 2940.00 mg/kg in *O. gratissimum* leavesfollowed byG.R.A Damboa road (1797.30 mg/kg) in *O. basilicum* leaves and Awgu town(973.70 mg/kg) in *O. gratissimum* leaves. 707Housing Estate had the lowest concentration of Fe(526.00 mg/kg) in *O. basilicum* leaves. The WHO/FAO standard of Fe show that theleaves samples of both*O. basilicum* and *O. gratissimum* confirmed Fe above the limit permitted in vegetables and also for NAFDAC standard in food as shown in Table 4.3.Low concentration of Fe in *O. basilicum* leaves was reported by Agunbiade *et*

al.(2015); also low concentration of Fe in *O. gratissimum* leaves was reported by Idris *et al.*(2011) and Asaolu *et al.*(2012). These results disagreed with our results. In soil samples, Nsukka town had the highest concentration of Fe(35480.20 mg/kg) followed by Awgu town (22016.70 mg/kg)and G.R.A Damboa road (5745.00 mg/kg). 707Housing Estate had the lowest concentration of Fe(2866.10 mg/kg). The WHO/FAO standard of Fe in soil show that the concentrations in all the soil samples were above the permissible limit in soil at Awgu town, Nsukka town and G.R.A Damboa road. Therefore, the soil samples were contaminatedat these sites. Only 707Housing Estate soil sample was within the permissible limit as shown in Table 4.5. Both *Ocimum* species had high content of Fe in pants leaves this was due to the high content of Fe in soil.

Potassium was obtainedin leaves samples of both*O. gratissimum* and *O. basilicum* with Awgu town having the highest concentration (2350.00 mg/kg) in *O. gratissimum* leaves followed byG.R.A Damboa road (1795.00mg/kg) in *O. basilicum* leaves and 707Housing Estate (1497.50mg/kg) in *O. basilicum* leaves. Nsukka town had the lowest concentration of (1400.00 mg/kg) in *O. gratissimum* leaves. The WHO/FAO and NAFDAC standard of K were not available for vegetablesas shown in Table 4.3.High concentration of K in *O. basilicum* leaves was reported by Daniel *et al.*(2011) and high concentration of *O. gratissimum* leaves was reported by Idris *et al.*(2011).These agreed with our result. In soil samples 707Housing Estate had the highest concentration (4900.00 mg/kg) followed byG.R.A Damboa Road (3407.50 mg/kg) and Nsukka (2370.00 mg/kg). Awgu town had the lowest concentration(610.00 mg/kg). The WHO/FAO standard for K in soil was not availableas shown in Table 4.5.Both *Ocimum* species had high content of K in pants leaves this was due to the high content of K in soil.

Manganese was obtained in the leaves samples of both *O. basilicum* and *O. gratissimum* withG.R.A Damboa road havingthe highest concentration(101.60 mg/kg) in *O. basilicum* leaves followed by Nsukka town(84.80 mg/kg) in *O. gratissimum* leaves and Awgu town (71.10 mg/kg) in *O. gratissimum* leaves. 707Housing Estate had the lowest concentration(54.00 mg/kg) of Mn in *O. basilicum* leaves. The WHO/FAO and NAFDAC standard of Mn show that the entire leaves sample werenot above the permissible limits as shown in Table 4.3.Low concentration of *O. gratissimum* was reported by Idris *et al.*(2011) and Asaolu *et al.* (2012). In soil samples Nsukka town had the highest concentration (338.60 mg/kg) in soil followed by Awgu town (200.30 mg/kg) and 707Housing Estate (100.00 mg/kg). G.R.A Damboa road had the lowest concentration of Mn (76.80 mg/kg). The WHO/FAO standard of Mn in soil show that all soil samples were lower than the permissible limit as shown in Table 4.5.

Zinc was obtained in all the leavessamples of *O. basilicum* and *O. gratissimum* with Awgu town havingthe highest concentration(98.80 mg/kg)in *O. gratissimum*followed by Nsukka town (88.20 mg/kg) in *O. gratissimum* and G.R.A Damboa road(59.10 mg/kg) in *O. basilicum*. 707Housing Estate had the lowest concentration of Zn(37.30 mg/kg) in *O. basilicum* leaves. The WHO/FAO and of Zn show that the entire samples were lower than the permissible limit in vegetable. With the exception of 707Housing Estate all leaves samples had Zn content above NAFDAC permissible limit in food as illustrated in Table 4.3.High concentration of Zn in *O. basilicum* leaves was reported by Agunbiade *et al.*(2015);also high concentration in *O. gratissimum* leaves was reported by Idris *et al.*(2011) and Asaolu *et al.*(2012) which might be due to contamination of the soil with Zn. In soil samples Nsukka town had the highest concentration of Zn(211.20 mg/kg) followed

byG.R.A Damboa road(69.10 mg/kg) and Awgu town (37.70 mg/kg) in soil. 707Housing Estate had the lowest concentration of Zn (28.40 mg/kg) in soil. The WHO/FAO standard show that the entire soil samples were below the permissible limit in soilas shown in Table 4.5.

Phosphorus was obtained in the leaves of *O.basilicum* and *O. gratissimum* with Nsukka town having the highest concentration (4123.23mg/kg) in *O. gratissimum* leaves followed by 707Housing Estate (3580.66 mg/kg) in *O. basilicum* leaves and G.R.A Damboa road (3219.02 mg/kg) in *O. basilicum* leaves. Awgu town had the lowest concentration (1530.49 mg/kg) in *O. gratissimum* leaves shown in Table 4.4. Low concentration of P in*O. gratissimum* was reported by Idris *et al.* (2011) and Asaolu *et al.* (2012); high concentration of P in *O. basilicum* leaves was reported by Agunbiade *et al.* (2015). In soil samples Nsukka had the highest concentration of P(1012.72 mg/kg) followed by707Housing Estate (694.89 mg/kg) and G.R.A Damboa road (643.00 mg/kg) in *O. basilicum* leaves. Awgu town had the lowest concentration of P(658.28 mg/kg) as shown in Table 4.6. The high concentration of phosphorus in leaves at Awgu town was due to the high concentration of phosphorus in the soil.

Sulphur was obtained in the leaves samples of *O. basilicum* and *O. gratissimum* with Awgu town having the highest concentration (5091.33 mg/kg) in *O. gratissimum* leaves followed by Nsukka town (3467.72 mg/kg) in *O. gratissimum* leaves and 707Housing Estate (2351.00 mg/kg) in *O. basilicum*leaves. G.R.A Damboa road had the lowest concentration of S (2057.15 mg/kg) in *O. basilicum* leavesas shown in Table 4.4. In soil samples Awgu town had the highestconcentration (1988.23 mg/kg) followed by Nsukka

town(1684.88 mg/kg) and G.R.A Damboa road(305.63 mg/kg). 707Housing Estate had the lowest concentration of S (301.71 mg/kg) as shown in Table 4.6. The high concentration of sulphur in the leaves at Awgu town might be due to the high concentration of sulphur in the soil.

Nitrogen was obtained in all the leaves samples of *O.basilicum* and *O. gratissimum* with 707Housing Estate having the highest concentration of 34.05 mg/ kg in *O. basilicum* leaves followed by Nsukka town of 29.10 mg/kg in*O. gratissimum* leaves and Awgu town(12.42mg/kg) in *O. gratissimum* leaves. G.R.A Damboa road had the lowest concentration of N (6.75 mg/kg) in *O. basilicum* leavesas shown in Table 4.4. In soil samples Awgu town had the highestconcentration(2.27 mg/kg) followed by Nsukka town(1.31 mg/kg) and 707Housing Estate (1.08 mg/kg). G.R.A Damboa road had the lowest concentration (1.04 mg/kg) as shown in Table 4.6.

5.2Proximate composition

Scent leaves contains nutritional constituents such as fats, protein, carbohydrates etc. (Nwankwo *etal.,* 2014). Vegetables constitute an important part of the human diet since they contain carbohydrates, proteins, vitamins, minerals as well as trace elements (Saeid, 2012).

For carbohydrate contents in leaves samples 707Housing Estate had the highest percentage(31.58%) in *O. basilicum* leaves follow by Awgu town(30.25%) in *O. gratissimum* leaves and Nsukka town(25%) in *O. gratissimum* leaves. G.R.A Damboa road had the lowest percentage of carbohydrate content (23.28%) in *O. basilicum* leaves as shown in Figure 4.1. High percentage content of carbohydrate was obtain in *O. basilicum*

leaves and *O. gratissimum* leaves as reported by Agunbaide *et al.,* (2015) and Nwankwo *et al.,* (2014), Asaolu *et al.,* (2012) and Idris *et al.,* (2011).

