IN VITRO ANTIOXIDANT AND BLOOD CLOTTING POTENTIALS OF METHANOL EXTRACTS OF FRESH LEAVES OF *Ocimum basilicum, Ocimum gratissimum* AND *Ocimum suave*

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA IN PARTIAL FULFILMENT FOR THE AWARD OF MASTERS OF SCIENCE IN BIOCHEMISTRY

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ZARIA

FEBUARY, 2016

DECLARATION

I declare that this thesis entitled "***IN VITRO*** **ANTIOXIDANT AND BLOOD CLOTTING POTENTIALS OF METHANOL EXTRACTS OF FRESH LEAVES OF *Ocimum basilicum, Ocimum gratissimum* AND *Ocimum suave***" is a record of my own research work under the supervision of Prof. H. C. Nzelibe and G. C. Njoku.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis has been presented elsewhere for award of a degree in any University.

Abdulwahab, Rabiat		Febuary, 2016
Name	Signature	Date

CERTIFICATION

This project titled "***IN VITRO* ANTIOXIDANT AND BLOOD CLOTTING POTENTIALS OF METHANOL EXTRACTS OF FRESH LEAVES OF *Ocimum basilicum, Ocimum gratissimum* AND *Ocimum suave***" by RABIAT ABDULWAHAB meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

Prof. H. C. Nzelibe Chairman, Supervisory Committee	________________ (Signature)	________________ Date
G. C. Njoku Member, Supervisory Committee	________________ (Signature)	________________ Date
Prof. N. M. Shuaibu Head of Biochemistry Department	________________ (Signature)	________________ Date
Prof. K. Bala Dean, School of Postgraduate Studies	________________ (Signature)	________________ Date

DEDICATION

This work is dedicated to my beloved husband Maruf Badmasi, who by all means stood by me vividly to the completion of this work and to my lovely children Abdulmu'izz, Abdulmumin and Abdulmujib.

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ABBREVIATIONS

4HR	4-Hexylresorcinol
ABU:	Ahmadu Bello University
ANOVA	Analysis of Variance
BHA	Butylated Hydroxyanisole
DPPH	Diphenylpicrylhydrazyl
IC_{50}	50% Inhibitory Concentration
NDGA	Nordihydroguaiaretic Acid
OG	Octyl Gallate
PG	Propyl Gallate
ROS	Reactive Oxygen Specie
SD:	Standard Deviation
TBHQ	Tertiary Butyl Hydroquinone
THBP	2,4,5-Trihydroxybutyrophenone

ABSTRACT

The genus *Ocimum* is ranked high and considered one of the important genus of the *lamiaceae* family due to the extensive use of many of its specie in traditional medicine. The plants extracts are used traditionally for the treatment and management of several diseases such as infections of the skin, urinary and gastrointestinal tracts, nasal bleeding, headache, diarrhea, wart, worms, kidney function, anticonvulsant, cough, catarrh, cold, fever and chest pains. The DPPH method was used to evaluate the *in vitro* free radical scavenging activities of the plant extracts. Standard methods were used to study the phytochemical constituents, isolation and identification of active compounds as well as the study of the effect of the plants extracts on blood clotting. The preliminary phytochemistry revealed the presence of several phytochemicals such as flavonoids, tannins, saponins, steroids, anthraquinone, carbohydrate, cardiac glycosides and triterpenes. The quantitative phytochemistry showed that all the plants have flavonoids and saponin in abundance with methanol extract of *O. basilicum* registering the highest percentage of 88.28% and 72.38% respectively. The aqueous extract of *O. basilicum* registered the lowest percentage of flavonoid (54.84%) while the aqueous extract of *O. suave* had lowest saponin (13.47%). Tannins were less abundant in all the plant extracts. The Radical Scavenging Activities of the extracts revealed that the methanol fraction of *O. basilicum* has more activity when compared to other extracts as it had the lowest IC_{50} of 0.58mg/ml. The GC-MS analysis of the chromatographic fractions obtained from the fractionation of *O. basilicum* provided peaks of different bioactive compounds showing 9-Octadecenal, 1-fluorodecane and 4-methyl-1-decene as the major compounds constituting 37.93%, 11.96% and 10.88% respectively in fraction 1 while the major compounds identified in fraction 2 were Heptanoic acid 2-methyl-2-butyl ester (32.21%), 9-Octadecenoic acid (Z)- (27.77%) and n-Hexadecanoic acid

(19.10%). The effect of the plants extract on clotting time revealed that the methanolic extracts of *O. basilicum* and *O. suave* are potent blood coagulant as they had clotting time similar to the standards used for the study. The study has validated the traditional use of *O. basilicum, O. gratissimum* and *O. suave* in the treatment of several diseases. It also shows that the use of the plant extracts in the treatment and management of nasal bleeding and wound healing is accurate.

CHAPTER ONE

1.0 INTRODUCTION

Many species of herbs and shrubs found in the tropical regions of Asia, Africa and Central and South America belong to the genus *Ocimum* (Javanmardi *et al.,* 2002). More than 60 species of *Ocimum* have been recognized and numerous varieties of these belong to the Family *Lamiaceae*. Essential oils rich in terpenoid compounds are found in most cultivated members of the *Lamiaceae* family (Lawrence, 1993).

In the Southern and Northern part of Nigeria, about four varieties of *Ocimum namely; Ocimum suave*, *Ocimum gratissimum, Ocimum basilium* and *Ocimum camium* are known to occur naturally (Muhammed *et al*., 2007). Several authors have reported the traditional use of the *Ocimum* species as medicinal plants in the treatment of headache, diarrhea, wart, worms, anticonvulsant, diaphoretic and carminative. It cures cough, catarrh, cold, fever and chest pains. Others are ear ache, ringworm, nasal bleeding, anti-spasmolytic, bronchitis, gouts and relief of pains of the colon (Muhammed *et al*., 2007; Seung-Jo-Lee *et al*., 2004). In addition to health benefit, these plants are used widely as a condiment or spice and as a source of flavor.

Medicinal plants are commonly used in treating and prevention of specific ailments and diseases. Generally, they are herbs valued for their medicinal, aromatic or savory qualities and considered to play a beneficial role in healthcare. Sodipo *et al.,* 2012, reported that the demand for these plants is steadily increasing both in the developing countries and the industrialized nations. Herbs produce and contain a variety of chemical substances that act upon the body (Anonymous, 2012). The use of plant materials as spices, condiments and for medicinal purposes is old as the history of mankind (Nweze *et al.,* 2004). Recently, the use of plants for medicinal purposes has gained acceptance in many countries of the world and most national governments have established the traditional medicine regulatory council under the supervision of their various health ministries to tap the numerous potentials of herbs (Nweze and Eze, 2009). This could be because traditional medicine has long been practiced even before the orthodox medical practice appeared (Okafor *et al.,* 2001).

In spite of the great achievements in modern medicine, plants still make an important contribution to health care. The value of traditional medical systems has been recognized and medicinal plants from indigenous pharmacopoeias having significant healing power have been identified (Holetz *et al.,* 2003). Medicinal plants contain chemical compounds that mediate their effect on human body by binding to receptor molecules present in the body. The processes through which these chemical compounds mediate their effects are identical to the already understood mechanism of actions of conventional drugs and as such herbal medicines do not differ greatly from conventional drugs in terms of how they work. In principle, medicinal plants are just as effective as conventional medicines and thus, have the same potential to cause harmful side effects. Many of the herbs and spices used by humans to season food have medicinal properties (Lai and Roy, 2004). Different kinds of acute and chronic inflammatory diseases like wound healing, edema and rheumatoid arthritis have been cured using medicinal plants and their products for many centuries (Gayathri *et al.,* 2007).

In the last three decades, especially in the developed countries of Europe and America, scientists have shown increasing interest in plant research. Sixty percent of the total world population has been estimated to rely on herbs and natural products that are recognized as important sources of drugs (Harvey, 2000). An important place among these plants is occupied by aromatic plants (which include *Ocimum* species) whose aroma is associated with the presence of essential oils, complex mixtures of volatile compounds dominated by mono and sesquiterpenes. In addition to essential oils, aromatic plants contain phenolic compounds which are primarily coumarins and phenyl propanoids that have been shown to possess multiple pharmacological activities (Kaurinovic, 2011).

Free radicals are highly reactive atoms with one or more unpaired electrons in their outer orbital and are capable of independent existence (Jesberger and Richardson, 1991). These species become very unstable and very reactive with other molecules due to the presence of unpaired electron(s) as they try to pair their electron(s) and generate a more stable compound (Karlsson, 1997). The most dangerous of these free radicals are the atomic and molecular varieties of oxygen which are known as Reactive Oxygen Species (ROS). Technically they are not free radicals but, they are highly reactive with the molecules around them (Sharma and Clark, 1998). ROS is a collective term, which includes both the oxygen radicals ($\ddot{O}_2$ and OH) and also some non-radical derivatives of oxygen such as hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and ozone (O_3) (Sjodin *et al.*, 1990).

The most reactive form of activated oxygen is Hydroxyl radical (OH) which is a product of molecular oxygen reduction and it is the most responsible for cytotoxic effects (Imlay and Linn, 1988). Due to its extreme reactivity, hydroxyl radicals react immediately with bio-molecules and can effectively attack every molecule present in living cells such as sugars, amino acids, phospholipids, organic acids, pyrimidine and purine bases (Hippeli and Elstner, 1999).

Excessive production of oxygen radicals in the body initiates the oxidation and degradation of polyunsaturated fatty acids. Highly unsaturated fatty acid membrane systems are attacked by free radicals thereby inducing lipid peroxidation which is a key process in many pathological conditions and one of the causes of oxidative stress. The biological membrane lipids of the spinal cord and brain are more vulnerable to these attacks because of the high levels of polyunsaturated fatty acids they contain. Moreover, significant amounts of transitional prooxidant metals are found in the brain and it consumes a lot of oxygen (Kaurinovic *et al.,* 2011). If these are not effectively scavenged by cellular constituents, the formation of oxygen radicals is facilitated which have been reported by several authors (Halliwell, 1994; Bhuiyan *et al.,* 2009; Sharma and Clark, 1998) to be involved in the processes of aging, neurodegenerative diseases (e.g. Parkinson's and Alzheimer's disease), ischemic heart damage, arthritis, myocardial infarction, arteriosclerosis, cancer, Behcet's disease, Crohn's disease, cataracts, sunburn, ulcers, osteoporosis, rheumatoid arthritis, diabetes mellitus, emphysema, stroke, hemorrhagic shock, cardiovascular disorders, cystic fibrosis, gastrointestinal ulcerogenesis and even early senescence.

The disruption of living cells by free radicals occurs as a result of attack of the nearest stable molecule stealing its electrons. The attacked molecule loses its electron thus, it becomes a free radical leading to a cascade of reactions (Jesberger and Richardson, 1991; Goldfarb, 1999). Free radicals are normal by-products of oxygen metabolism during mitochondrial oxidative phosphorylation. Thus, the mitochondrion is the main source of free radicals (Przedborski and Jackson-Lewwis, 1998).

Antioxidants being the first line of defense against free radical damage are critical for maintaining optimum health and well being (Percival, 1998). An antioxidant retards or prevents deterioration, damage or destruction by oxidation (Bhuiyan *et al.,* 2009). They exert their effects through several mechanisms including enzymatic mechanisms (enzymes such as superoxide dismutase, catalase, and glutathione peroxidase and non-enzymatic mechanisms (macromolecules such as albumin, ceruloplasmin, transferrin and ferritin and an array of small molecules such as ascorbic acid, reduced glutathione, carotene, uric acid and bilirubin) (Rice-Evans and Miller, 1994; Yu, 1994). Phenolic antioxidants stabilize the free oxygen radicals and free radicals formed from the substrate by donating hydrogen atoms or electrons. Many plant species and aromatic plants have been reported to have antioxidant and antiradical activity (Gulcin *et al*., 2011).

Owing to high performance, low cost and wide availability of synthetic antioxidants such as butylated hydroxyanisole (BHA), tertiary butyl hydroquinone (TBHQ), 2,4,5-trihydroxybutyrophenone (THBP), di-tertbutyl-4hydroxymethylphenol, propyl gallate (PG), octyl gallate (OG), nordihydroguaiaretic acid (NDGA) and 4-hexylresorcinol (4HR), many food industries have resorted to using them as food additive to prevent rancidification (Xiu-Qin *et al.,* 2009). However, recent publications have noted their possible toxic effect on man and other animals (Wagensteen *et al.,* 2004).

Hemostasis is the arrest of blood loss from injured blood vessels and maintenance of the blood fluidity. A wound or cut on blood vessels leads to vasoconstriction and thrombin activation which is accompanied by adhesion and platelet activation, fibrin formation from soluble fibrinogen which serves as the main substrate and coagulation inactivation mechanism (Rang *et al.,* 1999). The haemostatic system involves a balance between pro-coagulant, anticoagulant mechanisms which is linked to a process of fibrinolysis (Lewis and Decie, 2002). Five major components are involved in the haemostatic system; platelets, coagulation factors, coagulation inhibitors, fibrinolytic system and blood vessels (Guyton and Hall, 2000).

1.1 Statement of Research Problem

In spite of the great achievements in modern medicine, plants still make an important contribution to health care. Over the years, there has been an increase in the use of the

Ocimum species in rural areas for management and treatment of nasal bleeding and various oxidative stress related diseases and ailments whose pathogenesis have been linked to the formation of free radicals such as heart diseases, hypertension, stroke, head ache and diabetes (Droge, 2002; Hippeli and Elstner, 1999; Maxwell, 1995).

However, most of these claims made are yet to be proved as the efficacies and toxicities of most of these Ocimum species used in these areas are yet to be validated scientifically.

1.2 Justification

The antioxidant potentials of plant extracts are linked to the presence of phenolic compounds such as Flavonoids, phenolic diterpenes, phenolic acids and tannins in the plants. Compounds such as tannins and Flavonoids are known to accelerate blood clotting which is a vital step in the wound healing process. They act as free radical scavengers that provide protection to living organisms from damage caused by uncontrolled production of reactive oxygen species.

Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), Propylgallate (PG) and butylated hydroquinone are used in the pharmaceutical and food industries. However, these synthetic antioxidants have side effects such as liver damage and are carcinogenic (Ramesh *et al.,* 2011; Sherwin *et al.,* 1990). Therefore, there is a need for the identification of natural antioxidant having little or no side effects for use in food and pharmaceutical industries in order to replace synthetic antioxidants.

1.3 Aim and Objectives

The aim of this research work was to investigate the antioxidant potentials of three different species of Ocimum and evaluate the effect of these plants extracts on blood clotting.

The specific objectives are;

1. Qualitative and Quantitative determination of the phytochemical constituents of the Ocimum species.
2. *In-vitro* antioxidant activity by measuring the radical scavenging activity.
3. Isolation and identification of the active constituents of the plant extracts.
4. Evaluation of the effect of methanol extracts of the fresh leaves of the three *Ocimum* species on clotting *in vitro*

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Genus *Ocimum*

The genus *Ocimum* belonging to the *lamiaceae* family is considered a very important genus of this family due to the extensive use of many of its specie for therapeutic and culinary purposes (Prakash and Gupta, 2005). They originate from Central Africa and South East Asia and are probably one of the most commonly used herbs in cooking (Umar *et al.,* 2012). Previous studies reported that large number of species ranging between 50 to 150 and distinct varieties of herbs and shrubs fall into this genus which makes them very special and are widely spread over three main centers of biodiversity such as the tropical and sub-tropical regions of Africa, the tropical Asia and the tropical parts of America which encompasses the Central & Southern America (Kalita and Khan, 2013; Vani *et al.,* 2009; Javanmardi *et al.,* 2002; Darrah, 1980; Bailey, 1924)

The major species of *Ocimum* are (i) *Ocimum gratissimum*, (ii) *Ocimum basilicum*, (iii) *Ocimum americanum*, (iv) *Ocimum sanctum* and (v) *Ocimum canum* (Tanko *et al.,* 2008). Species of the genus *Ocimum* are characterized based on their leaves and habitat (Grayer *et al.,* 2002). Plants of the *Ocimum* are collectively called Basil.

Basils (*Ocimum spp. L*) are small plants with several branches which are commonly found in many gardens, road sides and wastelands in Nigeria (Umar *et al.,* 2012; Arhoghro *et al.,* 2009). The leaf color of basils varies from bright green to purple-green and sometimes almost black. The fresh leaves of basils have a strong and characteristic aroma that makes it incomparable to any other spice although it has a hint of clove (Vani *et al.,* 2009). Many local and foreign recipes are enhanced by the use of the basils. Studies have shown that many of the common cultivated members of the *Ocimum* specie accumulate essential oils rich in terpenoid and phenolic compounds and a wide array of other natural products including polyphenols such as flavonoids and anthocyanins (Lawrence, 1993; Phippen and Simon, 1998). Many studies have been conducted to monitor the developmental regulation of the biosynthesis of the terpenoid essential oil components. However, the non-terpenoid components have received much less attention (Gershenzon *et al*., 2000; Lewinsohn *et al*., 2000; Rohloff, 1999).

According to Paton, 1992, the genus *Ocimum* is divided into three sections;

1) Those with the appendiculate posterior stamens which are subdivided based on their calix morphology into; i) Subsection *Ocimum* consisting of *O. basilicum, O. americanum, O. kilimandscharicum, O. minimum,* and *O. xcitriodorum* which are the most economically important species of the genus *Ocimum* and ii) Subsection *gratissima* containing *O. gratissimum*.
2) *Hierocymum* which constitute those with fascicles of hairs at the base of their posterior stamens such as *O. selloi Benth* and *O. tenuiflorum L.*
3) *Gymnocymum* containing specie with glabrous posterior stamens *O. campechianum Mill*.

The genus *Ocimum* is cultivated for this extraordinary essential oil which has many therapeutic usages such as in medicinal application, herbs, culinary, perfume for herbal toiletries, aromatherapy treatment and as flavouring agent (Kashyap *et al*., 2011). The *Ocimum* species have attracted the attention of several researches due to its high contents of natural antioxidants (Singh *et al.,* 2011).

2.2 *Ocimum basilicum*

O. basilicum is an important plant particularly in the sub Saharan Africa region where it is appreciated for food security, medicinal and traditional usage (Ojo *et al.,* 2012). It is a member of the *Lamiaceae* family which is used both as a culinary and ornamental herb. It is a small shrub with many branches commonly found in many gardens around village huts in Nigeria (Umar *et al.,* 2012). Generally, *O. basilicum* is commonly known as scent leaf but it has different native names. In Yoruba language, it is known as Efirin ajase, Nchu-anwu in Igbo, daidoya in Hausa, Ireru in Ebira, Ebaubokho in Benin, ufuo-yibo in Urhobo and ntion in Efik (Iwu, 1993).

2.2.1 Plant description and distribution

O. basilicum is an erect, tender summer and herbaceous plant which grows about 30 to 90cm in height with white-purple flowers that are ovate, acuminate, toothed or entirely glaborous on both sides originating from tropical and warm areas such as India, Africa and Southern Asia (Verma and Kothiyal, 2012; Chang *et al*., 2009: kilmankova *et al.*, 2008). It is a hairy plant with a square stem and shiny oval leaves that are grayish-green beneath which are dotted dark oil cells. The leaves are soft and cool to touch which gives a delightful scent if slightly bruised (Tanko *et al.*, 2012).

Figure I: *Ocimum basilicum*
m.wikipedia.org

Different cultivars of *O. basilicum* have been recognized and each variety have been distinguished based on their leaf color (green or purple), flower color (white, red or purple), taste, aroma, height and colour of foliage (Tanko *et al*., 2012; Morales and Simon 1996).

2.2.2 Cultivation

O. basilicum grows in annual temperatures between 6 - 24°C in the cool moist and tropical rain forest zones. *O. basilicum* is cultivated in different climatic and ecological conditions however, the most favorable conditions are found in countries with a warm climate. The basic ecological requirements for *O. basilicum* cultivation are; Warmth, light, temperature and moisture (Caliskan *et al.,* 2009). The optimum day and night temperature for the germination of *Ocimum basilicum* seed in the laboratory has been determined to be 24 -27ºC and 19 - 22°C respectively (Putievsky and Galambosi, 1999). It has also been reported to develop best under long days in sunny conditions (Caliskan *et al.,* 2009).

Various chemotypes of *O. basilicum* are being traded internationally such as the European basil, which have linalool and methyl chavicol as its main components and it is often considered to have the finest flavour, the tropical chemotype with methy cinnamate as the main component, the Reunion basil which has high levels of methyl chavicol and the chemotype grown in northern Africa, Russia, eastern Europe, and parts of Asia Another type that is high in eugenol (Marotti *et al.,* 1996).

2.2.3 Scientific classification

Kingdom:	Plantae
Phylum:	Magnoliophyta
Class:	Magnoliopsida
Order:	Lamiales
Family:	Lamiaceae

Genus: *Ocimum*

Species: *O. basilicum*

Binomial Name: *Ocimum basilicum*

2.2.4 Medicinal uses

O. basilicum is one of the important medicinal plant species (Omidbiagi *et al.,* 2010) which is graded high among some of the astonishing herbs for having tremendous medicinal potentialities (Kashyap, 2011). It is widely used in traditional medicine and ayurveda. In traditional Chinese medicine, it is used for treatment cardiovascular diseases including hypertention (Umar *et al.,* 2010). Externally, *ocimum basilicum* is used to cure problems as diverse as earache, rheumatoid arthritis, acne, loss of smell, skin infections, snake bites and insect stings (Martin and Ernst, 2004). It is used as an embalming and preserving herb found in mummies of ancient Egypt (Marwat *et al.,* 2011).

Ocimum basilicum tea is used traditionally to cure digestion problems such as expelling of gas, constipation, stomach cramps, diarrhea and vomiting (Ramesh and Satakopan, 2010). The leaves and flowers of *Ocimum basilicum* have been reported to be used as antispasmodic, carminative, galactogogue and tonic agent (Bunrathep *et al.,* 2007; Meyers, 2003). They have also been used to treat fever, pile, colic, dizziness, paralysis, ulcer, hiccups, heart problems, migrane, insomnia, headache, dysentery, tooth ache, gonorrhea, numbness and nervous temperament (Marwat *et al.,* 2011; Sanganuwan, 2010). The plant have also been reported to have antiviral (Chiang *et al.,* 2005) and antimicrobial (Baratta *et al.,* 1998) activities.

The essential oil of sweet basil has also been found to have a pronounced antimicrobial, antiviral and antifungal action that varies significantly depending on the time of harvesting and extraction of essential oil (Maria *et al.,* 2012; Banchio *et al*., 2009; Hussain *et al*., 2008; Almeida *et al.,* 2007; Chiang *et al.,* 2005;). One of the major components of the essential oil of sweet basil is estragole, a non-terpenoid allylphenol derivative (Lewinsohn *et al*., 2000). Terpenic and phenolic compounds synthesized and accumulated by basil are also appreciated in the pharmaceutical industries due to their antioxidant potentials (Maria *et al.*, 2012).

A study carried out in 2003 by Momen *et al*., reported that *O. basilicum* showed high mortality rate of some predacious mites when compared to other insecticides. This is of great importance due to the increasing use of biodegradable organic insecticides as compared to the conventional ones (Ojo *et al.*, 2012)

O. basilicum is also widely used for flavoring confectionaries, baked foods and meat products (Wan, 1998) due to its rich, spicy and mildly peppery flavor (Xie, 1995). *O. basilicum* is used both fresh and dried to spice up various kinds of meals (kilmankova *et al.,* 2008). It is a common ingredient in Thai cuisine, used to flavour curries and stir-fries (Narwal *et al*., 2011).

2.3 *Ocimum gratissimum*

Ocimum gratissimum also known as tea bush or fever plant is a shrub which is highly exploited in folk medicine (Gege-Adebayo *et al.,* 2013). It also belongs to the family of plant known as Lamiaceae with characteristic pleasant aroma due to the presence of volatile oil (Dalziel, 1993). It is a widely used local plant for both nutritional and therapeutic purposes (Arhoghro *et al.,* 2009). In Nigeria, *O.gratissimum* is called “Efinrin-nla” in Yoruba, “Ahuji” in Igbo, “Aramogbo” in Edo, “Tan-motsungi-wawagi” in Nupe and “Bunsuru daji” in Hausa (Onaolapo and Onaolapo, 2012; Effraim *et al.*, 2003). It is known by various names in different parts of the world for instance, In India it is known by several vernacular names such as Vriddhutulsi, Ram tulsi and Nimma tulasi (Prabhu *et al.,* 2009).

2.3.1 Plant description and distribution

Ocimum gratissimum is a perennial and odoriferous herb which grows to a height of about 1-2 feets with branched stems. It has 1-2 inches long, oval, pointed, sharp and pale greenish yellow leaves which are sub-acuminate to acuminate at apex, cuneate at base with a coarsely crenate, serrated at the margin and dotted on both the sides. It has small black elongated seeds that are round at one end and flattened at the other (Verma and Kothiyal, 2012; Choudhury *et al.,* 2011). The peduncles are densely pubescent with Calyx which is 5-7mm long, campanulate and greenish-white to greenish-yellow in colour (Bhat, 2003). The 2 surfaces of the leaf epidermal cells are typical of irregular contours with diacytic stomata and secretory glands mostly abundant in the leaf. These are presented in simple pluricellular hairs on the leaf veins (Garcia *et al.,* 1998).

Figure II: *Ocimum gratissimum*
m.wikipedia.org

The plant is indigenous to the savannah, tropical rain forest and coastal areas of West Africa especially in Kenya and Nigeria. It is also found in the tropical Asia such as India (Gege-Adebayo *et al.,* 2013; Oguanobi *et al*., 2012; Akinjogunla *et al*., 2011). In

Nigeria, it is found in the Savannah and coastal areas (Illoh and Awojide, 2000). It has been found to be cultivated in Ceylon, South Sea Islands and also within Nepal, Bengal, Chittagong and Deccan (Nadkarni and Nadkarni, 1999).

2.3.2 Scientific classification

Kingdom:	Plantae
Order:	Lamiales
Family:	Lamiaceae
Genus:	*Ocimum*
Species:	*O. gratissimum*
Binomial Name:	*Ocimum gratissimum*

2.3.3 Cultivation

Ocimum gratissimum is not a problem in cultivation, it is mostly a weed of roadsides and wasteland. However, it is also an important pasture. The basic requirements for its growth are moist and fertile soils but it can tolerate drought after flowering (Arhoghro *et al.*, 2009). *Ocimum gratissimum* is grown for the essential oils in its leaves and stems (Sulistiarini *et al*., 1999).

2.3.4 Medicinal uses

Ocimum gratissimum is extensively used clinically throughout the world compared to the other various species of *Ocimum*. It has extremely broad therapeutic use which varies from one country to the other (Kpoviessi *et al.*, 2012). In Kenyan and the sub Saharan African communities, this plant is used for various purposes. The leaves are used for abdominal pains, sore eyes, ear infections, coughs, barrenness, fever, convulsions, tooth gargle, regulation of menstruation and they are rubbed between the palms and sniffed as a treatment for blocked nostrils (Matasyoh *et al.,* 2007). The whole plant of *Ocimum gratissimum* has been used for the treatment of sunstroke, headache and influenza in India (Tania *et al*., 2006). A decoction of the root of *Ocimum gratissimum* is used as a sedative for children by the people of Brazilian tropical forest (Cristiana *et al.*, 2006). In Congo, a decoction of this plant is used for gonorrheal infection, vaginal douches for metritis and vaginitis and used in treatment of mental illness (Nwinyi *et al.*, 2009).