For Moisture contents of leaves samples G.R.A Damboa road had the highest percentage (26.66%) in *O. basilicum* leaves followed by Awgu town (25.33%) in *O. gratissimum* leaves and Nsukka town (22.00%) in *O. gratissimum* leaves. 707 Housing Estate hadthe lowest percentage(17.99%) in *O. basilicum* leavesas shown in Figure 4.2.

For ash content ofleaves samples, 707Housing Estate had the highest percentage(15.33%) in *O. basilicum* leaves followed byNsukka town (13.00%) in *O. gratissimum*leaves and G.R.A Damboa road (12.66%) in *O. basilicum* leaves. Awgu town had the lowest percentage of ash content (9.50%) in *O. gratissimum* leavesas shown in Figure 4.3. Low percentage content of ash was reported by Agunbaide *et al.,* (2015) and Idris *et al.,* (2011) in *O. basilicum* leaves and *O. gratissimum* leaves which were closer to the result obtained in our work.

For crude fibre content in leaves samples,G.R.A Damboa road had the highest percentage (32.00%) in *O.basilicum* leaves followed byNsukka town (31.99%) in *O. gratissimum* leaves and Awgu town (30.66%) in *O. gratissimum* leaves. 707Housing Estate had the lowest percentage of crude fibre (29.33%) in *O. basilicum* leavesas shown in Figure 4.4. Low percentage content of crude fibre was reported by Agunbaide *et al.,* (2015) and Nwankwo *et al.,* (2014) in *O. basilicum* leaves and *O. gratissimum* leaves which were closer with the result obtained.

The leaves samples of *O. basilicum* found at 707Housing Estate (6.90) and G.R.A Damboa road (6.93) were slightly acidic, while pH values of *O. gratissimum* leaves found at Nsukka town (7.48) and Awgu town (7.54) were slightly basic as shown in Figure 4.5.

For crude protein content of leaves samples, Nsukka town had the highest percentage (7.61%) in *O. gratissimum* leaves followed by 707Housing Estate (5.76%) in *O. basilicum* leaves and G.R.A Damboa road (5.39%). Awgu town had the lowest percentage of proteins (4.08%) in *O. basilicum* leaves as shown in Figure 4.6. High percentage content of protein was obtain in *O. basilicum* leaves and *O. gratissimum* leaves as reported by Agunbaide *et al.,* (2015) and Nwankwo *et al.,* (2014), Asaolu *et al.,* (2012) and Idris *et al.,* (2011) which were higher than the result obtained in our work.

For crude fats content in leaves samples, G.R.A Damboa road had the highest percentage (18.66%)in *O. basilicum* leaves followed by707Housing Estate (17.33%) in *O basilicum* leaves and Nsukka town (16.00%) in *O. gratissimum* leaves. Awgu town had the lowest percentage of crude fats (15.33%) in *O. gratissimum* lavesas shown in Figure 4.7. Low percentage content of crude fats was reported by Agunbaide *et al.,* (2015) and Nwankwo *et al.,* (2014) in *O. basilicum* leaves and *O. gratissimum* leaves which were closer with the result obtained.

5.3Phytochemical Compositions

Phytochemical compositions are mostly secondary metabolites which are produced naturally by plants (Demain and Fang, 2000). Phytochemicals are linked with the health benefits of foods (Manjula *et al*., 2009). From the resultswe obtained, it was found that the methanol extracts of both *O. basilicum* and *O. gratissimum* showed the presence Carbohydrate, tannins, cardiac glycosides,terpenoids and flavonoids but monosaccharide carbohydrate was absent. For the n-hexane extracts of both *O. basilicum* and *O. gratissimum* leaves carbohydrates, cardiac glycosides, terpenoids and Flavonoids were presentwhile tannins were absent; also monosaccharides were absent as shown in Table

4.1. The*O. basilicum* and*O. basilicum*leaves can serve asgood medicinal alternatives with respect to the phytochemical compositions in the sample extracts.

5.4Antioxidant properties

Antioxidant substance scavenges against free radicals within the body. It also protects the body from different diseases either by inhibiting the reactive oxygen species or stimulating the antioxidant defense mechanisms (Umamaheswari and Chatterjee, 2008). Antioxidants are also important in autoimmune disorders such as rheumatoid arthritis etc. (Beckman and Ames, 1998).

Researchers had studied the presence of flavonoid and related polyphenol constituents in medicinal plants and their reducing activities on phosphomolybdate (Sharififar *et al.,* 2009; Khan *et al.,* 2012; Saeed *et al.,* 2012). Phenolic contents are secondary metabolites in plants which possess ability to inhibit free radicals and are used in therapeutics for anti-carcinogenic, anti-mutagenic and antioxidant activities (Yen *et al.,* 1993). The ability of phenol to inhibit free radicals is due to the presence of hydroxyl group, and also the presence of flavonoid content in plant contributes directly to the antioxidant activity in inhibiting the free radical formation (Umamaheswari and Chatterjee, 2008). Results obtained in the present work showed that *O. basilicum* leaves had higher phenolic content, in both n-hexane and methanol extracts (102 mg GAE/g and 99 mg GAE/g) than the values recorded for n-hexane and methanol extracts of *O. gratissimum* (11.75 mg GAE/g and of 10.25 mg GAE/g) respectively as shown in Figure 4.8.

From the results obtained, it was found that both n-hexane and methanol extracts of *O. basilicum, O. gratissimum* showed maximum percentage DPPH radical scavenging effect at 250 µg/cm^3. The n-hexane extracts of*O. gratissimum* leaves had maximum DPPH radical scavenging effect of 95.63 % and*O. basilicum* leaves of94.38% as shown in Figure 4.9. In the methanol extract,*O. basilicum* leaves showed a maximum DPPH radical scavenging effect of 98.56% and*O. gratissimum* of 98.13% as shown in Figure 4.10. Ascorbic acid was used as areference standard and it recorded maximum DPPH radical scavenging effect of 99.56%. These results indicate that the extracts of the two *Ocimum* species have good DPPH radical scavenging effect comparable to that of ascorbic acid.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

The determination of elemental composition and nutritional contents of *O. basilicum* and *O. gratissimum* leaves was performed using atomic absorption spectrometry after digestion for elemental composition (Ca, Cu, Co, Fe, Mn, K, Zn, P, S, and N) and proximate analysis (carbohydrate, moisture content, crude fiber, ash content pH value and protein contents) for nutritional contents. N-hexane and methanol extracts were used for phytochemical composition (carbohydrate, cardiac glycoside, tannins, terpenoid and flavonoid composition) and for antioxidant properties (DPPHand total phenol content assay). Elemental Composition of soil samples from study area(s) was also determined.

The elemental analysis ofleavessamples revealed that Ca, Cu, K, Zn, S were highest in leaves found at Awgu town, while Fe and P were highest in leaves found at Nsukka town. Mn was highest in leaves found at G. R. A Damboa Road and N had highest concentration in leaves found at 707Housing Estate. Cobalt was not in any of the leaves samples.The WHO/FAO standard in vegetables showedthat the elementsfound in both leaves were within the stipulated limits with the exceptionof Fe. The soils where both *O. basilicum* and *O. gratissimum*planted were grown were also analyzed and results showed that Ca and K had the highest concentration in 707Housing Estate soil, Co, Cu, Fe, Mn, Zn, and P were highest in Nsukka town sample and S and N were highest in samples from Awgu town. The WHO/FAO standard in soil which was compared with the elements in the soil from the locations where both *O. basilicum* and *O. gratissimum* were planted showed that the

soils were not contaminated with the exception of samples from Nsukka town, Awgu town and G.R.A Damboa road which were contaminated with Fe. Proximate analysis indicated that carbohydrate content, ash content were highest in*O. basilicum* leaves found at 707Housing Estate; moisture content, crude fiber and crude fat were highest in*O. basilicum* leaves found at G.R.A Damboa road and protein content was highest in*O. gratissimum* leaves found in Nsukka town. The pH value of *O. basilicum* leaves was slightly acidic while that of *O. gratissimum* leaves was slightly basic.

The phytochemical composition was determined using n-hexane and methanol solvent to extract the leaves of *O. basilicum* and *O. gratissimum*, and the extracts were subjected to screeningfor carbohydrate, cardiac glycoside, tannins, terpenoid and flavonoid compositions. The n-hexane extracts of both leavesshowed the presence of all the analytes except tannins while the methanol extracts of both leaves revealed the presence of all the constituents.

The analysis of antioxidant properties was done using n-hexane and methanol extracts of *O. basilicum* and *O. gratissimum* leaves in which the n-hexane extract of*O. basilicum* leaves showed higher effect in total phenol content assay. The methanol extract of *O. basilicum* leaves showed the highest reducing effectin DPPH assay. From the analysis of these results, both leaves of *O. basilicum* and *O. gratissimum* showed better antioxidant potency inn-hexane and methanol extracts. However, both leaves of *O. basilicum* and *O. gratissimum* had lower antioxidant activity than the ascorbic acid used as referencestandard as shown by the DPPH and total phenolic content assay results.

6.2 Conclusion

The determination of phytochemical compositions, nutritional contents and antioxidant properties of leaves of *O. basilicum* and *O. gratissimum* showed that

i. *O. gratissimum* had higher concentrations of Ca, Cu, K, Zn, Fe, S and P while *O.basilicum* had higher concentration of Mn and N in the essential element concentration. Fe concentration in both leaves was higher thanWHO/FAO standard in vegetable and NAFDAC standard for food.
ii. *O. basilicum* had higher contents of carbohydrate, moisture,ash, fibre and fat content and was slightly acidic while *O. gratissimum* had higher protein content and was slightly basic. Both leaves showed good nutritionalcontent with respect to the elemental composition,and proximate contents.
iii. The phytochemical screening showed the presence of the entire screened phytochemicals in methanol extract of both leaves while in n-hexane extract, tannins were absent.
iv. N-hexane extracts had higher activity in total phenol content assay of antioxidant potency while the methanol extracts showed higher activity in DPPH. These results show the potential effectiveness of both leaves in health and medicinal applications.
v. Statistically both leaves showed no differences in all the content checked in respect of the antioxidant properties and nutritional contents.

6.3 Recommendation

From the analysis carried out, both leaves of *O. basilicum* and *O. gratissimum* have good nutritional and medicinal values. Therefore;

i. Applying scent leaves in daily meal will help in building and boosting the body system; also the consumption of both leaves have to be regulated due to the high content of iron.
ii. Since the n-hexane extract had the highest inhibition in both of the leaves of *O. basilicum* and *O. gratissimum*,it is good to check itsanticorrosion ability with metals; the ability of both leaves to detoxify heavy metals at different concentrations and also determine other composition responsible for inhibition of free radicals in the extracts of n-hexane in both leaves.

REFERENCES

Abbaspour, N., Hurrell, R., and Kelishadi, R. (2014). Review on iron and its importance for human health. *Journal of Research in Medical Sciences : The Official Journal of Isfahan University of Medical Sciences*. ***19***(2): 164–174.

Ackerman, Cheri M. and Chang, Christopher J. (2017).Copper signaling in the brain and beyond.Retrieved October 30, 2017, From http://www.jbc.org

Afolabi, O. B., Oloyede, O. I., Olayide, I. I. and Obafemi, T. O. (2015). Antioxidant enhancing ability of different solvents extractable components of *Talinum triangulare* in some selected tissue homogenates of albino rats -In vitro. *Journal of Applied Pharmaceutical Science*. **5**(9): 56-61.

Agunbiade, S.O., Ojezele, M.O. and Alao, O.O. (2015).Evaluation of the nutritional, phytochemical compositions and likely medicinal benefits of *Vernomia amygdalina, Talinum triangulare* and *Ocimum basilicum*leavesy-vegetables.*Advances in Biological Research*. **9**(3): 151-155.

Ahmed, Ch, Mueen Naz Batool Syeda, Sharif Asifa, Akram Mai moona and Saeed Muhammad Asad (2015). Biological and pharmacological properties of sweat basil (*Ocimum basilicum*). *British Journal of Pharmaceutical research.***7**(5): 330-339.

Ajiboye, A. T., Lasisi T. O., Adebayo, Z. F., Hassan, A. T. and Abdusalam, Z. A. (2021). Phytochemical and antioxidant potential of some indigenous vegetables cultivated in malete, Kwara State, Nigeria. *International Journal of Ayurveda*. **6**(3): 30-36.

Akpabio, U. D. and Ikpe, E. E. (2013).Proximate composition and nutrient analysis of *Aneilema aequinoctiale* leaves.*Asian Journal of Plant Science and Research.***3**(2):55-61.

Anonymous. (2013). Nutrition and health the importance of potassium. Retrieved from International Potash Institute (IPI), 2013. From http://www.ipipotash.org/udocs/410human-health-ipi.pdf

Anonymous. (2016). Cobalt-related exposures, cobalt and cobalt compound that release cobalt ions in vivo. Retrieved from National Toxicology Program, Department of Health and Human Services Report on Carcinogens. 14thEdition.from http://ntp.niehs.nih.gov/ntp/roc/content/profiles/cobalt.pdf

AOAC. (1970).Official methods of analysis.11th Edition.Association of Official Analytical Chemists, Washington DC, USA.

AOAC. (1990).Official methods of analysis.15th Edition.Association of Official Analytical

Chemists, Washington DC, USA.

Asaolu, S. S., Adefemi, O. S., Oyakilome, I. G., Ajibulu, K. E., and Asaolu, M. F. (2012).Proximate and mineral composition of Nigerian leavesy vegetables.*Journal of Food Research*. **1**(3): 214–218.

Avila, Daiana Silva, Puntel Robson Luiz and Aschner Michael (2013).Manganese in health and disease. *Metal Ions in Life Sciences*. **13**:199-227.

Bailey, LH. (1924). Manual of Cultivated plants. Macmillan Co. New York. p. 101-3.

Beckman, K. B. and Ames, B. N. (1998). The free radical theory of aging matures. *Physiological Reviews*.**78**: 547-581.

Bolanle, A. O., Olumuyiwa, S. F., Onome, U., Bridget, O. O., Adewale, O. and Steve, R. A. A. (2004).The effect of seasoning salts and local condiments on mineral availability from two Nigeria vegetables.*Pakistan Journal of Nutrition.* **3**(3): 146 – 153.

Bolanle, Ajayi Olubunmi, Akomolafe Seun Funmilola and Adefioye Adedayo (2014). Proximate analysis, mineral contents, amino Acid composition, anti-nutrients and phytochemical screening of *Brachystegia Eurycoma* harms and *Pipper Guineense* schum and thonn.*American Journal of Food and Nutrition.* **2**(1):11-17.

Bosta, Muriel, Houdartb Sabine, Oberlib Marion, Kalonjib Esther, Huneauc Jean-Francoisand Margaritisb Irène (2016). Review dietary copper and human health. *Journal of Trace Elements in Medicine and Biology*. **35**: 107–115.

Calvo, M. S. and Uribarri,J. (2013a). Contributions to total phosphorus intake: all sources considered.*Seminars in Dialysis*. **26**(1): 54–61.

Calvo, M. S. and Uribarri,J.(2013). Public health impact of dietary phosphorus excess on bone and cardiovascular health in the general population. *American Journal of Clinical Nutrition*. **98**(1): 6–15.

Chibuzor, S., Ike A., Ahamefula, I., and Francess, O. (2014). The nutritional and anti - nutritional values of two culinary herbs – uziza leaves (*Piper guineense*) and scent leaves (*Ocimum gratissimum*) popularly used in Nigeria.*International Journal of Scientific and Engineering Research.**5***(12): 1160 – 1163.

Christopher, Hobbs and Elson Haas. (2017). Sulfur: A nutrient for supporting cartilage. Retrieved on October 2017, dummies: A WileyBrand Site from http://www.dummies.com/health/nutrition/sulfur-a-nutrient-for-supporting-cartilage/

Clementson, Jan. (2014). Macronutrients vs micronutrients. Retrieved (Pdf) 20thjune 2014 from

http://borderharriers.co.uk/app/webroot/upload/File/Nutrition/Nutrition%204%20Jun%2014

Daniel, V.N., Daniang, I. E., and Nimyel, N.D. (2011). Phytochemical analysis and mineral elements composition of *Ocimum basilicum* obtained in Jos metropolis plateau state, Nigeria. *International Journal of Engineering and Technology*. **11**(6): 135-137.

Darrah, H.H. (1980). The cultivated basils. Buckeye Printing Company, Karachi, India. p 112- 120.

Demain, A. l. and Fang, A. (2000). Review article on the natural function of secondary metabolites. *AdvanceBiochemical Engineering and Biotechnology*.**69**:1-39.

Devika, R. and Koilpillai, Justin (2014).In vitro quantification study of flavonoids from *Tagetes erecta*. *Asian Journal of Biotechnology*. **6**:21-24.

Effrain, I. O., Salami, H. A. and T. S. (2000). The effect of aqueous leaves extract of *Ocimum gratissimum* on hematological and Biological parameters in Rabbits. *African Journal of Biomedical Research.* **1**: 175-179

Emmanuel, Mukwevho, Zané, Ferreira and Ademola, Ayeleso (2014).Potential role of sulfur-containing antioxidant systems in highly oxidative environments.*Molecules*. **19**: 19376-19389

Ezekwesili, C.N., Obiora, K. A. and Ugwu, O.P. (2004). Evaluation of anti-diarrhoeal property of crude aqueous extracts of *Ocimum gratissimum* in rats. *Biokemistri.* **16**(2): 122-131.