In Nigeria, the use of various parts of *Ocimum gratissimum* to cure different ailments has shown remarkable success. In the southeast, *Ocimum gratissimum* is used in the management of the baby's cord to keep the surfaces sterile (Onaolapo and Onaolapo, 2012). In the coastal areas of Nigeria, the plant is used for treatment of epilepsy, high fever and diarrhoea (Effraim *et al*., 2003) while in the Savannah areas decoctions of the leaves are used to treat mental illness (Akinmoladun *et al*., 2007). In some parts of Nigeria, *O. gratissimum* was reported to be used in the treatment of shigellosis, trypanosomiasis, convulsion, pile and anaemia (Mann, 2012) and It has been implicated in oral hygiene and veterinary in Nigeria (Abu *et al.*, 2009; Matekaire and Bwakur 2004).

The review of the comprehensive biological activities of *O. gratissimum* suggests that it is associated with antibacterial, antifungal, hypoglycaemic, antipyretic, anti-nociceptive, antioxidant, anti-inflammatory, anthelmintic, chemo-preventive, anti-carcinogenic, free radical scavenging, radio protective, antidermatophytic activities, and numerous others pharmacological use (Asuquo *et al., 2010;* Aprioku and Obianime, 2008; Nwanjo and Oze, 2007; Leal *et al., 2006;* Ngassoum et al., 2003; Cimanga *et al., 2002;* Njoku and Asuzu, 1998). Smooth muscle contraction and antimutagenic activity as well as the anti-diarrhoeal effects of *Ocimum gratissimum* in experimental animals has also been reported (Offiah and Chikwendu, 1999; Onajobi, 1986).

The antimicrobial, antifungal, antinociceptive, germicidal and antibacterial properties of the essential oils of *O. gratissimum* have also been reported (Prasannabalaji *et al.*, 2012; Ladipo *et al*., 2010; Holetz *et al*., 2003; Pessoa *et al*., 2002; Rabelo *et al.*, 2003; Dubey *et al*., 2000; Nakamura *et al*., 1999; Sofowora 1982). It essential oils are also used in a variety of bases as topical antiseptics and in treatment of wounds, pimples and boils (Orafidiya *et al*., 2001). The *Ocimum* oil of this plant is active against both Gram positive bacteria (*Staphylococcus aureus, Listeria monocytogenes*) and Gram negative bacteria (*Escherichia coli, Shigella spp., Salmonella spp.* and *Proteus spp*.) and also on *Trichophyton rubrum, T. mentagrophytes, Cryptococcus neoformans, Penicillium spp*. and *Candida albicans* (Lopez *et al.,* 2005; Akinyemi *et al.,* 2004; Nwosu and Okafor 1995).

Ocimum gratissimum is very effective in the management of upper respiratory tract infection, diarrhoea, headache, skin disease, pneumonia, fever, haemorrhoids, rheumatism, paralysis, epilepsy, high fever, sunstroke, influenza, conjuctivities and mental illness (Sulistiarini, 1999; Dekkers *et al.*, 1996; Sofowora, 1993; Oliver, 1980; Dhawan *et al.*, 1977). It has proved to be useful in the medication for people living with Human Immunodeficiency Virus (HIV) and Acquired Immuno Deficiency Syndrome virus AIDs and also for the treatment of gonorrheal infection and mental illness (Akinjogunla *et al.*, 2011). Aguiyi *et al*., 2000 and Egusie *et al.*, 2006, reported the hypoglycaemic effects of plant extracts of Ocimum gratissimum on alloxan-induced and streptozotocin (STZ) induced type-1 model diabetic rats respectively. A recent research by Oguanobi *et al.,* 2012, reported the hypoglycaemic effect of the aqueous extract of *Ocimum gratissimum* on Type-2 model rats. Mann, 2012, reported the antimicrobial and grain protectant potentials of this plant. A combined formulation of *Ocimum* *gratissimum* and Xylopia aethiopica has been reported to be used in the preparation of teas and portions for women during peuperium (Ijeh *et al*., 2005).

Clinical test involving a range of concentrations of *Ocimum gratissimum* oil in comparison to 10% benzoyl peroxide and a placebo for the reduction of acne lesions during a period of four weeks carried out on some students by Orafidiya *et al.,* 2002 showed that 2% *Ocimum gratissimum* in Cetomacrogol is suitable for the management of acne since it had similar acivity as the reference product used. Another clinical test carried out by the same people in 2004 which involved a preparation of *Ocimum gratissimum* and aloe vera gel in 84 subjects with clinically significant acne vulgaris showed that the oil of *Ocimum gratissimum* or its combination with aloe vera gel is effective in the treatment acne vulgaris than1% clindamycin.

Many pharmacological activities of extracts of the *Ocimum gratissimum* plants and its essential oil have been reported by many researchers over the years. The antibacterial activity of different leaves extracts of *O. gratissimum* was tested against *Staphylococcus aureus, Escherichia coli, Salmonella typhi* and *Salmonella typhimurium* have been reported (Adebolu and Salau, 2005). Akinyemi *et al.,* 2004, also reported the antimicrobial activity of both ethanol and aqueous extracts of *Ocimum gratissimum* against *S. aureus* and methicilin resistant *S. aureus* (Nakamura *et al.,* 1999). Alabi *et al.,* 2005, tested the fungitoxic activity of four botanicals and they observed that *O. gratissimum* exhibited mild activity compared to the other three botanicals. Mbata and Saikia, 2007, also reported that *O. gratissimum* oils inhibited the growth of psychrophils and heat resistant organisms and suggested that *Ocimum gratissimum* and its derivatives could be used primarily as food flavouring and for antimicrobial activities (Mbata and Saikia, 2007).

A study carried out to evaluate the ovacidal activity of the essential oils of *Ocimum gratissimum* has also been reported. The results obtained from the study suggested the possible utilization of the essential oil of *O. gratissimum* as an aid to the control of gastrointestinal helmintosis of small ruminants (Pessoa *et al.*, 2002). Another study carried out by Luize *et al.*, 2005, showed that hydroalcholic extract of *O. gratissimum* showed good leishmanicidal activity against *Leishmania amazonensis*. *Ocimum gratissimum* extract at a concentration of 100µg/ml showed a 91.5% inhibition.

The oil of *Ocimum gratissimum* has been reported to be active against several species of bacteria and fungi such as *Listeria monocytogenes, Shigella, Salmonella, Proteus, Trichophyton rubrum, Trichophyton mentagrophytes, Cryptococcus neoformans, Penicillum islandi cum* and *Candida albicans* (Janine de Aquino Lemos *et al*., 2005; Lopez *et al.,* 2005; Akinyemi *et al*., 2004; Nwosu and Okafor, 1995; Begum *et al*., 1993).

In addition to the medicinal uses of *Ocimum gratissimum*, the plant also has culinary uses. In the Southern part of Nigeria, the plant is used as a spice and condiment (Akinmoladun *et al.,* 2007). The leaves of *Ocimum gratissimum* taste like cloves, hence they are widely used for flavoring of vegetables (Choudhury *et al.,* 2011).

2.4 *Ocimum suave*

Ocimum suave is a seasonal, ruderal and anthropophilic plant which is sometimes cultivated (Tan *et al.,* 2011). This aromatic shrub grows specifically at high altitudes (Tan *et al.,* 2013). It is a culinary herb plant that belongs to the family lamiaceae (Umar *et al.,* 2012) which contains aromatics valued for their medicinal and culinary uses. It is commonly called scent leaf. The local Nigerian names are: “Daidoya ta gida” (Hausa), “Efirin-ajija” (Yoruba) and “Ncha-anwu” (Ibo) (Umar *et al.,* 2012).

2.4.1 Plant description and distribution

Ocimum suave is a branched erect shrub which grows about 50-100cm in height. It is often densely and profusely branched with hairy opposite leaves and small, white and sweet-scented flowers (Fichtl and Adi, 1994). It is an exotic West African specie which is highly pubescent, perennial and an under shrub (Bhasin, 2012). The branches and stems of *Ocimum suave* are villous or sub-glabrous which turn red-brown when dry (Tan *et al.,* 2011). Its geographical distribution stretches from tropical Asia to tropical West and East Africa where it is found widespread on mountainous and open waste areas. It is Common in the upland forest areas of East Africa (Hassanali, 1990).

Figure III: *Ocimum suave*
m.wikipedia.org

2.4.2 Scientific classification

Kingdom:	Plantae
Order:	Lamiales
Family:	Lamiaceae
Genus:	*Ocimum*
Species:	*O. suave*
Binomial Name:	*Ocimum suave*

2.4.3 Cultivation

Ocimum suave is an annual shrub which grows at high altitude. It is annually propagated by wind and grazing animals which increases its availability for large scale medicinal use especially since it is not consumed by man or animals as food (Tan *et al.,* 2011)

2.4.4 Medicinal uses

Like other members of the *Ocimum* genus, *Ocimum suave* is also appreciated for its wide array of medicinal uses. The plant has been reported to be used for the treatment of cough, oral thrush, abdominal pain, nasal congestion and inflammation of ear and eye (Bhasin, 2012; Omori *et al.,* 2012).

Ocimum suave is used traditionally to cure different diseases and ailment based on the different countries where it is cultivated. In Ethiopia, fresh leaves of *Ocimum suave* are squeezed and the juice is then snuffed, drunk or inhaled after boiling in water to relieve pain, fever and inflammatory disorders (Makonnen *et al.,* 2003a). In Uganda, *Ocimum suave* is used traditionally to heal wounds while in East Africa, it is used as a cure for ulcers (Hassan *et al.,* 2011; Tan *et al.,* 2008). A formulation containing leaves of *Ocimum suave* and other plants is used to treat fever in children, stomach ache, broncho-pneumonic affections and menstrual pains by the people of Congo Brazaville (Tan *et al.,* 2011). In Kenya, bark of the *Ocimum sauve* stem is used to oral thrush and diarrheoa (Omori *et al.,* 2012)

As reported by Mwagi *et al.,* 1995, *Ocimum suave* was found to have repellant and acaricidal properties against *Rhipicephalus appendiculatus* ticks. The analgesic property of *Ocimum suave* has been reported by Makonnen *et al.,* 2003a using tail flick and hot plate as well as writhing method by Debella *et al.,* 2003. Aqueous and ethanol leaves extracts of *Ocimum suave* have also been reported to exhibit antipyretic activity in yeast induced fever (Makonnen *et al.*, 2003b). It has also been reported to have antioxidant, cytoprotective and ulcer healing potential (Tan *et al.,* 2013), mosquito repellent (Seyoum *et al.,* 2002), wound healing (Hassan *et al.,* 2011), anti-inflammatory (Masresha, 2012), insecticidal (Ojianwuna *et al.,* 2011; Kweka, 2008), hypolipidemic and anti-diabetic (Umar *et al.,* 2012), antimicrobial (Runyoro *et al.,* 2010), Anti-Helicobacter and gastroduodenal cytoprotective (Tan *et al.*, 2011) actions. The effects of the leaf extracts of *Ocimum suave* against HCl/ethanol, absolute ethanol, indomethacin and pylorus ligation ulcer models have also been reported (Tan *et al.,* 2002)

Kitula, 2007 reported that the roots of *Ocimum suave* are boiled to cure abdominal pain, mixture of the pounded leaves of *Ocimum suave* and sheep tail fat is inserted into the anus to cure back ache after delivery and skin rashes is cured by rubbing or drinking pounded leaves of the shrub. Extract of the leaves and succulent stems of *Ocimum suave* have been reported to provide an effective repellence against stored product insect and pests (Bekele *et al.,* 1996). The leaves and seeds of *Ocimum suave* have been found to be very effective repellents against *Anopheles gambiae* Giles while thermal expulsion of *Ocimum suave* showed significant repellence against the main vectors of malaria in Africa (Seyoum et al., 2003; Seyoum et al., 2002). The essential oils of *Ocimum suave* plants have been reported to have a significant impact on repellence and inhibition of feeding against malaria vectors and other insect both in the laboratory and on field (Kweka *et al.,* 2008; Odalo *et al.,* 2005; Omolo *et al.,* 2004).The strongly scented leaves of *O. suave* are used as disinfectant and insecticide (Kokwaro, 1976).

2.5 ANTIOXIDANTS

Antioxidants are chemicals that prevent oxidation. They neutralize the damaging effects of free radicals formed by the normal cell metabolism (Badarinath *et al.,* 2010). A biological antioxidant even when present in low concentration will prevent or delay the oxidation of an oxidizable substrate (Benzie and Strian, 1999). In the late 19th century, antioxidants were used in industrial processes such as the prevention of metal corrosion, the vulcanization of rubber and the polymerization of fuels (Matill, 1947). Antioxidants act using different mechanisms including removal of oxygen or decreasing local oxygen concentrations, removal of catalytic metal ions, removal of reactive oxygen species (ROS) such as the superoxide anions and hydrogen peroxide, scavenging of free radicals that initiate series of reactions such as hydroxyl free radicals and breaking of oxidation sequences (Gutteridge, 1993).

The antioxidant system is made up of the endogenous antioxidant system which comprises of the antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) and the non-enzymatic enzymes (α-toopherol, vitamin C and E). In addition to endogenous antioxidant system, some exogenous antioxidant compounds obtained from diet have been reported to exhibit antioxidant properties which effectively ameliorate the oxidative damage caused by formation of free radicals (Vertuani *et al.*, 2004).

In the 20th century, Phenolic compounds provided the stepping stone for the production of synthetic antioxidants such as gallates, 2,6-di-tert-butyl-4-methylphenol (BHT), tertiary-butyl hydroxyanisole (BHA) and tertiary-butyl hydroquinone (TBHQ) (Sherwin *et al.,* 1990). However, the safety and toxicity of these synthetic antioxidants has often been a point of concern to consumers especially with synthetic anti-oxidants reportedly being implicated in causing liver damage and carcinogenesis (Wichi, 1988; Grice, 1986). These safety concerns prompted an increase in the research and use in natural antioxidants other than the synthetic ones which have been found to be carcinogenic (Ramesh *et al.,* 2011; Sherwin *et al.,* 1990). Research has shown that plants offer a wide range of secondary metabolites whose antioxidant potentials are high (Sanchez-Moreno *et al.*, 1999; Demo *et al.*, 1998).

The prevention of diseases such as cardiovascular diseases, cancer and also decrease in the risk of sudden death from diseases such as arteriosclerosis, diabetes and acute hypertension have all been linked with the intake of many plants products such as vegetables, fruits and teas which are rich in natural antioxidants (Lim *et al.,* 2002; Johnson, 2001).

2.5.1 Natural antioxidants

Recently, manufacturers and researchers have taken advantage of the ability of natural antioxidants to prevent cancer and heart diseases. The health benefits of consuming these natural antioxidants have been touted since each of these natural antioxidants also has potential negative side effects. For instance, Vitamin C which is the most popular and commonly consumed natural antioxidant has many positive health effects (McClain and Jochen, 2003) . Recent studies revealed that it also prevent the oxidation of DNA thus, leading to certain cancers and cardiovascular disease (Goodman, 1980).

The overdose of natural antioxidants is very rare however, it is a definite possibility. When natural antioxidants are taken in greater than certain concentrations, they produce significant and even deadly physiological effects. The concentration of Vitamin C to be ingested to produce an overdose-like effect is approximately 3000mg/day. Exceeding this dose leads to some negative physiological effects. Consuming a dose larger than 3000 mg/day for several days have been reported to lead to kidney stones and an increased need for oxygen. Also increased consumption of Vitamin C can lead to excess uric acid excretion and erosion of dental enamel (Williamson *et al.,* 2000).

Additionally, all polyphenols have exhibited pro-oxidant behavior while some polyphenols have been shown to interfere with the metabolism of certain medicines when taken in excess (Shahidi, 2008). overdoses of tocopherols is one of the more severe forms of dietary supplement overdoses. In doses of 1600-3200 mg/day for extended periods of time, Vitamin A overdoses can lead to symptoms such as fatigue, breast soreness, gastrointestinal stress, vascular inflammation, and thyroid problems (Goodman, 1980). It has been reported that in very high doses for extended periods of time, excess Vitamin A consumption can lead to hypercalacemia,. This condition can be a very serious disease, leading to nausea, dementia, renal failure, and sometimes even death (Kahl, 1984).

Negative side effects of Coq10 have also been reported if taken in excess. It may cause severe hemorrhages if taken in very large quantities and just as with polyphenols, it could act as a pro-oxidant in high concentrations.

2.5.1.1 Endogenous antioxidants

Endogenous antioxidants in cells are classified into the enzymatic antioxidants and the non-enzymatic antioxidants.

Many antioxidant enzymes are directly involved in the neutralization of free radicals such as the Reactive Oxygen Species (ROS) and the Reactive Nitrogen Species (RNS). Superoxide dismutase (SOD) is the first line of action against free radicals. It catalyse the dismutation of superoxide anion radical (O_2^-) into hydrogen peroxide (H_2O_2) through a reduction reaction. The SOD enzymes are found in extracellular fluids and almost all aerobic cells (Bannister *et al.*, 1987). There are different classifications of SOD enzymes which are based on the prosthetic metal they contain such as Manganese SOD which is found in the mitochondrion and Cupper/Zinc SOD found in the cytosol. Others are Iron and Nickel containing SOD (Ho *et al*., 1998).

The next reaction is carried out by the catalases (CAT) which catalyzes the transformation of hydrogen peroxide formed into water and oxygen using an iron or manganese cofactor. It is localized to peroxisomes in most eukaryotic cells (Chelikani *et al.,* 2004; Zamocky and Koller, 1999).

Glutathione peroxidase which is a selenium containing protein removes hydrogen peroxide by using it to oxidize glutathione (GSH) into an oxidized form of glutathione (GSSG), glutathione reductase in turn reduces the oxidized glutathione back to glutathione (GSH) (Pacher *et al*., 2007; Bahorun *et al.*, 2006; Droge, 2002; Young and Woodside 2001).

The non-enzymatic antioxidants which are also known as the metabolic antioxidants are produced by normal metabolism in the body. These are, glutathione, L-arginine, coenzyme Q10, melatonin, uric acid, bilirubin, metal-chelating proteins, transferrin (Willcox *et al.,* 2004; Droge, 2002).

2.5.1.2 Exogenous antioxidants

These are compounds which cannot be produced in the body hence they are obtained from foods or supplements such as vitamin E, vitamin C, carotenoids, trace metals (selenium, manganese and zinc), flavonoids, omega-3 and omega-6 fatty acids, etc. They help the endogenous antioxidants in the neutralization of oxidative stress (Pham-huy *et al.,* 2008). Deficiency of these exogenous antioxidants is one of the numerous causes of chronic and degenerative pathologies. These nutrients have unique structures and antioxidant functions they perform (Donaldson, 2004; Willcox *et al.,* 2004).

Vitamin E is a fat-soluble vitamin with high antioxidant potency which protect cell membranes from damage by free radicals by protection against lipid peroxidation by scavenging lipid peroxyl radical (Niki, 1996). Vitamin E has been reported to prevent some cancers such as the colon, prostate and breast and some cardiovascular diseases such as ischemia, cataract, arthritis and certain neurological disorders (Pham-huy *et al.*, 2008).

Vitamin C, also known as ascorbic acid is a water-soluble vitamin. It is essential for collagen, carnitine and neurotransmitters biosynthesis (Li and Schellhorn, 2007). Vitamin C works synergistically with vitamin E to prevent effects of free radicals and also regenerates the reduced form of vitamin E (Pham-huy *et al*., 2008). Under most physiologic conditions, Vitamin C is present as its deprotonated state known as ascorbate (Sies and Stahl, 1995). Ascorbate functions as metal chelator and oxygen scavenger. It has been reported that, Approximately 3.5 mg of ascorbic acid scavenges the oxygen contained in a $1cm^3$ headspace. It is also effective in trapping both singlet oxygen and superoxide (Kitts, 1997).

Beta-carotene is a fat soluble carotenoids and it is considered a provitamin because it is readily converted to active vitamin A which is essential for vision. It is a strong antioxidant and it works against the singlet oxygen (Pham-huy *et al.,* 2008).

Flavonoids are polyphenolic compounds which are present in most plants. Most plants contain a unique combination of flavonoids, which is why different herbs have very different effects on the body (Hanneken *et al.,* 2006). Over 4000 flavonoids have been identified and classified into different classes such as flavanols, flavanones, flavones, isoflavones, catechins, anthocyanins and proanthocyanidins. Beneficial effects of flavonoids on human health mainly reside in their potent antioxidant activity (Rice-Evans *et al.,* 1996).

2.5.2 Synthetic antioxidants

Synthetic antioxidants are chemically synthesized and are added to food as preservatives to help prevent lipid oxidation (Shahidi *et al.,* 1992). Synthetic antioxidants are of two major categories, the Primary antioxidants and Secondary antioxidants depending on their mode of action. The primary antioxidants react with lipid peroxy radicals converting them to stable products. This group includes chain breakers (or free radical inhibitors) and peroxide decomposers. Secondary antioxidants such as oxygen scavengers suppress the formation of free radicals thereby reducing the rate of chain initiation as well as providing protection against oxidative damage (Lim *et al.,* 2007; Juntachote and Berghofer, 2005; Duh, 1998).

Ever since free radicals were found to be responsible for lipid oxidation, a lot of natural and synthetic compounds have been evaluated for their efficacy as free radical scavengers or inhibitory effects on free radicals. Among all the synthetic compounds evaluated, only four synthetic antioxidants are widely used in food industries such as, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tert-butylhydroquinone (TBHQ) (Buck and Edwards, 1997). Many studies have been carried out towards the development of novel synthetic antioxidants aimed at retarding the effects of free radical induced damage in food products as well as in the human body cells (Hussain *et al.,* 2003).

The most prevalent synthetic antioxidants used in food industries as reported by the National Research Council Food Additive Committee are BHT and BHA. BHA is a mixture of two isomers 2-tertiary-butyl-4-hydroxyanisole and 3-tertiary-butyl-4-hydroxyanisole in a ratio of 9:1. The 3-tertiary-butyl-4-hydroxyanisole isomer is generally considered to be a better antioxidant (Buck and Edwards, 1997). BHA is sold in the form of a white waxy solid available as flakes or tablets while BHT is presented as a white crystalline solid. They are both highly fat-soluble monohydric phenolic antioxidant with bulky hydrocarbon side chains. Both of these additives maintain their functionality even after undergoing processing steps such as baking and frying. They are extensively used in bulk oils as well as oil-in-water emulsions (Devlieghere *et al.*, 2004; Reische, 1998; Pratt, 1996). BHT and BHA are also used in food packaging materials either by direct addition in the waxed inner linings or indirectly as emulsions. BHA and BHT are fairly volatile with their melting points ranging between 48-55ºC and 69-72ºC respectively (Rangan and Barceloux, 2009; Shahidi, 2003).

Arguments about the safety of the use of synthetic antioxidants (Shahidi and Wanasundara, 1992) have lead to a decrease in the use of BHA and BHT in foods due to their potential action as promoters of carcinogenesis (Farag *et al*., 2003). Many researchers have reported the carcinogenicity of BHA in the fore stomach of rodents as well as the cytotoxicity of BHA and BHT (Saito *et al.*, 2003; Sarafian *et al*., 2002; Verhagen *et al.*, 1991). Suggestions have been made on the withdrawal of BHT from use in all food because of its possible adverse effects on the kidney and liver as well as lung tissues of rat (Lanigan and Yamarik, 2002; Siman and Eriksson, 1996). However, some scientists have reported that the metabolism of BHT in rat and man are entirely different to allow a proper hazard assessment of BHT in humans (Verhagen *et al*., 1989).

However it is generally considered that permitted food antioxidants have a considerable safety margin. For instance, the dose for enhancement of carcinogenesis is at least 1500-fold greater than that in human exposure (Whysner *et al.*, 1994; lessof, 1992). Furthermore, several studies showed that BHA and BHT pose no cancer hazard but health benefits in relations to their anticarcinogenic and antimutagenic effects to humans as well as cholesteral oxidation inhibition (Slamenova *et al.,* 2003; Valenzuela *et al.,* 2003; Hirose *et al.,* 1999; Williams *et al.,* 1999).

The negative and positive reports on these synthetic antioxidants have lead many countries to adopted regulations systems to monitor their use such as the Food and Drug Administration (FDA) and the U.S. Department of Agriculture (USDA), the Food and Drug Regulations (National Health and Welfare) of Canada, The European Economic Community (EEC) in Europe and the Food Sanitation Law in Japan (Reische *et al*., 1998; Pratt, 1996).

Tertiary butyl hydroquinone (TBHQ) is also an important synthetic antioxidant used in food industries for the preservation of food items especially those containing frying oils. It is a diphenolic antioxidant which is commercially available as beige colored or white to tan solid. It has similar structural features as BHT and BHA but it is less volatile with a melting point ranging between 126.5–128.5ºC (Shahidi, 2003). TBHQ is an effective replacement to the expensive process of liquid oil hydrogenation which provides increased oxidative stability and color improvement. In addition to its use as a singular additive, TBHQ has been shown to have good synergistic effects when used in combination with BHT and/or BHA which increases the smoke point of fats and oils (Shahidi, 2003; Reische *et al.,* 1998; Yen, 1997; Pratt, 1996). Its stabilizing effects on the food lipids has also been found to be enhanced when used in combination with chelating agents like citric acid in substances such as vegetable oils, animal fats and shortenings in particular. A ternary mixture containing TBHQ, monoacylglycerol citrate (MGC), and ascorbyl palmitate (AP) have been reported to exhibit the highest thermal stability and provides optimum protection for oil during high-temperature processing (Allam and Muhammed, 2002).

Propyl gallate (PG) is the most important and widely used antioxidant among all the other gallates. Just like BHA and BHT, propyl gallate is also a phenol and is prepared by treating of gallic acid with propyl alcohol and which is then distilled to get rid of the excess alcohol. It is available as a white crystalline solid which is barely soluble in water due to the presence of a number of hydroxyl groups in its structure whereas the higher octyl and deodecyl gallate are practically insoluble in water but dissolve easily in fats and oils (Pratt, 1996). When compared to BHT, BHA and TBHQ, Propyl gallate is the most volatile with a melting point range of 146-150ºC (Shahidi, 2003). PG is effective as a stabilizer in vegetable oils and animal fats, and is found in products such as meats, spices and snacks.

However, it is less effective as compared to TBHQ in the preservation of vegetable oils. Just like TBHQ, it has been shown to have good synergistic effects with BHA and/or BHA. It is also used along with chelating agents like citric acid because in the absence of these chelating agents, PG chelates metal ions forming an aesthetically unappealing blue-black complex (Reische *et al.,* 1998; Buck and Edward, 1997).

Octyl gallate (OG) and dodecyl gallate (DG) are odorless white to creamy white crystalline solids. They are the other two gallates used as antioxidants in food (Benzie, 2003; Threlfall, 2002). Like TBHQ, Octyl gallate (OG) is also a diphenol and similarities in their sizes and structures have been reported. Both of these synthetic antioxidants are insoluble in water like TBHQ, BHT and BHA. The boiling points of OG and DG range between 442.9-522.9ºC and 476.7-566.7ºC respectively. They are the least volatile among all other gallates (Naidu, 2000)

As in other synthetic antioxidants, the safeties of TBHQ and gallates have also been questioned. Both drawbacks and benefits of TBHQ and gallates have been reported. TBHQ have been reported to exhibit cell death which has been found to be non typical and it has proved to be cytotoxic toward human monocytic leukaemia cells (Okubo *et al.,* 2003). It has been reported to cause apoptosis and promot DNA damage (Badary *et al.*, 2003; Li *et al.*, 2002; Kong *et al.*, 1998). PG has been reported to suppress humoral immunity (Kim *et al.,* 1996). The co-administration of TBHQ or PG with sodium nitrite promoted fore-stomach carcinogenesis (Miyauchi *et al*., 2002). However both of them have been reported to be beneficial to human health. TBHQ was reported to be effective in the inhibition of cholesterol oxidation (Valenzuela *et al.,* 2003) while PG provided inhibition of food borne pathogens (Kong *et al*., 1998). Anticarcinogenic and antimutagenic activities of TBHQ and PG have also been reported (Hirose *et al.,* 1999; Hirose *et al*., 1998).