Falah, S. Al-Fartusie and Saja, N. Mohssan., (2017).Essential trace elements and their vital roles in human body.*Indian Journal of Advances in Chemical Science*. **5**(3): 127-136.

Gad, N., Aziz E. E., Bekbayeva, L. K., and Surif, M. (2013). Role of cobalt in sweet basil (*Ocimum basilicum L.)*plants B. endogenous hormones, chemical and nutritional contents.*American Eurasian Journal of Agricultural and Environmental Science*. **13**(1): 16–21.

Gal, Nancy, J. and Dahl, Wendy J. (2017).Facts about phosphorus.Food science and human nutrition department, UF/IFAS Extension university of Florida Revised May 2017, Retrieved from http://edis.ifas.ufl.edu.

Gideon, O. Okunlola, Mahboob, A. Jimoh, Olusanya, A. Olatunji, Abdulfatai, B. Rufai andAdepeju, O. Omidiran (2019). Proximate analysis, mineral composition, and antioxidant properties of bitter leaves and scent leaves. *International Journal of*

Vegetable Science. **25**(4):346-354.

Gul, shumaila and Mahpara, Safdar (2009).Proximate composition and mineral analysis of cinnamon.*Pakistan Journal of Nutrition.* **8**(9):1456-1460

Gulcin, Ilham. (2005). The antioxidant and medicinal scavenging activities of black pepper (*Piper nigrum*) seed. *International Journal of food Sciences and Nutrition***7**(56): 491-499.

Idris, S., Iyaka, Y.A., Ndamitso, M. M. and Paiko, Y.B. (2011). Nutritional composition of the leaves and stems of *Ocimum gratissimum*.*Journal of Emerging Trends in Engineering and Applied Sciences*. **2**(5): 801-805.

Jumare, Fatima M. (2018). Qualitative and quantitative phytochemical screening of Nigerian indigenous *Ocimum gratissimum linn* (Clove Scented Basil Leaves). *Journal of Science, Technology & Education*. **6**(4): 338-344.

Karp, Heini. (2013). Dietary phosphorus sources and their acute effects on mineral metabolism in healthy women.Department of food and environmental sciences, University of Helsinki, Finland.Retrieved on October 2016, from http://www.pubmed.com/nutrition/

Kasote, D. M., Katyare, S. S., Hegde, M. V.and Bae, H. (2015).Significance of antioxidant potential of plants and its relevance to therapeutic applications.*International Journal of Biological Sciences.* **11**(8): 982-991.

Katarzyna, Dzida. (2010). Nutrients contents in sweet basil (*Ocimum basilicum L.*) Herb depending on calcium carbonate dose and cultivar.*Acta Scientiarum Polonorum Hortorum Cultus*. ***9***(4): 143–151.

Khan, Barkat Ali, Akhtar Naveed, Rasul Akhtar, Khan Haroon, Murtaza Ghulam, Ali Atif, Ahmad Kamran Khan, Shahiq-uz-Zaman, Jameel Adnan, Waseem Khalid and Mahmood Tariq (2012).Human skin, aging and antioxidants. *Journal of Medicinal Plants Research*.**6**(1): 1-6.

Ladipo, M.K.,Doherty, V.F. and Kanife, U.C. (2011).Phytochemical screening and antibacterial investigation of extract of *Ocimum gratissimum* (Scent Leaves) On Selected enterobacteriaceae.*Patnsuk Journal.* **6**(2): 75-84.

Lo, A. W. and Wang, J. (2000). Trading Volume : Definitions, data analysis, and implications of portfolio Theory.*National Bureau of Economic Research.**13***(2): 257–300.

Manjula, C., Rajaguru,P. and Muthuselvam,M. (2009). Screening for antibiotic sensitivity of free and immobilized *Actinomycetes* isolated from India. *Advance in Biological Research.* **3**: 84-88.

Markham, R. Kenneth and Given, R. David.(1988). The major flavonoids of an Antarctic bryum. *Phytochemistry.* **27** (9): 2843-2845.

Markus, Heckenmüller, Daiju Narita and Gernot Klepper. (2014). Global availability of phosphorus and its implications for global food supply: An economic overview. Retrieved from Kiel Institute for the World Economy, Hindenburgufer, Germany, on September 2017, from www.ifw-kiel.de.

Mathews, Christopher K. and Van, Holde K. E. (2013). Chapter 20 metabolism of nitrogenous compounds I. Biochemistry. 4th ed. Toronto, Canada: Pearson, p822-48.

Matos, M. J., Santana L., Uriarte, E., Abreu O. A., Molina, E. and Yordi, E. G. (2015) Coumarins—an important class of phytochemicals. In: Rao V., Rao L. G. (5^{th}Ed) Phytochemicals—isolation, characterization and role in human health. p113–140.

Mullins, Briana. (2014). Nitrogen metabolism and excretion in the human body. Retrieved December 8th, 2014, Biochemistry I Term Project From https://www.academia.edu/9770370/Nitrogen_Metabolism_and_Excretion_in_the_Human_Body/

Niki, E. (2016). Oxidative stress and antioxidant: distress or eustress.*Archive Biochemistryand Biophysics.***1**(595):19-24

Njoku, Obioma, U., Elijah, P., Victor, C., and Nzubechukwu, C. (2011).Antioxidant properties of*Ocimum gratissimum* (scent leaves).*New York Science Journal*.**4**(5): 98–103.

Nwankwo, Chibuzo S., Ebenezer, Ike A., Ikpeama, Ahamefula I. and Asuzu, Francess O. (2014). The nutritional and anti – nutritional values of two culinary herbs – uziza leaves (*Piper guineense*) and scent leaves (O*cimum gratissimum*) popularly used in Nigeria. *International Journal of Scientific and Engineering Research*.**5**(12):1160-1163.

Nyamai, D. W., Arika, W., Ogola, P. E., Njagi, E. N. M. and Ngugi, M. P. (2016). Research and Reviews: *Journal of Pharmacognosy and Phytochemistry Medicinally Important Phytochemicals*.**4**(1): 35-49.

Oluwafemi, A. Ojo, Omotade Oloyede, Oladimeji Tugbobo, Olaide Olarewaju and Adebola, Ojo.(2014). Antioxidant and inhibitory effect of scent leaves (*Ocimum gratissimum*) on Fe^{2+} and Sodium nitropusside induced lipid peroxidation in rat brain in vitro.*Advances in Biological Research.***8**(1): 08-17.

Omale, James Olajide Joseph Eniola and Okafor, Polycarp Nnacheta.(2008).Comparative evaluation of antioxidant capacity and cytotoxicity of two Nigerian *Ocimum* Species.*International Journal of Chemical Sciences*.**6**(4):1742–1751.

Oriakhi, Kelly, Oikeh Ehigbai Igbuan, Ezeugwu Nkeiruka, Anoliefo Ogechukwu, Aguebor Omorede and Omoregie Ehimwenma Sheena (2014).Comparative antioxidant activities of extracts of *Vernonia amygdalina* and *Ocimumgratissimum* leaves.*Journal of Agricultural Science*.**6**(1): 13-20.

Owoso, O. and Ogunmoyela, O. A. (2001).Proximate constituents of food. In:Chemical analysis of foods- an outline. Concept Publishers Limited, Lagos Nigeria.

Paul, E. D., Adamu, A.U., Gimba, C.E. and Ndukwe, G. I. (2014).Proximate composition and elemental analysis in *Borreria Stachydea* and *Cassia AbsusLinn*.*Nigerian Journal of Scientific Research*.**13** (2): 79-82.

Prasad, R. (2014). Major sulphur compounds in plants and their role in human nutrition and health – An Overview.*Proceedings of the Indian National Science Academy*.**80**(5): 1045-1054.

Pu, Fan, Chenb, Ningand Xuea, Shenghui (2016).Calcium intake, calcium homeostasis and health.*Food Science and Human Wellness*.**5**: 8–16.

Renata, Szymańska, Beatrycze Nowicka, Michał Gabruk, Sława Glińska Sylwia, Michlewska Jolanta Dłużewska, Anna Sawicka Jerzy Krukand Roosa Laitinen.(2015). Physiological and antioxidant responses of two accession of *Arabidopsis thaliana* in different light and temperature.*Physiologia Plantarum.***154**(2): 194-209.

Roohani, N., Hurrell R., Kelishadi, R. and Schulin, R. (2013). Zinc and its importance for human health: An integrative review. *Journal of Research in Medical Sciences : The Official Journal of Isfahan University of Medical Sciences*. **18**(2): 144–157.

Saeed, Naima, Khan R. Muhammad and Maria Shabbir. (2012). Antioxidant activity, total phenolic and total flavonoid content of whole plant extracts of *Torilis leptophylla l.BMC Complementary and Alternative Medicine.* **12**(221):1-12.

Saeid, Nazemi. (2012). Concentration of heavy metal in edible vegetables widely consumed in shahroud, the North east of Iran.*J. Appl. Environ. Biol. Sci*.**2**(8): 386-391.

Sharififar, F., Dehghn-Nudeh, G. and Mirtajaldini, M. (2009). Major flavonoidswith antioxidant activity from *Teucrium polium L*. *Food Chem*. **112**:885-888.

Shovon, Bhattacharjee, Abida Sultana, Muhammad Hasnan Sazzad, Muhammed Ariful Islam, Ahtashom M. M. and Asaduzzaman (2013). Analysis of the proximate composition and energy values of two varieties of Onion (*Allium cepa L*.) bulbs of different origin: A comparative study. *International Journal of Nutrition and Food Sciences.***2**(5): 246-253.

Simon, J.E., Morales,M.R.,Phippen,W.B., Vieira,R.F. and Hao,Z. (1999).A source of aroma compounds and a popular culinary and ornamental herb.In J. Janick(Ed.), Perspectives on new crops and new uses. Alexandria. VA: ASHS Press, p 499-505.

Siver, L. G., Lee, I. S. and Kinghom, D. A., (1998). Special problem with the extraction of plant. In: Natural products isolation (Cannel, RJP Ed) Humana Press999. Review drive, Suite 208, Totawa New Jessy, USA 072512. p343-364.

Sodamade, A., Bolaji, O. S. and Adeboye, O. O. (2013).Proximate analysis, mineral contents and functional properties of *Moringa oleifera* leaves protein concentrate.*IOSR Journal of Applied Chemistry*.**4**(6): 47-51.

Staff, L.H. (1976). Bailey Hortorium. Hortus III. MacMillan, New York.

Szymanska, R., Pospisil, P. and Kruk, J. (2016).Plant-derived antioxidants in disease prevention. *Oxidative Medicine and Cellular Longevity*.**2016**: 1-2.

Talabi, Y. Justina and Makanjuola Akinremi Solomon (2017).Proximate, phytochemical and in vitro antimicrobial properties of dried leaves from *Ocimum gratissimum*.*Prev. Nutr. Food Sci.***22**(3):191-194.

Tasrina, R. C., Rowshon, A., Mustafizur, A. M. R., Rafiqul, I. and Ali, M. P. (2015).Heavy metals contamination in vegetables and its growing soil.*Journal of Environ Anal Chem.***2**(30): 1-6.

Teofilović, B., Grujić-Letić, N. and Gligorić, E. (2021). Experimental and computational evaluation of extraction procedure and scavenging capacity of sweet basil extracts (*Ocimum basilicum L.*). *Plant Foods Hum Nutr*. 76(2): 21-34.

Trease,G. E. and Evans, W. C. (2002).Pharmacognocy.15th Edition.Sanders publishers, London. Pp. 42-44, 221-229, 246-249, 304-306, 331-332, 339-393.

Uba, S., Uzairu, A., Harrison, S.F.G., Balarebe, L. M. and Okunola, J. O. (2013).Assessment of heavy metals bioavailability in dumpsites of Zaria metropolis, Nigeria.*Africa Journal of Biotechnology*.**7**(2): 122-130.

Udo, E.J. and Ogunwele, D.A. (1986).Laboratory manual for analysis in soil plants and water Analysis 3rd Edition.Ilorin, University of Ilorin, Kwara State Nigeria.p 131-152.

Umamaheswari, M. and Chatterjee, T. K. (2008).Invitro antioxidant activities of the fractions of *Coccinia grandis l.* leaves extract.*African Journal TraditionalComplementary and Alternative Medicines*.**5**(1): 61-73.

Vishnoi, N. R. (1979). Advanced practical chemistry. Ghaziabad- India: Yikas Publication House, PVT Ltd. p 447-449.

Wadood, A., Ghufran, M., Jamal, S. B., Naeem, M. and Khan, A. (2013).Phytochemical analysis of medicinal plants occurring in local area of Mardan.*Biochemistry and Analytical Biochemistry*.**2**(4):1000144-1000148.

Xiao, Q., Murphy, R. A., Houston, D. K., Harris, T. B., Chow, W. H. and Park, Y. (2013). Dietary and supplemental calcium intake and cardiovascular disease Mortality: The National Institutes of Health-AARP Diet and Health Study. *JAMA Internal Medicine*.**4**:1-8.

Yen, G. C., Duh, P. D. and Tsai, C. L. (1993).Relationship between antioxidant activities and maturity of peanut hulls.*J. Agric. Food Chem*. **41**: 67-70.

Zhou, Y., Xue, S. and Yang, J.J. (2013) Calciomics: integrative studies ofCa^{2+} binding proteins and their interactomes in biological systems.*Metallomics*.**5**(1): 29–42.

Appendices

Appendix I

Spiking Analysis

Table A1-1: Mean concentration ± STD of spiking analysis for MESS leaves and soil.

Metal	Spiked blank	Spikedleaves	Spiked soil	Un-spikedleaves	Un-spiked soil
Calcium R^2=0.999	3289.1 ± 0.36	23878.0 ± 118.61	11902.9 ± 52.44	19761.4 ± 68.53	7991.2 ± 31.52
Copper R^2=0.998	1193.0 ± 4.48	853.7 ± 7.16	767.8 ± 5.67	12.3 ± 0.22	9.3 ± 0.94
Cobalt R^2=1.0	1962.8 ± 31.26	1214.6 ± 66.52	1180 ± 59.61	ND	ND
Iron R^2=0.998	1899.2 ± 1.40	2221.5 ± 34.78	4617.9 ± 157.81	1037.3 ± 120.95	2866.1 ± 19.75
Manganese R^2=0.998	1117 ± 10.16	945.5 ± 0.18	980.2 ± 49.62	101.6 ± 0.41	100 ± 4.80
Potassium R^2=0.999	1060 ± 28.28	2455 ± 84.14	1385 ± 79.90	1497.5 ± 85.20	490 ± 29.69
Zinc R^2= 0.999	2777.1 ± 19.89	2289.1 ± 28.10	1615.3 ± 71.44	59.1 ± 1.52	69.2 ± 5.11

Key: ND= Not Detected

Calculation of each metal from its salt solutions

The weight of each metal of study was calculated from 50 ppm of its salt solution

1 ppm = 1 mg/l

1000 ppm =1 g/l

Therefore for 50 ppm

50 ppm = x

To find x

$X=\frac{50 \times 1}{1000}$ = 0.05 g

X= 0.05 g

Using a 1000 cm^3 of volumetric flask

Therefore 0.05 g =1000 cm^3

Xg = 500 cm^3

$X= \frac{0.05 \times 500}{1000}$= 0.025g

Formula for calculating the weight of salt

$Wg = \frac{A \times 0.025}{M}$

Wg = Weigh of Salt Wg

A = Molar mass of element (g/mol)

M = molecular mass of specific salt

0.025 g = Expected gram of element in 50 ppm

Calculation of percentage recovery for element in plant leaves sample

$$\%R \text{ for plant leaves metals} = \frac{mean\ concentration\ \ for\ spike\ leaf - mean\ concentration\ \ of\ unspike\ leaf}{mean\ concentration\ \ for\ spike\ blank\ MESS} \text{x } 100$$

For %recovery of Ca in leaves= $\frac{23878.0 - 19761.4}{3289.1}$x 100 = 125.1%

For %recovery of Cuin leaves= $\frac{853.7 - 12.3}{1193}$x 100 = 70.5%

For %recovery of Coin leaves= $\frac{1214.6 - 0.00}{1962.8}$x 100 = 61.8%

For %recovery of Fein leaves= $\frac{2221.5 - 1037.3}{1899.2}$x 100 = 59.9%

For %recovery of Kin leaves= $\frac{2455 - 1497.5}{1060}$x 100 = 90.3%

For %recovery of Mnin leaves= $\frac{945.5 - 101.6}{1117}$x 100 = 75.5%

For %recovery of Znin leaves= $\frac{2289.1 - 59.1}{2777.1}$x 100 = 80.2%

% Recovery for metals in soil

$$= \frac{mean\ \ concentration\ \ \ of\ spike\ soil - mean\ \ concentration\ \ \ of\ unspike\ \ soil}{mean\ \ concentration\ \ \ of\ spike\ blank} \text{x } 100$$