2.6 HAEMOSTASIS

The word hemostasis is derived from a Greek word which means the stoppage of blood flow. The process of hemostasis involves cellular and biochemical reactions working together thereby keeping blood in the fluidy state within the veins and arteries and also preventing blood loss by forming blood clot following injury (Hermening, 1997; Rodak, 2002). It consists of a cascade of regulated system which includes the vascular system, coagulation system, fibrinolytic system, platelets, kinin system, serine protease inhibitors, and the complement system (Stiene-Martin *et al.,* 1998). These systems work together when the blood vessel endothelial lining is disrupted by mechanical trauma, physical agents, or chemical trauma to produce clots. The formation of clots stops bleeding which is eventually dissolved through the fibrinolytic process.

The process of hemostasis is classified into primary or secondary process; Primary hemostasis takes place when there are injuries to small vessels. During this process, the vessels injured contract to seal off the wound and then platelets are mobilized, aggregated and they adhere to components of the subendothelium of the vasculature. In other words, the process involves the response of the vascular system and platelets to vessel injury which leads to the formation of a platelet plug. However, the secondary hemostasis is a continuation of the primary hemostatic mechanisms. It involves the response of the coagulation system to vessel injury. It controls bleeding from large wounds and its outcome unlike the primary process is the formation of a thrombus (Stiene-Martin *et al.,* 1998; Ogedegbe, 2002).

2.6.1 Systems of Hemostasis

The hemostatic system is made up of several systems working together as a team namely; the vascular system, coagulation system, fibrinolytic system, platelets, kinin system, serine protease inhibitors, and the complement system (Stiene-Martin *et al.*, 1998).

2.6.1.1 The Vascular System

This system is made up of blood vessels that have endothelial cells in their innermost lining which which forms smooth and unbroken surface that promotes the passage of blood. It also prevents turbulence that may trigger the activation of platelets and plasma proteins. The vascular system has procoagulant, anticoagulant and fibrinolytic properties. Any damage in the vascular system is rapidly repaired so as to maintain blood flow and the integrity of the vasculature. Bleeding through vessel contraction is prevented by the vascular system. It also prevents the diversion of blood flow from damaged vessels and initiation of contact activation of platelets and the activation of coagulation (Hermening, 1997).

2.6.1.2 Coagulation System

The coagulation system involves the interaction between the coagulation factors which leads to the formation of a fibrin clot. It is involved in the conversion of a major component of the acute inflammatory exudates known as the soluble fibrinogen into fibrin. This reinforces the platelet plug formed during primary hemostasis. There are several protein factors which are present in their inactive state in the blood participating in the coagulation system. At least five different components are involved in coagulation process; blood vessels, platelets, plasma coagulation factors and their inhibitors and then the fibrinolytic system (Lewis *et al.,* 2006; Baklaja *et al.,* 2008).

2.6.1.3 Fibrinolytic System

Fibrinolytic system involves the physiological process in which plasmin removes insoluble fibrin clots through enzymatic digestion of the cross-linked fibrin polymers. Plasmin breaks down fibrin and fibrinogen through hydrolysis into fibrin degradation products. This causes local effects on vascular permeability. This process occurs at the same time that healing occurs. Eventually, the cells of the mononuclear phagocytic system phagocytize the fibrin degredation products of the hydrolytic digestion (Turgeon, 1999).

2.6.1.3 Platelets

The hemostatic functions of Platelets are elicited through nuclear fragments derived from the bone marrow megakaryocytes. Platelets consist of 2 major intracellular granules knowns as the α-granules which contains the platelet thrombospondin, fibrinogen, fibronectin, platelet factor 4, vWF, platelet derived growth factor, β-thromboglobulin and coagulation factors V and VIII. The dense granules are made up of Adenosine DiPhosphate (ADP), Adenosine TriPhosphate (ATP) and serotonin. Both intracellular granules are released through the open canalicular system whenever the platelets are stimulated (Esmon, 2001).

The process of plasma aggregation leads to the use of energy sources, lost of their membrane integrity and formation of an unstructured mass known as syncytium. In addition to the plug formation, platelet aggregation releases micro-platelet membrane particles rich in phospholipids and various coagulation proteins which provide a localized environment suitable for plasma coagulation (Rodak, 2002).

2.6.1.4 Kinin System

The activation of the kinin system occurs through the action of the coagulation factor XII. The kinins are peptides made up of 9 to 11 amino acids. The most important vascular permeability factor of theses peptides is known as bradykinin (BK). Bradykinin is a chemical mediator of pain which acts as a cardinal feature of acute inflammation. It is capable of reproducing many inflammatory state characteristics such as changes in local blood pressure, edema and pain which results in vasodilation and increased microvessel permeability (Colman and Schmaier, 1997).

2.6.1.5 Complement System

Complement system play an important role in the inflammation and normal functions of the immune system. Activated complement fragments have the capacity to bind and damage self-tissues. The complement system consists of about 22 serum proteins. These proteins with the aid of antibodies and clotting factors perform an essential role as mediators of both immune and allergic reactions. The complement proteins are involved in reactions which lead to the lysis of cells (Ogedegbe, 2002).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 chemicals/ reagents:

2,2-Diphenyl-2-picrylhydrazyl (DPPH), Vanillin and all other reagents and chemicals were of analytical grade purchased from Sigma-Aldrich chemical Company St. Louis U.S.A.

3.1.2 Equipments

6405 UV-Visible Spectrophotometer (Jenway Ltd, United Kingdom), GF-2000 weighing balance (A&D Instrument limited , Japan), soxhlet apparatus, TLC Plates, Economy Incubator (Gallenkamp, England), RS-232C Weighing balance (A&D Instrument limited , Japan), Water Bath (Grant Instrument Ltd, England), (10×20cm, kieselgel $60F_{254,}$ Merck, Germany), Chromatography Column and Tanks, Gas Chromatography-Mass Spectrophotometer and TLC UV Visualiser.

3.1.3 Plant samples

The leaves of *O. basilicum*, *Ocimum gratissimum* and *Ocimum suave* were collected from gardens around Samaru, Zaria and authenticated at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria where the voucher numbers 1055, 1285 and 1873 were assigned respectively and deposited.

3.1.4 Preparation of plant extracts

The plant samples were rinsed in clean water and homogenized using a blender to obtain a slurry. These were divided into two groups of one kilogram each. The first group of each of the homogenized leaves was poured into 2.5L of distilled water separately for 24 hours at room temperature and filtered.

The second group of plant samples was extracted in a soxhlet extractor using methanol as the extracting solvent.

The filtrates were then concentrated by drying in a water bath maintained at a temperature of 45ºC. These were kept in sealed containers and refrigerated at 2-4ºC until required.

3.2 Methods

3.2.1 Determination of phytochemicals

A) Qualitative Determination of Phytochemicals

Chemical tests were carried out on the plants extracts using standard procedure as described by Sofowora (1993) and Trease and Evans (2002).

Test for Saponins:

Frothing test

The extract (0.5g) was dissolved in 10ml of distilled water. The test tube was stoppered and shaken vigorously for 30 seconds and allowed to stand for 45 minutes. The appearance of frothing which persist on warming indicate the presence of saponins

Test for alkaloids

i) Mayer’s test

The extract (0.5g) was stirred with 5ml of 1% aqueous Hydrochloric acid on a steam bath. 1ml of the filtrate was mixed with a few drops of Mayer’s reagent. The presence of alkaloids was confirmed by the formation of precipitate or turbidity.

ii) Dragendorf test

The extract (0.5g) was stirred with 5ml of 1% aqueous Hydrochloric acid on a steam bath. 1ml of the filtrate was mixed with a few drops of Drangendorf reagent. The presence of alkaloids was confirmed by the formation of a rose red precipitate.

Test for carbohydrates

Molisch's test

Few drops of molisch reagent were added to a little quantity of extract in a test tube and a small quantity of concentrated sulphuric acid was allowed to run down the side of the tube to form a lower purple to violet colour at the interface indicating the presence of carbohydrates.

Test for cardiac glycosides

i) Kella-killiani test

Small amount of the extract was dissolved in glacial acetic acid containing traces of ferric chloride. The test tube was held at an angle of 45º and 1ml of concentrated sulphuric acid was added down the side. A purple ring at the interface confirmed the presence of cardiac glycosides.

ii) Salwoski's test

A 0.5g of extract was dissolved in 2ml of chloroform and a few drops of concentrated sulphuric acid were added to form a lower layer. A reddish-brown colour at interface confirmed the presence of a steroidal ring.

Test for tannins

i) Ferric chloride test

The extract (0.5g) was dissolved in 5ml of water followed by a few drops of 10% ferric chloride. The presence of tannin was confirmed by the formation of a blue-black, green or blue-green precipitate.

ii) Lead sub-acetate test

Few drops of lead sub-acetate were added to a solution of the extract. A coloured precipitate indicated the presence of tannins.

Test for anthraquinones

Borntrager's test

Small portion of the extract was shook with 10ml of benzene and filtered. 5ml of 10% of ammonia solution was added to the filtrate and stirred. The production of a pink-red colour confirmed the presence of free anthraquinones.

Test for flavonoids

i) Shinoda test

A 0.5g extract was dissolved in 1-2ml of 50% methanol. Metallic magnesium chips and 4 to 5 drops of concentrated hydrochloric acid were added. A red or orange colour confirmed the presence of Flavonoids aglycones.

ii) Sodium hydroxide test

Few drops of aqueous sodium hydroxide were added to 5ml of extract, a yellow colouration confirmed the presence of flavonoid.

Test for steroid and triterpenes

i) Lieberman-burchards test

Equal volume of acetic anhydride was added to the extract and 1ml sulphuric acid was added down the side of the test tube. The colour change was observed immediately. Red, pink or purple colour change confirmed the presence of triterpenes while blue or blue-green colour change confirmed the presence of steroids.

B) Quantitative determination of phytochemicals

i) Determination of alkaloids

The alkaloid content of the plant extract was estimated using by the method described by Harbone (1973) with slight modification.

Procedure: The sample (2g) was weighed into a 250ml beaker and 80ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 4hrs. This was filtered and the extract concentrated on a water bath to ¼ of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation is complete. The whole solution was allowed to settle and the precipitate collected, washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid which was dried and weighed.

$$\% \text{ Alkaloid} = \frac{\text{Wt of Alkaloid}}{\text{Wt of Alkaloid}} \times 100$$

ii) Determination of tannins

The tannin content of the plant extracts was determined by the method of Van-Burden and Robinson (1981).

Procedure: The sample (0.5g) was stirred into 50ml of distilled water and shaken for 1hr using a mechanical shaker. This was filtered and made up to 50ml. 5ml of the mixture was pipetted and mixed with 2ml 0.1M $FeCl_3$ in 0.1N HCl and 0.008M Potassium Ferrocyanide. Absorbance of the mixture was measured at 620nm within 10min and this was compared on a standard tannic acid curve.

$$\% \text{ Tannin} = \frac{\text{Conc of Tannic acid} \times \text{Extract Volume} \times 100}{\text{Aliquot Volume} \times \text{Wt of Sample}}$$

3.2.6 Measurement of the free radical scavenging activity using 2,2-diphenyl-2-picrylhydrazyl

The free radical scavenging activity of the plant extracts was determined using a stable free radical 2,2-Diphenyl-2-picrylhydrazyl (DPPH) as described by Brand-Williams *et al.*, (1995).

Principle**:** α,α-Diphenyl-2-picrylhydrazyl (DPPH) contains an odd number of electrons in its structure and is one of the standard stable free radicals widely used for testing preliminary radical scavenging activity of a compound or a plant extract. Antioxidants quench DPPH free radical by providing hydrogen atom or by electron transfer possibly through a free radical attack on the DPPH molecules and converting the purple coloured radical to a yellow coloured product known as 2,2-diphenyl-1-hydrazine or a substituted analogous hydrazine resulting in a decrease in absorbance at 517nm (Yamaguchi *et al.*, 1998). The more rapidly the absorbance decreases, the more potent the antioxidant in the extract is.

Procedure: To 4ml of 0.004% methanol solution of DPPH was added 1ml of various concentration of the plant extract (1000, 500, 250, 125. 62.5, 31.25 µg/ml) and incubated for 30minutes at room temperature after which the absorbance was read at 520nm. The absorbance of the control solution (DPPH solution in the absence of the plant) was also measured. The radical scavenging activity of the plant extracts in percentage at different plant extract concentrations was calculated as follows;

$$\%RSA = \frac{\text{Abs of Control} - \text{Abs of Sample}}{\text{Abs of control}} \times 100$$

The 50% inhibitory concentration IC_{50} was extrapolated from a graph of the percentage radical scavenging activity (% RSA) against the extract concentrations.

3.2.7 Column Chromatography

Column chromatography was carried out as described by Roge *et al.*, 2011. Briefly, a glass column was packed with a slurry of silica gel in hexane and loaded on to the column. The extract to be separated was mixed with small amount of methanol and adsorbed to in silica gel. This was allowed to dry at room temperature then loaded on to the column. This was eluted with different concentrations of hexane, ethyl acetate and methanol in an increasing gradient starting with 100% Hexane.

3.2.8 Thin Layer Chromatography

Thin layer chromatography was performed on a silica gel plate (10×20cm, kieselgel $60F_{254,}$ Merck, Germany) as described by Corpuz et al., 2013.

3.2.9 Gas Chromatography-Mass Spectometry

GC-MS analysis was carried out at the National Research Institute for Chemical Technology (NARICT), Zaria. Interpretation of the mass spectrum obtained from the GC-MS was done using the database of National Research Institute for Chemical Technology (NARICT), Zaria. The mass spectrums of the unknown components were compared with the spectrum of the already known components stored in the NARICT library. The name, molecular weight and structure of the component based on percentage similarity were then ascertained.

3.2.6 Clotting Assay

The determination of the clotting effect of the plant extracts was carried out as described by Tabudlo (1996).

PROCEDURE: The methanolic extract (1g) of each of the plant samples was added differently to 5ml of blood sample in a test tube. This was allowed to stand until clotting was observed and the time noted. This was done in triplicate.

The same procedure was repeated with 2g each of the different extracts. Talcum powder and zinc oxide were used as positive controls.