For %recovery of Ca in soil = $\frac{11902.9 - 7991.2}{3289.1}$x 100 = 118.9%

For %recovery of Cu in soil = $\frac{767.8 - 9.3}{1193}$x 100 = 63.5%

For %recovery of Coin soil= $\frac{1180 - 0.00}{1962.8}$x 100 = 60.1%

For %recovery of Fein soil= $\frac{4617.9 - 2866.1}{1899.2}$x 100 = 88.0%

For %recovery of Kin soil= $\frac{1385 - 490}{1060}$x 100 = 84.4%

For %recovery of Mn in soil = $\frac{980.2 - 100.0}{1177}$x 100 = 78.7%

For %recovery of Zn in soil = $\frac{1615.3 - 69.2}{2277.1}$x 100 = 55.6%

Appendix II

Percentage recovery of extracts from plant leaves

Grams of leaves weigh

O. basilicum leaves=250g

O. gratissimum leaves= 250g

n-hexane extraction

O. basilicum leaves=18.95g

O. gratissimum leaves= 16.16

Methanol extraction

O. basilicum leaves=20.24g

O. gratissimum leaves= 8.2g

Weight of plant leaves after extraction

O. basilicum leaves=208.22g

O. gratissimum leaves= 224.97g

Percentage recovery

For n-hexane extraction

O. basilicum leaves=7.58%

O. gratissimum leaves= 6.46%

For methanol extraction

O. basilicum leaves=8.10%

O. gratissimum leaves= 3.28%

For the total extraction

O. basilicum leaves=16.71%

O. gratissimum leaves= 10.01%

Appendix III

Table A3-1: Mean percentage concentration ± STD of proximate analysis in leave of *O. basilicum* and *O. gratissimum* found in Maiduguri and Enugu

Proximate Analysis	*Ocimum basilicum*		*Ocimum gratissimum*	
	707Housing Estate Maiduguri	Damboa Road Maiduguri	Nsukka town Enugu	Awgu town Enugu
Carbohydrate content (non-fats extract)	31.58 ± 0.69	23.28 ± 0.63	25.39 ± 0.38	30.25 ± 1.85
Moisture content (%)	17.99 ± 0.94	26.66 ± 1.88	22.00 ± 2.82	25.33 ± 1.88
Ash content (%)	15.33 ± 2.82	12.66 ± 0.94	13.00 ± 4.24	9.50 ± 2.12
Crude fiber (%)	29.33 ± 1.88	32.00 ± 0.00	31.99 ± 0.94	30.66 ± 1.88
pH	6.90 ± 0.09	6.93 ± 0.06	7.48 ± 0.11	7.54 ± 0.08
Protein content (%)	5.76 ± 0.68	5.39 ± 0.31	7.61 ± 0.09	4.08 ± 0.44
Crude fats (%)	17.33 ± 2.82	18.66 ± 1.88	16.00 ± 2.82	15.33 ± 2.82

Appendix IV

Antioxidant properties

Table A4.-1: Mean concentration ± STD of total phenolic content assay of n-hexane and methanol extract of *O. basilicum* and *O. gratissimum* leaves.

Concentration of extract solution(μg/cm^3)	n-hexane extract mgGAC/g *O. gratissimum*	n-hexane extract mgGAC/g*O. basilicum*	Methanol extract mgGAC/g *O. gratissimum*	Methanol e mgGAC/g*O. b*
50	11.75 ± 0.00	102.0 ± 0.02	10.25 ± 0.00	99.0 ± 0.02

Table A4-2: Mean percentage inhibition ± STD of DPPH assay of n-hexane and methanol extract of *O. basilicum* and *O. gratissimum* leaves.

Concentration of extract solution ($\mu g/cm^3$)	Standard ascorbic acid	n-hexane extract (%I) *O. gratissimum*	n-hexane extract (%I) *O. basilicum*	Methanol extract (%I) *O. gratissimum*	Methanol extract (%I) *O. basilicum*
50	73.22 ± 0.62	70.04 ± 1.06	54.49 ± 2.03	65.92 ± 3.35	71.29 ± 0.71
100	81.02 ± 1.41	72.66 ± 4.41	59.68 ± 0.35	76.34 ± 0.79	86.27 ± 0.18
150	91.01 ± 0.00	85.27 ± 0.18	74.78 ± 0.71	93.07 ± 0.44	95.26 ± 0.18
200	96.94 ± 0.62	92.57 ± 0.25	86.95 ± 0.26	95.69 ± 0.62	97.13 ± 0.00
250	99.56 ± 0.09	95.63 ± 0.18	94.38 ± 0.53	98.13 ± 0.18	98.56 ± 0.09

Appendix V

Statistical analysis tables for the Data of nutritional contents and antioxidant properties of *Ocimum basilicum* and *Ocimum gratissimum* leaves with soil.

Table A5-1: p= 0.956 for f (3, 36) = 0.106, for mean concentration for elements of *Ocimum basilicum* and *Ocimum gratissimum* leaves in the four sampling site.

Elemental Analysis for Leaves	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	694609.186	3	231536.395	.106	.956
Within Groups	7.889E7	36	2191427.351		
Total	7.959E7	39			

Table A5-2: p=0.505 for f (3, 36) = 0.795, for mean concentration of elements for soil in the four sampling sites.

Elemental Analysis for soil	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1333134.182	3	444378.061	.795	.505
Within Groups	2.013E7	36	559041.227		
Total	2.146E7	39			

Table A5-3: p=1.00 for f (3, 24) = 0 002, for the mean percentage proximate content of *Ocimum basilicum* and *Ocimum gratissimum* leaves in the four sampling sites

Proximate Analysis	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.694	3	.231	.002	1.000
Within Groups	2471.882	24	102.995		
Total	2472.576	27			

Table A5-4: p=0.409 for f (3, 16) = 1.023, for the mean percentage inhibition of *Ocimum basilicum* and *Ocimum gratissimum* plant leaves for the four sample extracts of n-hexane and methanol extracts for DPPH assay

Percentage Inhibition for DPPH Assay	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	587.275	3	195.758	1.023	.409
Within Groups	3061.279	16	191.330		
Total	3648.554	19			