3.2.7 Statistical Analysis

The data was analyzed by the analysis of variance (ANOVA). The differences between the various extracts were compared using the Duncan Multiple Range Test. The results were expressed as mean ± standard deviation (SD) except where otherwise stated. P value less than 0.05 was taken as significant ($P < 0.05$).

CHAPTER FOUR

4.0 RESULTS

4.1 Recovered Yield of Extracts

The three *Ocimum* species used for this study were extracted differently using methanol and distilled water. Each extraction was carried out in triplicate and the yield of each extraction was determined as a percentage of the net weight of the plant species used for the extraction and is presented in Table 4.1. The aqueous extractions yielded an average percentage of 3.2%, 2.6% and 3.5% for *Ocimum basilicum, Ocimum gratissimum and Ocimum suave* respectively. While the recovered yield for the methanol extractions were 4.9% for *Ocimum basilicum*, 3.8% for *Ocimum gratissimum* and 3.0% for *Ocimum suave.*

Table 4.1: Yield of Aqueous and Methanol Extracts of the Three *Ocimum* species (%)

Plants	Aquoeus Extracts (%)	Methanol Extracts (%)
O. basilicum	3.18 ± 0.02^{b}	4.88 ± 0.30^{c}
O. gratissimum	2.57 ± 0.05^{a}	3.79 ± 0.14^{b}
O. suave	3.50 ± 0.16^{c}	2.98 ± 0.05^{a}

Values are expressed as the Mean±SD of three different determinations (n=3).
Values with different superscripts down the column are significantly different at P<0.05.

4.2 Qualitative Phytochemistry

Chemical tests were carried out to check the presence of different phytochemicals in the extracts (Table 4.2). Test for carbohydrates yielded a positive result in all the plant extracts except the aqueous extract of *O. suave* where it was found negative. Cardiac glycosides, flavonoids, tannins and alkaloids were found to be present in all the plant extracts and none of the plant extracts contain anthraquinones.

Table 4.2: Qualitative Phytochemistry of Aqueous and Methanol Extracts of the Three *Ocimum* Species

Constituents	Inference					
	O. basilicum		*O. gratissimum*		*O. suave*	
	Aqueous Extract	methanol Extract	Aqueous Extract	methanol Extract	Aqueous Extract	methanol Extract
Alkaloids	++	+	++	+	+	+
Anthraquinone	-	-	-	-	-	-
carbohydrate	+	+	+	+	-	+
Cardiac Glycoside	++	++	+	+	+	+
Flavonoids	++	++	++	+	++	+
Saponins	+	+	+	+	+	+
Steroids and Triterpenes	-	+	-	-	-	-
Tannins	++	++	++	++	+	++

++= Positive for two tests   + = Positive for one test   -= Negative for one test

4.3 Quantitative Phytochemistry

Quantitative phytochemistry of flavonoids, alkaloids and tannins were carried out on the aqueous and methanol extracts of the three *Ocimum* species, as shown in Table 4.3. All the plant extracts showed significant ($P<0.05$) difference in the phytochemical content in them. Methanol extract of *Ocimum basilicum* registered the highest flavonoid content (88.28%) while the aqueous extract of *Ocimum basilicum* registered the least flavonoid content (58.84%). The Methanol and Aqueous extracts of *Ocimum suave* showed higher alkaloids and tannins contents of 8.32% and 9.45% respectively.

Table 4.3: Quantitative Phytochemistry of Aqueous and Methanol Extracts of the Three *Ocimum* Species (%)

Extracts	Flavonoids	Alkaloids	Tannins
O. basilicum Aqueous Extract	58.84 ± 0.17^{a}	2.29 ± 0.00^{a}	1.45 ± 0.00^{b}
O. basilicum Methanol Extract	88.28 ± 0.08^{f}	6.71 ± 0.00^{d}	1.05 ± 0.01^{a}
O. gratissimum Aqueous Extract	70.42 ± 0.02^{d}	4.48 ± 0.02^{c}	6.05 ± 0.02^{e}
O. gratissimum Methanol Extract	64.64 ± 0.00^{b}	7.56 ± 0.00^{e}	2.75 ± 0.00^{d}
O. suave Aqueous Extract	80.18 ± 0.07^{e}	2.64 ± 0.04^{b}	9.45 ± 0.04^{f}
O. suave Methanol Extract	67.56 ± 0.00^{c}	8.32 ± 0.01^{f}	2.25 ± 0.00^{c}

Values are expressed as the Mean±SD of three different determinations (n=3). Values with different superscripts down the column are significantly different at $P<0.05$.

4.4 Free Radical Scavenging Ability Using DPPH of the *Ocimum* Species

The antioxidant power of a plant is based on its ability to scavenge DPPH radical which is depicted from a plot of radical scavenging ability in percentage against the concentration of the plant extract. The antioxidant potential of the plant is inversely proportional to the inhibitory concentration in mg/ml (IC_{50}) which is the effective concentration of a plant extract which scavenges 50% of the DPPH radical (Gupta *et al*., 2003).

The IC_{50} of the three *Ocimum* species in comparison to a standard antioxidant (Vitamin C) is shown in table 4.4. Vitamin C possessed an IC_{50} of 0.049mg/ml which is significantly ($P<0.05$) different from the IC_{50} of all the studied *Ocimum* species extracts except the methanol extract of *Ocimum basilicum* which have an IC_{50} (0.580mg/ml) that is significantly ($P>0.05$) similar to that of Vitamin C.

Aqueous extract of *O. basilicum* recorded the highest IC_{50} (79.393mg/ml) while the least IC_{50} (0.580mg/ml).

Table 4.4: Inhibitory Concentrations (IC_{50}) of the Three *Ocimum* Plants Extracted with Distilled Water and Methanol on DPPH

Extracts/Vitamin C	Aqueous Extracts (mg/ml)	Methanol Extracts (mg/ml)
O. basilicum	79.39 ± 16.25^{c}	0.58 ± 0.00^{a}
O. gratissimum	13.48 ± 0.32^{b}	2.30 ± 0.06^{b}
O. suave	72.85 ± 2.06^{c}	4.61 ± 0.03^{C}
Vitamin C	0.05 ± 0.01^{a}	0.05 ± 0.01^{a}

Values are expressed as the Mean±SD of three different determinations (n=3).
Values with different superscripts down the column are significantly different at $P<0.05$.

4.5 Free Radical Scavenging Ability of Column Chromatographic Fractions of *O. Basilicum* Using DPPH.

The IC_{50} of the different fractions obtained from column chromatography of *Ocimum basilicum* is shown on Table 4.5.

Vitamin C, the standard antioxidant used in this study recorded an IC_{50} of 0.067mg/ml and this was found to be significantly ($P<0.05$) different from some of the fractions evaluated. The IC_{50} of fractions A, D, E, F, G, H and I were found to be significantly ($P<0.05$) similar to that of the standard (Vitamin C) used.

The least IC_{50} were observed in fractions F and H with 0.506mg/ml and 0.541mg/ml respectively while fractions B recorded the highest IC_{50} of 47.398mg/ml.

Table 4.5: Inhibitory Concentration (IC_{50}) of the Different Column Fractions of *Ocimum Basilicum* on DPPH

Vitamin C/ Fractions	IC_{50} (mg/ml)
A	$0.95±0.05^{a,b}$
B	$47.40±13.48^{e}$
C	$13.87±1.86^{c}$
D	$2.16±0.01^{a,b}$
E	$1.09±0.00^{a,b}$
F	$0.51±0.28^{a,b}$
G	$1.34±0.57^{a,b}$
H	$0.54±0.00^{a,b}$
I	$1.45±0.01^{a,b}$
J	$10.92±0.37^{c}$
K	$7.65±0.15^{b,c}$
L	$41.00±1.30^{d}$
Vitamin C	$0.07±0.00^{a}$

Values are expressed as the Mean±SD of three different determinations (n=3). Values with different superscripts down the column are significantly different at $P<0.05$.

4. 6 TLC Screening for Antioxidant Activity of Fractions Obtained from Fraction F of *Ocimum Basilicum*

The different fractions obtained from column chromatography of fraction F were spotted on TLC plates. The TLC chromatograms were developed in the following solvent systems

1) Hexane : Ethyl acetate 3:1
2) Hexane : Ethyl acetate 2:3
3) Hexane : Ethyl acetate : Methanol 2:8:1

The spots were visualized by spraying the plates with spray solutions. The spray solutions used include vanillin in sulphuric acid and 10% sulphuric acid.

Plate IV: TLC Chromatogram Showing Column Fractions 3 to 11of Fraction F Sprayed With 10% Sulphuric Acid with Arrows showing Areas of Suspected Antioxidant Compounds

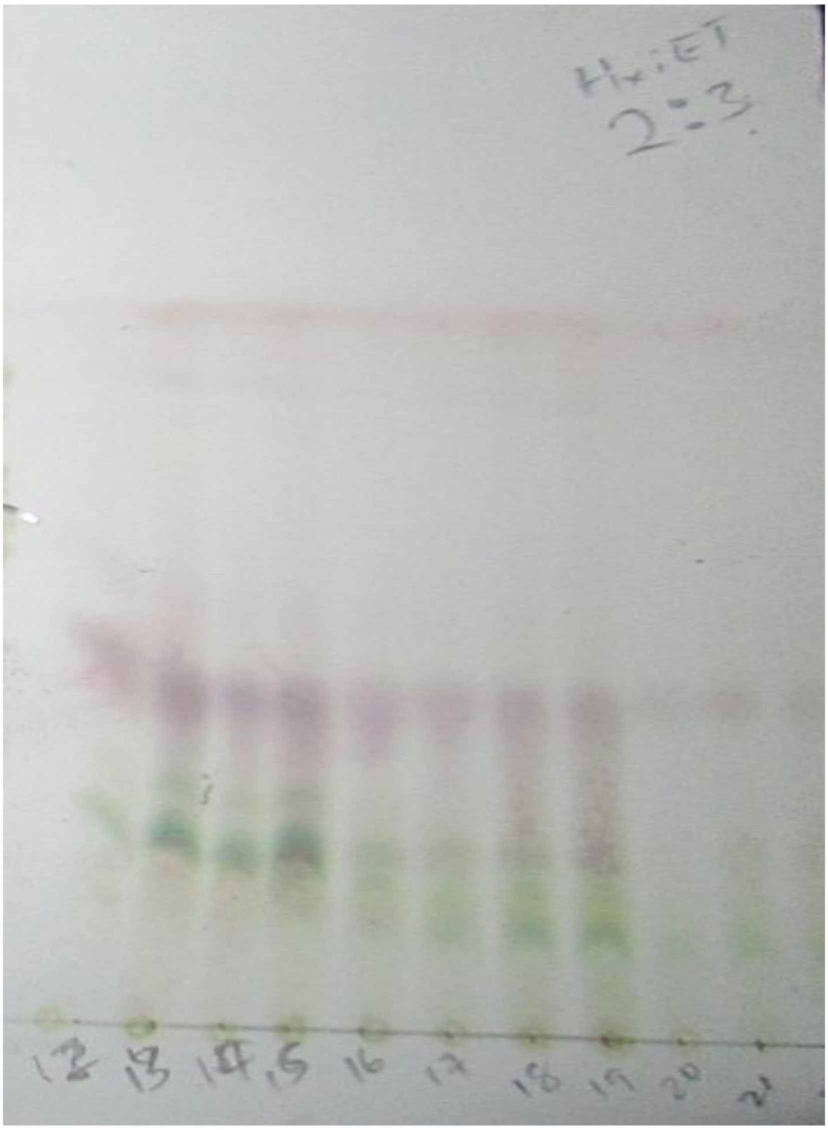


Plate V: TLC Chromatogram Showing Column Fractions 12 to 21 of Fraction F Sprayed with 10% Sulphuric Acid.

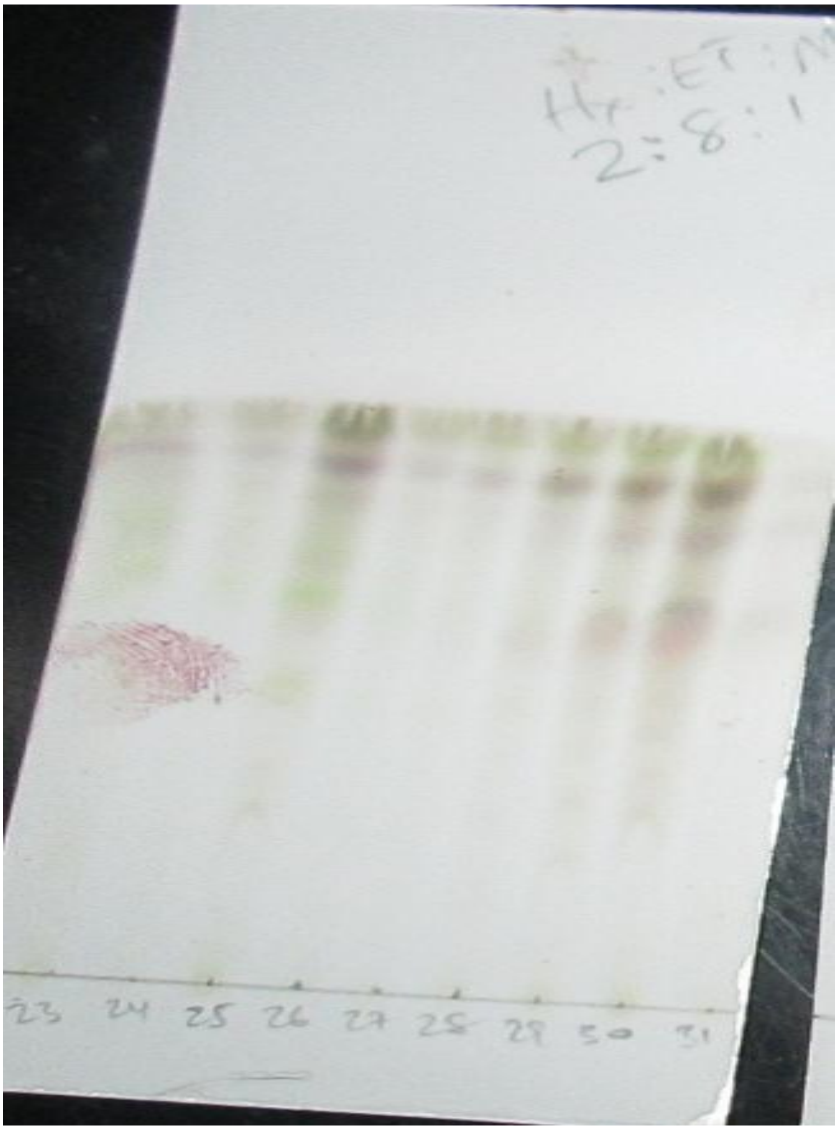


Plate VI: TLC Chromatogram Showing Column Fractions 23 to 31 of Fraction F Sprayed with 10% Sulphuric Acid.