Appendix VI

Calibration curve of elements

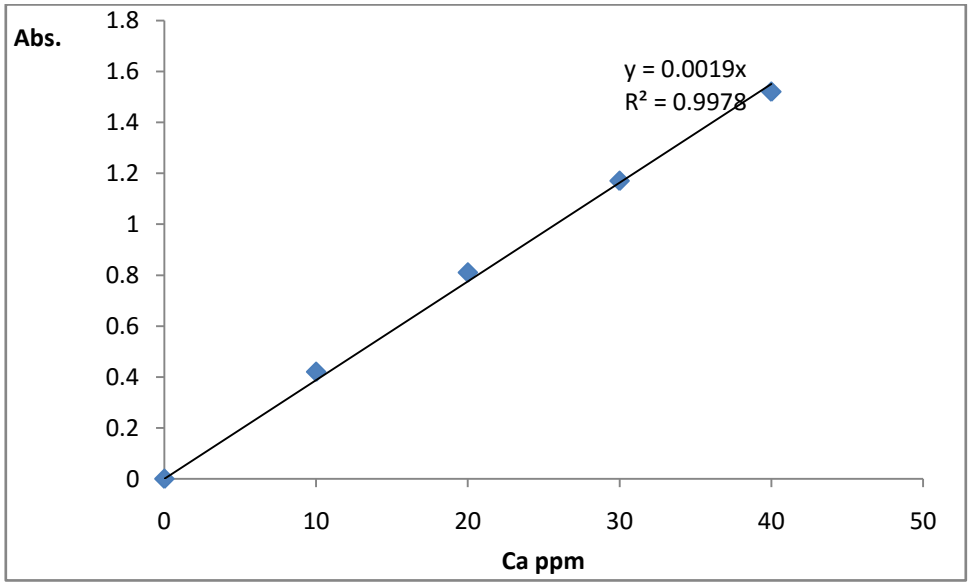


Figure A6-1: Calibration Curve of Calcium

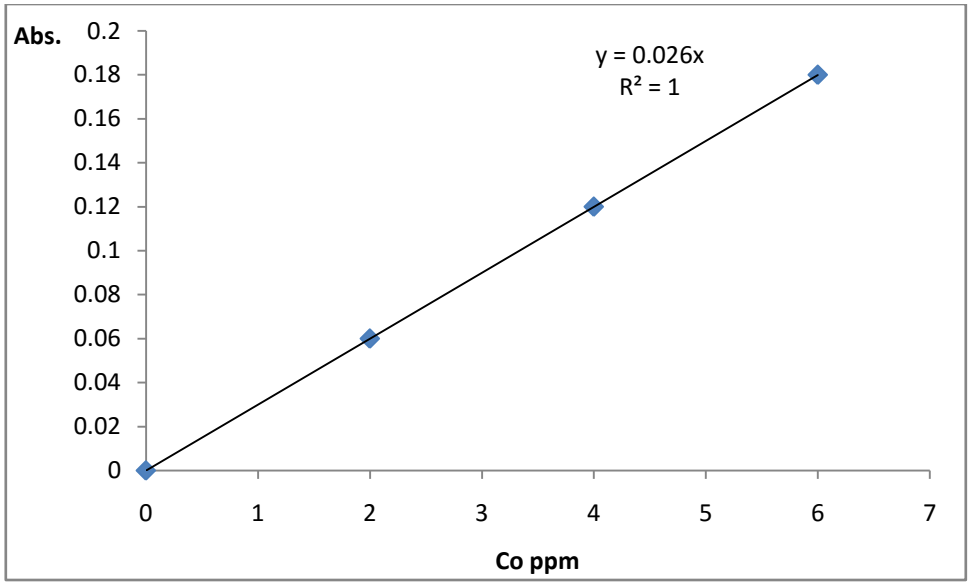


Figure A6-2: Calibration Curve of Cobalt

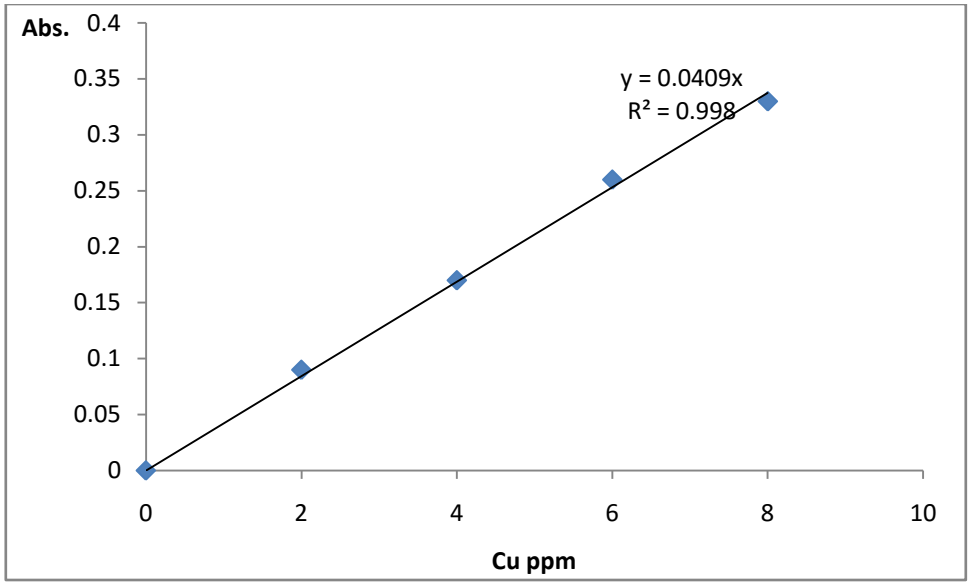


Figure A6-3: Calibration Curve of Cupper

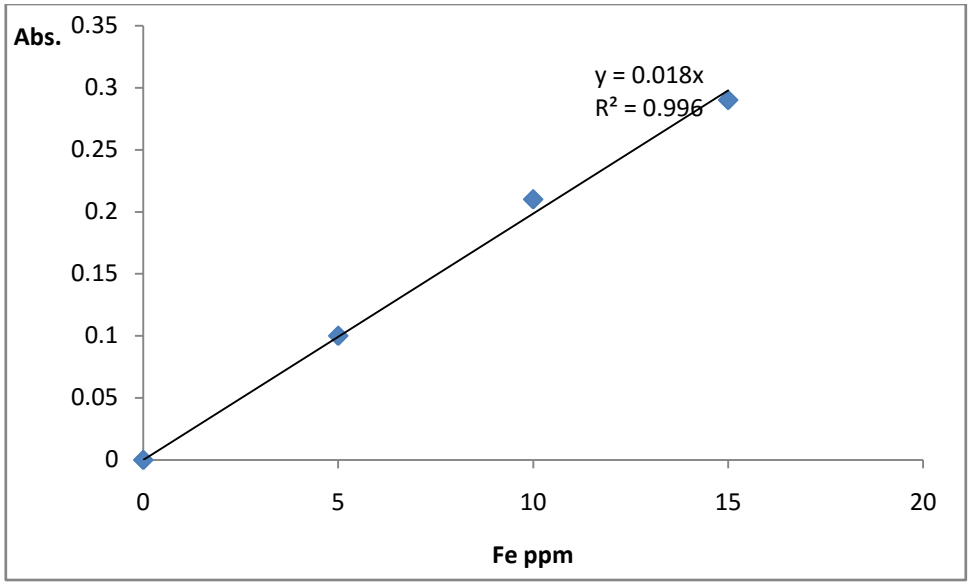


Figure A6-4: Calibration Curve of Iron

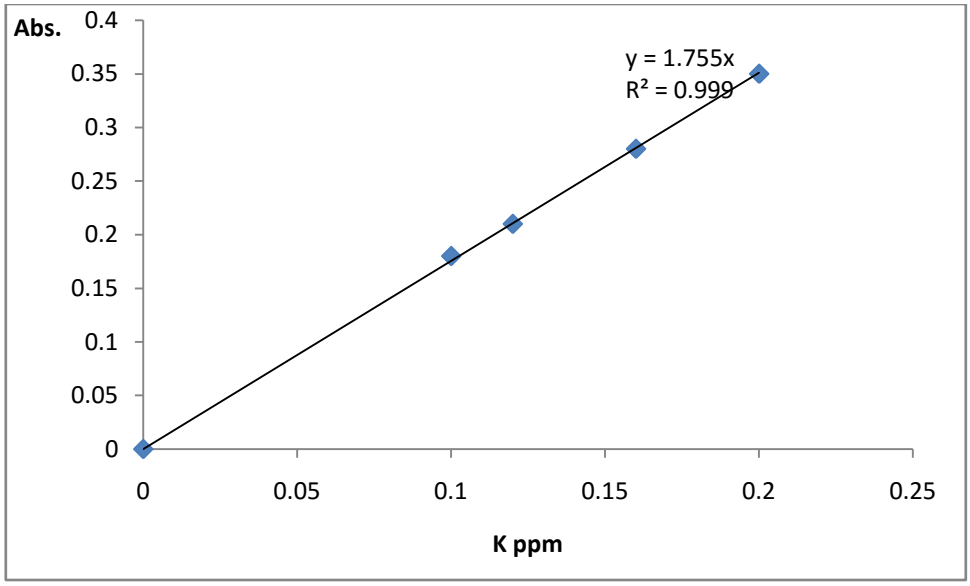


Figure A6-5: Calibration Curve of Potassium

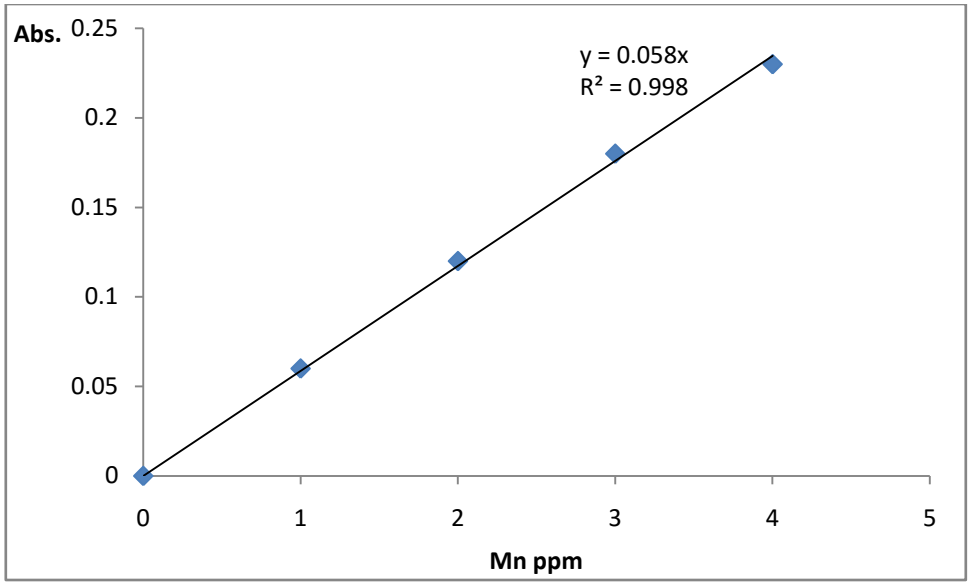


Figure A6-6: Calibration Curve of Manganese

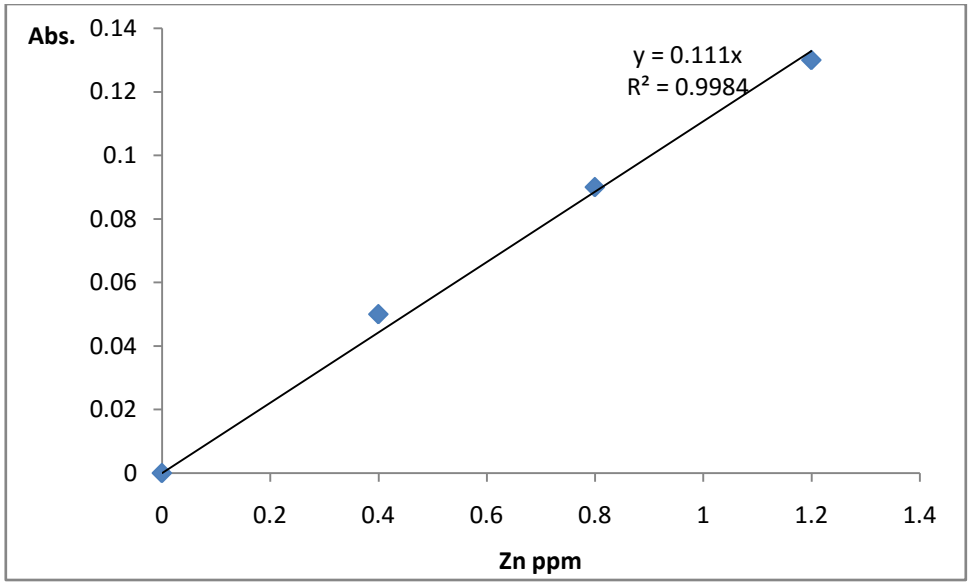
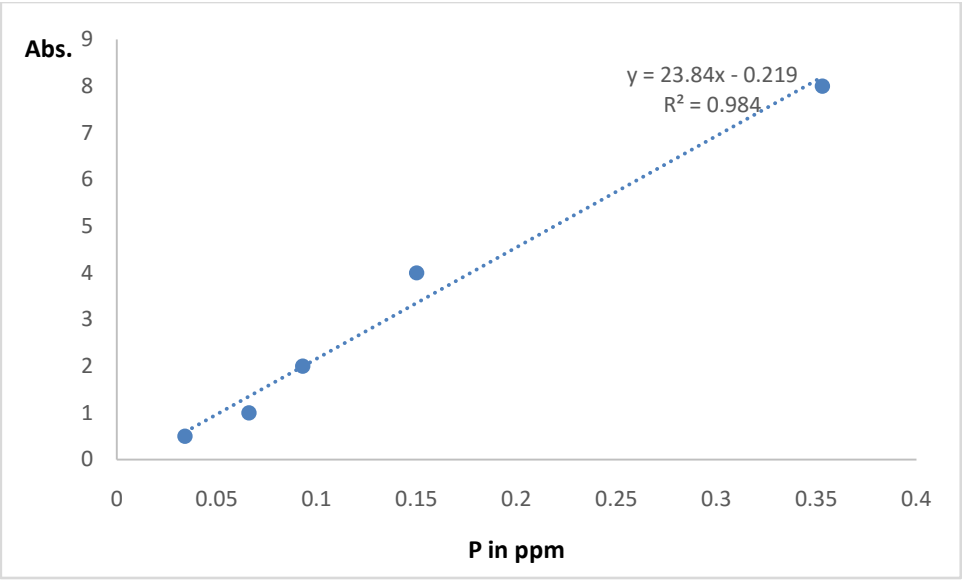
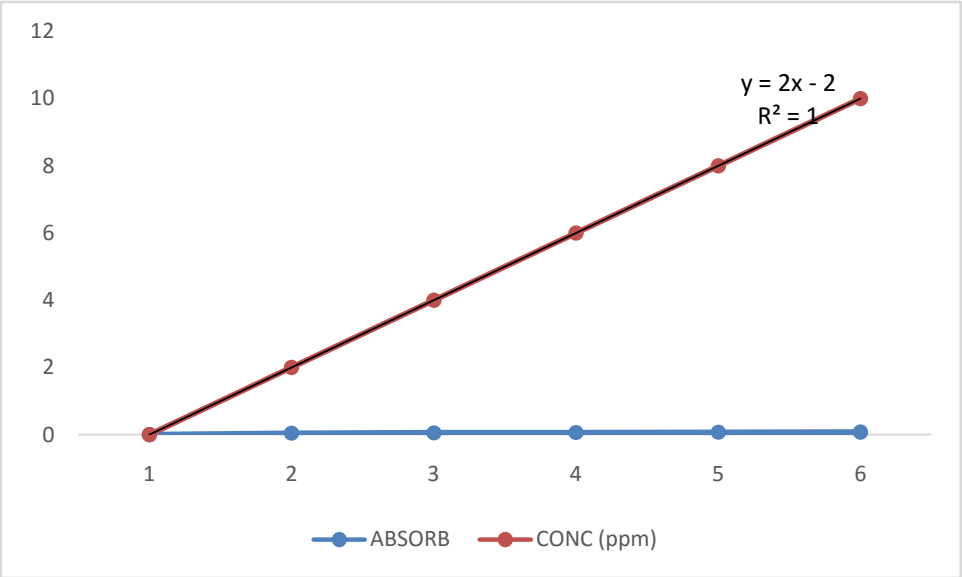


Figure A6-7: Calibration Curve of Zinc



Standard gradient for Phosphorus = 23.847

Figure A6-8: Calibration Curve of Phosphorus



Standard gradient for Sulphur = 94.04

Figure A6-9: Calibration Curve of Sulphur

Scientific name

Kingdom plantae

Class equisetopsida

Order lamiales

Family lamiaceae

Genius Ocimum

Species: *O. basilicum*

PLATE I: *Ocimum basilicum* plant

Scientific name

Kingdom plantae

Class magnoliopsida

Order lamiales

Family lamiaceae

Genius Ocimum

Species: *O. gratisimum*

PLATE II: *Ocimum gratissimum* plant

Appendix VIII

Antnutritional Contents

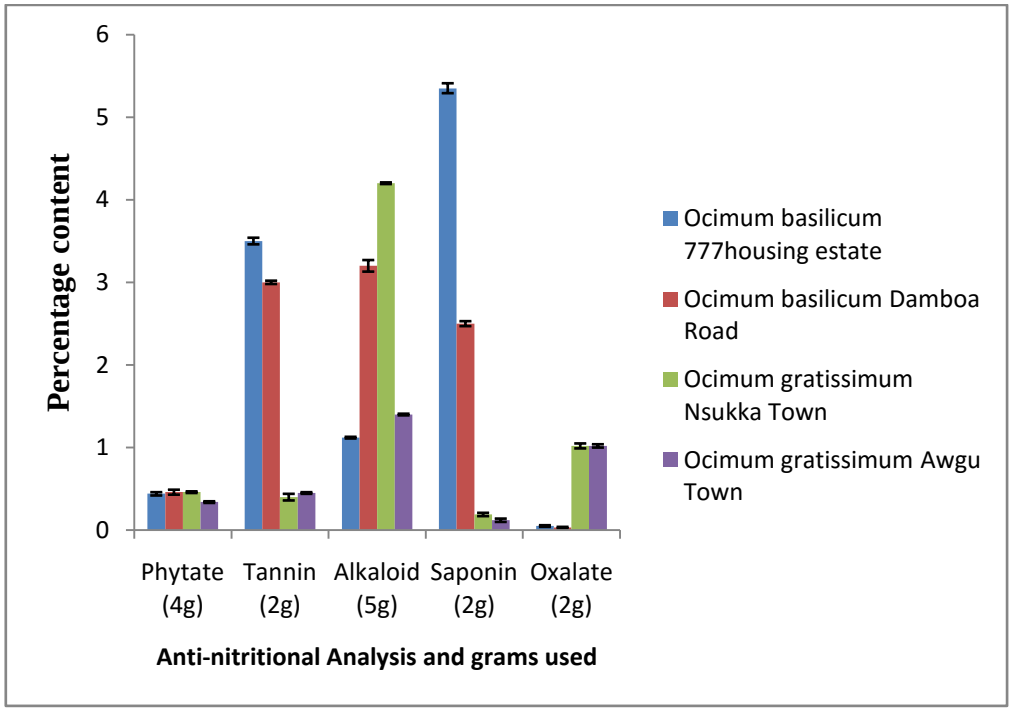


Figure A7-1: Anti nutritional contents of *Ocimum basilicum* and *Ocimum gratissimum* leaves.