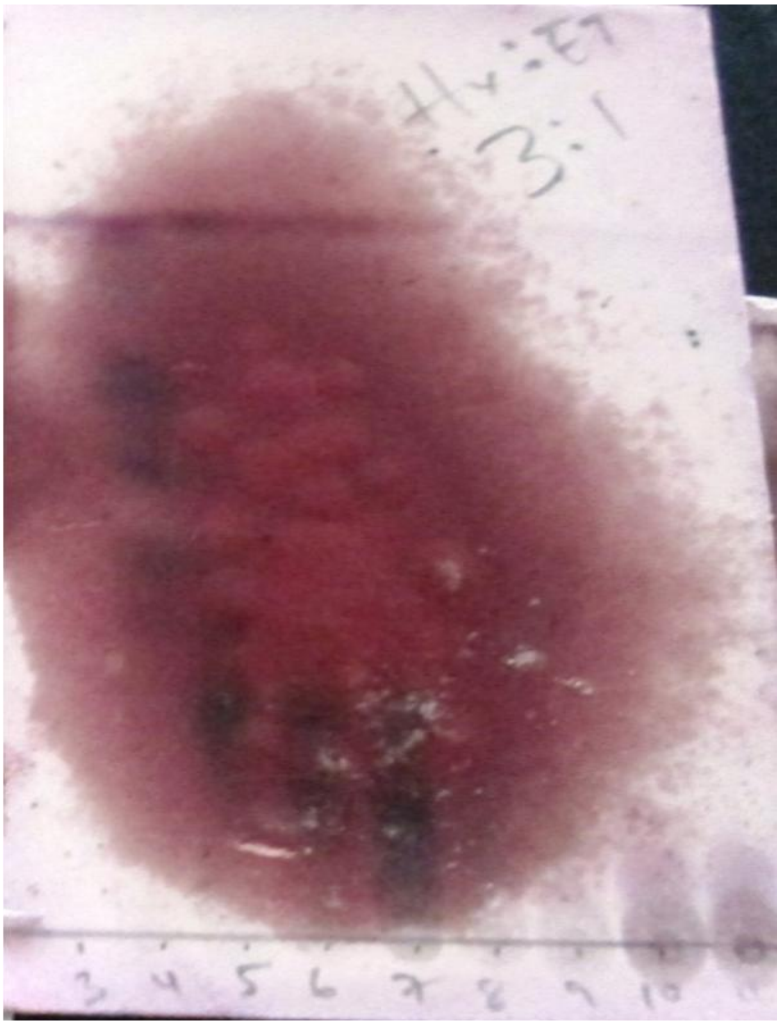


Figure VII: TLC Chromatogram Showing Column Fractions 1 to 11 of Fraction F Sprayed with Vanillin in Sulphuric Acid.

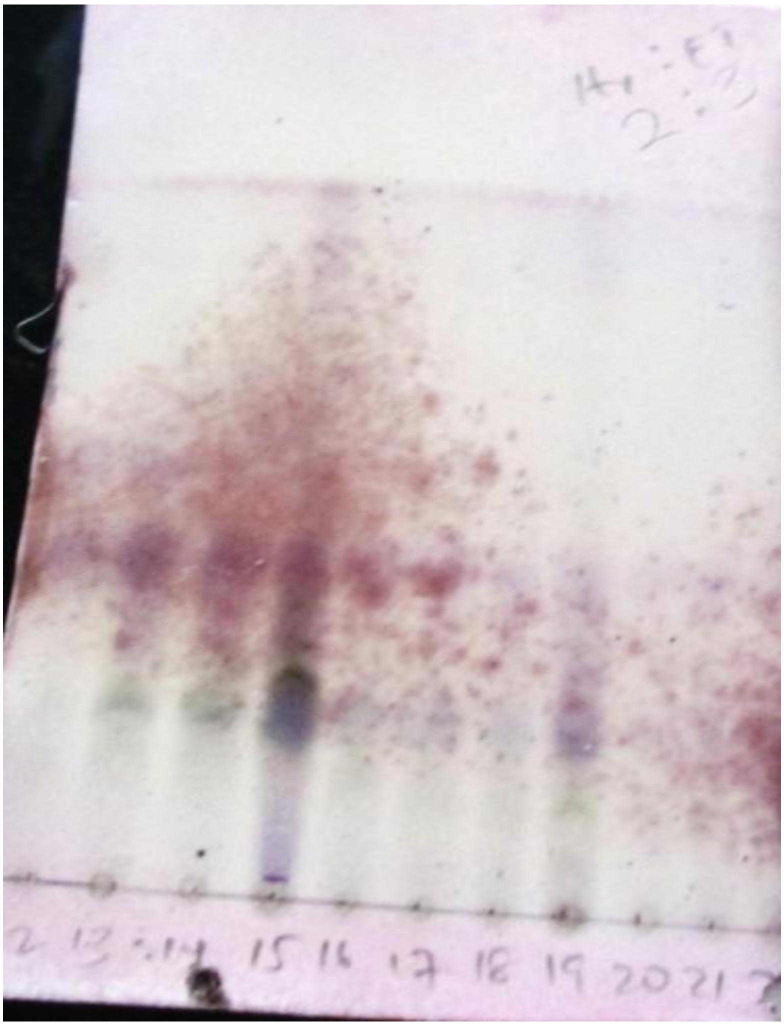


Figure VIII: TLC Chromatogram Showing Column Fractions 12 to 22 of Fraction F Sprayed with Vanillin in Sulphuric Acid.

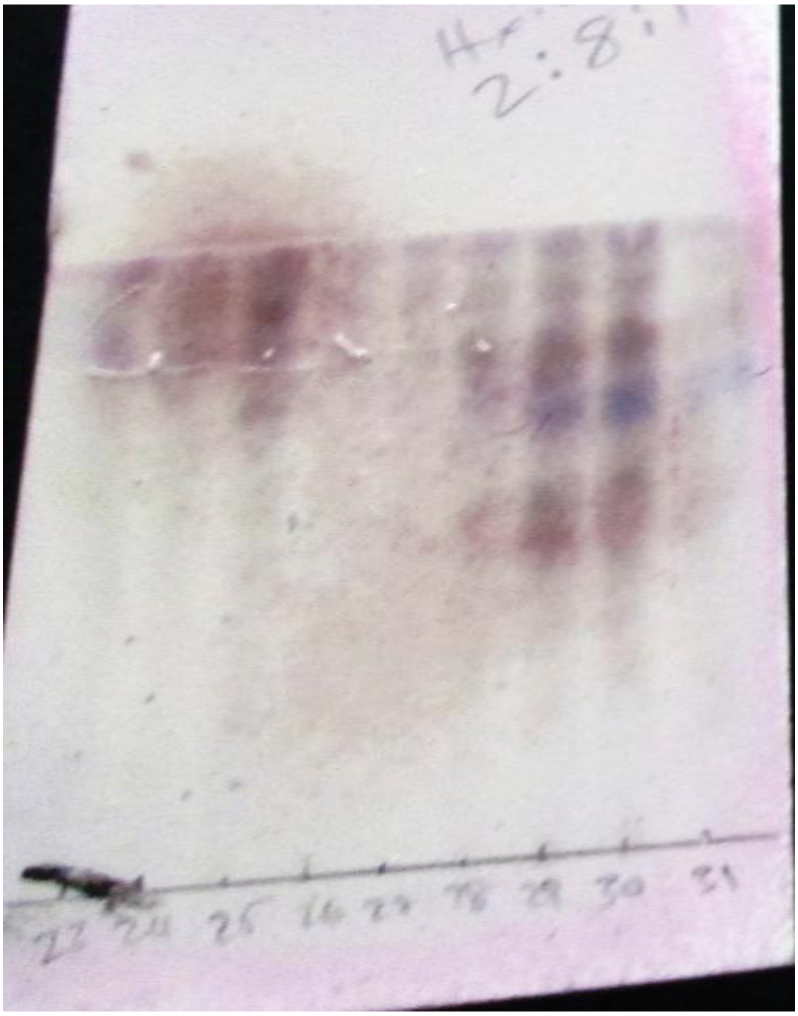


Figure IX: TLC Chromatogram Showing Column Fractions 23 to 31 of Fraction F Sprayed with Vanillin in Sulphuric Acid.

4.7 GC-MS Analysis of Compounds from Chromatographic Fraction F, Sub-Fraction 15 of *Ocimum Basilicum*

The GC-MS study of fresh leaves extract of *O. basilicum* has shown the presence of different groups of compounds such as phenols, esters, alcohols and hydrocarbons which have been linked to the medicinal activity of the plant.

Table 4.6 represents the compounds identified from sub-fraction 15 showing 9-Octadecenal, 1-fluorodecane and 4-methyl-1-decene as the major compounds constituting 37.93%, 11.96% and 10.88% respectively while 1-Dodecene and 1-Tetradecene constituting percentage area of 2.92% and 3.22% showed the least abundant compounds. Other compounds identified include; Phenol, 2,4-bis(1,1-dimethylethyl)- (4.50%), Trifluoroacetic acid n-heptadecyl ester (4.58%), 1-Tetracosanol (5.19%), 11-Octadecanoic acid methyl ester (5.02%), 3-Hexadecyloxycarbonyl-5-(2-hydrxyethyl)-4-methylimidazolium ion (6.72%), 9-Octadecenamide (Z)- (7.08%) in the 1st fraction .

The compounds identified from fraction 2 of the sub-fraction 15 are represented on table 4.7. The major compounds identified are Heptanoic acid 2-methyl-2-butyl ester (32.21%), 9-Octadecenoic acid (Z)- (27.77%) and n-Hexadecanoic acid (19.10%). Other compounds such as 3,4-Altrosan (5.33%), 3-Hydroxybezoic acid (4.88%), 1-(4,5-Dimethyl-2-nitrophenyl)-1H-tetraazole (2.59%), 11-Octadecenoic acid methyl ester (6.23%), Heptacosanoic acid methyl ester (1.24%) and Hexadecanoic acid 2,3-dihydroxypropyl ester (1.31%) were also identified having Heptacosanoic acid methyl ester and Hexadecanoic acid 2,3-dihydroxypropyl ester as the less abundant compounds.

Table 4.6: Compounds Identified from Chromatographic Fraction F,Sub Fraction 15a of *Ocimum basilicum*

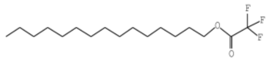
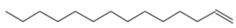
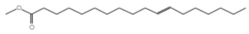
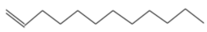
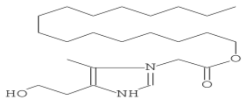
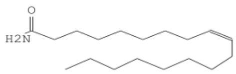
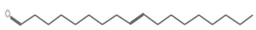
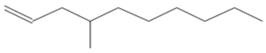
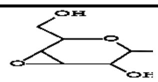
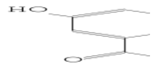
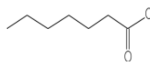
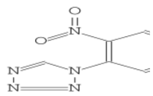
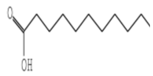
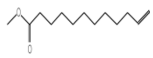
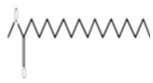
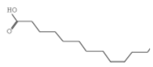
P/No	Retention time	% Intensity	Compound name	Similarity Index	Structure	Molecular weight
1	13.65	4.50	2,4-di-t-butylphenol	76		206
2	16.76	4.58	Trifluoroacetic acid, n-heptadecyl ester	93		324
3	20.54	3.22	1-Tetradecene	92		196
4	22.38	5.19	1-Tetracosanol	94		354
5	22.52	5.02	11-Octadecanoic acid, methyl ester	89		296
6	23.56	2.92	1-Dodecene	89		168
7	25.13	6.72	3-hexadecyloxy carbonyl-5-(2-hydr-oxyethyl)-4-methyl imidazolium ion	85		409
8	26.14	7.08	9-Octadecenamide, (Z)-	89		281
9	26.97	37.93	9-Octadecenal	91		266
10	27.16	11.96	1-flurodecane	84		160
11	27.65	10.88	4-methyl-1-Decene	83		154

Table 4.7: Compounds Identified from Chromatographic Fraction F, Sub-Fraction 15b of *Ocimum basilicum*

P/No	Retention time	% Intensity	Compound Name	Similarity Index	Structure	Molecular Weight
1	14.41	5.33	3,4-Altrosan	72		162
2	15.45	4.88	3-Hydroxybenzoic acid	78		138
3	16.61	32.21	Heptanoic acid,2-methyl-2-butyl ester	79		200
4	17.27	2.59	1-(4,5-dimethyl-2-nitrophenyl)1H-tetrazole	69		219
5	20.84	19.10	n-Hexadecanoic acid	93		256
6	22.51	6.23	11-Octadecanoic acid, methyl ester	90		296
7	22.84	1.24	Heptacosanoic acid, methy ester	90		424
8	23.62	27.11	9-Octadecanoic acid (Z)-	94		282
9	27.64	1.31	Hexadecanoic acid, 2,3-dihydroxy propoyl ester	90		330

4.8 Coagulating Effect of Crude Extracts of the Three *Ocimum* Species

The coagulative effect of the methanol extracts of the *Ocimum* species on blood of Swiss albino rats is presented in Table 4.8. For the control in which no plant extract was used the blood coagulation took an average of 101.33sec. The extracts presented an average coagulation time at different concentrations which ranged between 68.00sec in *O.*

suave to 100.00s in *O. gratissimum*. Hence, *O. suave* showed the most effect on the clotting time. The clotting time of the blood treated with Talcum powder was significantly (P<0.05) different from that of all the studied *Ocimum* species and Zinc Oxide which was also used as a standard for this study. The mean clotting time for the plant extracts were 81.00sec, 100.00sec and 77.00sec at a concentration of 0.2g/ml in *Ocimum basilicum, Ocimum gratissimum* and *Ocimum suave* respectively while at an increased concentration of 0.4g/ml, the mean clotting time of the extracts were 77.33sec, 84.0sec and 68.00sec in *Ocimum basilicum, Ocimum gratissimum* and *Ocimum suave* respectively.

The clotting time of Zinc Oxide was found not to be significantly (P>0.05) different from that of *Ocimum suave* at both concentrations.

Table 4.8: Coagulative Effects of Methanolic Extracts of the Three *Ocimum* Species (sec)

EXTRACTS	0.2g/ml	0.4g/ml
Normal Control	101.33 ± 2.52^{c}	101.33 ± 2.52^{e}
O. basilicum	81.00 ± 2.00^{b}	77.33 ± 2.08^{c}
O. gratissimum	100.00 ± 2.65^{c}	84.00 ± 1.53^{d}
O. suave	77.00 ± 2.00^{b}	68.00 ± 3.06^{b}
Zinc Oxide	81.00 ± 2.65^{b}	68.00 ± 1.00^{b}
Talcum powder	59.67 ± 1.53^{a}	50.00 ± 1.00^{a}

Values are expressed as the Mean±SD of three different determinations (n=3). Values with different superscripts down the column are significantly different at $P<0.05$.

CHAPTER FIVE

5.0 DISCUSSION

A wide range of extracting solvents have been known to hold better chances for the extraction and isolation of biologically active molecules for general screening of bioactivity (Kumar *et al.*, 2008). Thus, two solvents were used for extraction in this study. The Percentage yield of the extracts of *Ocimum* species used for this study using methanol and distilled water ranged between 2.57% to 4.88%. Extraction with methanol showed the highest yield in *Ocimum basilicum* (4.88%) followed by that of *Ocimum gratissimum* (3.79%). The least percentage yield was observed in aqueous extract of *Ocimum gratissimum* (2.57%). Extraction using methanol yielded the highest amount of extract in all the *Ocimum* species except *Ocimum suave* in which the aqueous extraction yielded more (3.50%) than methanol extraction (2.98%). This means that the *Ocimum* species used for this study contain mostly of lipophilic compounds such as waxes, cholorophyll and fatty acids.

Phytochemicals are products of plant metabolism or synthesized by plants as defense system which are either useful or toxic to the human body (Sale and Maji, 2006). Phytochemicals such as polyphenols, quinones, flavonoids, catechins, coumarins and terpenoids in addition to other smaller molecules like ascorbic acid (Vitamin C) and alpha-tocopherol (Vitamin E) have been reported to be the most important dietary antioxidants (Vilioglu *et al.,* 1998).

The result of the preliminary phytochemical screening revealed the presence of tannins, phenolic compounds, terpenoids, saponins and alkaloids in all the plants extracts. In particular the flavonoids, terpenoids and alkaloids have been detected in most of the extracts of the basil grown in Nigeria (Mann, 2012).

The quantitative phytochemistry revealed that all the plants have flavonoids in abundance. The flavonoid content in all the extracts ranged from 64.64% - 88.28% in methanol extract of *Ocimum suave* and *Ocimum basilicum* respectively. Flavonoids fall into the largest group of phytochemicals that have been reported to account for most of the antioxidant potential of many plants. The tannin and alkaloid content ranged between 1.05% - 9.45% and 2.29% - 8.32% respectively. The role of alkaloids and tannins in different types of antibiotics used for the treatment of common pathogens have being reported by many authors (Kubmarawa *et al.,* 2009; Ajayi *et al.,* 2011) and this could be the reason for the use of *Ocimum* species in the treatment of several pathogenic strains and the management of several ailments traditionally (Seung-Jo-Lee *et al*., 2004; Muhammed *et al*., 2007; Onaolapo and Onaolapo, 2012). Moreover, the results obtained from the phytochemical studies are similar to several phytochemical studies reported on *Ocimum basilicum* and *Ocimum gratissimum* grown in Nigeria (Omale et al., 2008). The presence of these phytochemicals could support the traditional medicine uses of most of these *Ocimum* species and the use of their edible leaves in the preparation of soup and tea (Mann, 2012).

Several researchers have reported that the consumption of vegetables and fruits reduce the risk of chronic diseases (Chu et al., 2002). This study was carried out to evaluate the free radical scavenging activity of some members of the *Ocimum* genus which are widely used in the Nigerian households for traditional medicine and culinary uses. The DPPH free radical scavenging activity of the *Ocimum* species ranged between 25.88%-96.39%. The IC_{50} of the standard antioxidant (Vitamin C) used for this study was found to be significantly ($P<0.05$) different from the IC_{50} of all the plant extracts. The IC_{50} of the methanolic extracts were found to be lower than that of the aqueous extracts hence, the methanolic extracts possess better radical scavenging potentials than the aqueous extracts since the antioxidant potential of a plant is inversely proportional to the inhibitory concentration. The differences observed in the free radical scavenging activities of these extracts may be due to insolubility of some of the active compounds of these plants in water (Nwiyi *et al.,* 2009). Methanol, ethanol and their aqueous mixtures (typically 20 - 40%) have been reported to be good antioxidant extracting solvents for a wide range of plants (Iqbal et al. 2005; Chatha et al., 2006; Katsube et al., 2009; Chan et al. 2009).

The DPPH radical scavenging potentials of the fractions obtained from the column chromatography ranged from 27.37%-89.05% in 0.078mg/ml-2.5mg/ml. The IC_{50} of Vitamin C was lower than that of all the bulked fractions. However, fractions A, D, E, F, G, H and I were observed to be significantly similar to the standard antioxidant with fraction F having the lowest IC_{50} next to the standard. Differences observed in the DPPH free radical scavenging activity of the column fractions is likely due to the difference in solubility of the different polyphenols which are responsible for the antioxidant potential of the plant in different solvents. Elution of these compounds is in accordance to their solubity rate.

Different compounds which are suspected to be responsible for the elicitation of different pharmacological effects by *O. basilicum* were identified. The GC-MS of spots 1 and 2 of sub-fraction 15 of the methanol extract of the fresh *O. basilicum* leaves detected 11 and 9 chemical compounds respectively each of which possesses different functions according to their physiological and chemical properties.

Phenolic compounds such as phenolic acids, Flavonoids and tannins have been reported to be the backbone behind the antioxidant potential of medicinal plants. These antioxidant compounds possess diverse biological activities such as anti-inflammatory, anti-atherosclerotic, anti-ageing and anti-carcinogenic potentials which contribute to their antioxidant activity (Chung et al., 1998). Phenols fall into a major group of non-essential dietary components that have been associated with the inhibition of several conditions such as age related degenerative brain disorders, atherosclerosis and cancer (Wang et al., 2009; Cheung et al., 2003). They have been reported to play an important role as antioxidant and antimicrobial (Dar et al., 2012). In the methanol extract of *O. basilicum*, 2,4-bis(1,1-dimethylethyl)-Phenol, 3-Hydroxybenzoic acid, 11-Octadecenoic acid methyl ester and 9-Octadecanoic acid (Z)- were identified. The antioxidant activities of these compounds have been reported by several authors and these have been identified in many plants with antioxidant activity. Hence, they could be part of the active principle of antioxidant activity of *O. basilicum* (Ajayi et al., 2011)

Abdelwahab et al., in 2010 reported 1-dodecene as one of the major components of Etlingera elatior (IC_{50} 0.995mg/ml) with a percentage of 24.31%. Similarly, different publications have reported that other compounds identified from the column fraction of *O.basilicum* such as 1-Tetradecene, 1-Tetracosanol, 1-Dodecene, 13-Docosenoic acid methyl ester, n-Hexadecenoic acid, 3-Hydroxybenzoic acid, 9-Octadecenamide (Z)- and 13-Octadecenoic acid methyl ester have antioxidant, anticancer, anti-inflamatory, fungicidal and anti-microbial (Devi and Muthu, 2014; Omotosho et al., 2014; Rajeswari et al., 2013; Cakmak et al., 2012; Jain and Yadav, 2012; Rajeswari et al., 2012;; Ravikumar et al., 2012; Kumar et al., 2010). Selective inhibition of eukaryotic DNA polymerase activities in vitro by 11-Octadecenoic acid methyl ester was reported by Yoshiyuki et al., in 1996.

The result obtained from the coagulating assay implies that the *Ocimum* species used for this study can shorten the clotting time of blood in vitro as compared to the control in which no plant extract was used. The significant ($p<0.05$) reduction in clotting time by the methanolic extracts of the plant extracts suggest that the extracts contains strong principles with hemostatic property and they may have achieved this effect of blood clotting lowering by stimulating the formation and activation of blood platelets.

A review by Chung et al., in 1998 revealed that tannins have been used to exert many physiological effects which includes the acceleration of blood clotting. Tannic acid is used as a popular home remedy to stop bleeding after the wisdom teeth extraction by applying tea bags in the back of the jaws which is chewed upon with the hope that the tannic acid in the tea stops the bleeding. Also, Ocimum gratissimum has been reported to be used for making of teas and portions which is given to stop post-partum bleeding (Ijeh et al., 2005). Several authors reported the use of *Ocimum suave* and *Ocimum basilicum* in acceleration of wound healing and treatment of ulcers (Tan et al., 2008; Marwat et al., 2011; Hassan et al., 2011).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

- The qualitative and quantitative phytochemistry revealed that all the plant extracts (*O. basilicum, O. gratissimum and O. suave*) are good reservoirs of bioactive compounds such as flavonoids, alkaloids, phenols amd tannins.
- The methanolic extracts of the plants samples showed good free radical scavenging avility on DPPH. However, the methanolic fraction of *O. basilicum* showed a better free radical scavenging activity than the methanolic fractions of *O.gratissimum* and *O. suave* as compared to the standard (Vitamin C).
- Some active compounds were identified from the methanolic fraction of *O. basilicum* which have been reported to have positive effects on free radical causing diseases thus, acting as antioxidants.
- All the methanolic fractions of the plant extracts (*O. basilicum, O. gratissimum and O. suave*) reduced the clotting time of blood obtained from swiss albino rats.

6.2 Conclusion

The *Ocimum* species studied showed some degree of variability in their DPPH radical scavenging activities. The methanolic extracts of the 3 *Ocimum* species (*O. basilicum, O. gratissimum* and *O. suave*) possessed higher antioxidant potentials than the aqueous extracts of the plants. However, the methanolic extract of *O. basilicum* showed higher antioxidant potential than the other two methanolic extracts (*O. gratissimum* and *O. suave*). The antioxidant potentials of all the extracts were significantly ($P<0.05$) different when compared to antioxidant potential of the standard (Vitamin C) with respect to their IC_{50} except the methanolic extract of *O. basilicum* which was statistically ($P>0.05$) similar to the standard. The antioxidant activity of the fractions obtained from column chromatography of *O. basilicum* were nonetheless significantly ($P<0.05$) lower than that of the standard.

The GC-MS study of the fractions obtained from fractionation of *O. basilicum* reveals that it is a good source of bioactive compounds such as Hydrocarbons, Fatty acid esters, Phenols and Phenolic acids. Some of these bioactive compounds identified from the methanol extract of *O. basilicum* have been reported to have good antioxidant potentials. These results hence support the use of *O. basilicum* in traditional medicine as a source of natural food occurring antioxidants.

Results obtained justifies the traditional claim that the plant extracts (*O. basilicum, O. gratissimum and O. suave*) stops nasal bleeding and post-partum bleeding as well as promotes wound healing, since stopping of bleeding may be the first line of action in the physiology of wound healing.

6.2 RECOMMENDATIONS

Over the years, there has been an increase in the use of medicinal plants for the management and treatment of several diseases and illness especially in the rural areas. However the efficacies of these plants are yet to be known. The *Ocimum* species are widely used in rural areas for management and treatment of various diseases and ailments. They have been celebrated for their different medicinal and culinary purposes. In fact, in some parts of the world the *Ocimum* plants are worshiped (Singh et al., 2012). A better understanding of the mechanism of action and the bioactivity of the pure compounds from these plants as well as the use of these compounds instead of their synthetic counterparts should be considered for further studies.

REFERENCES

A.O.A.C. (1984): *Official Methods of Analysis*. Association of Official Analytical Chemists. Washington D. C., USA.

Abayomi, O. E., Kenneth, E. and Mkparu, K. I. (2014). Chemometric Profiling Of Methanolic Leaf Extract of *Cnidoscolus Aconitifolius (Euphorbiaceae)* Using UV-VIS, FTIR and GC-MS Techniques. *Peak Journal of Medicinal Plants Research*, *2*(1), 6-12.

Abdelwhab, S. I., Faridah, Q. Z., Mariod, A. A., Yacoob, M., Abdelmageed, A. H. A. and Khamis, S. (2010). Chemical Composition, Antioxidant and Antibacterial Properties of The Essential Oils Of *Etlingera Elatior* And *Cinnamomum Pubescens Kochummen. Journal of the Science of Food and Agriculture, 90,* 2682-2668.

Abdullahi, M. (2012). Phytochemical Constituents and Antimicrobial and Grain Protectant Activities of Clove Basil (*Ocimum gratissimum L.*) Grown in Nigeria. *International Journal of Plant Research*, *2*(1), 51-58.

Abu, A. H., Ofukwu, R. A., and Mazawaje, D. (2009). A Study of Traditional Animal Health Care in Nasarawa State, Nigeria. *American-Eurasian Journal Sustainaible Agriculture, 3*(3), 468-472.

Adebolu, T. T. and Salau, A. O. (2005). Antimicrobial Activity of Leaf Extracts of Ocimum Gratissimum on Selected Diarrhoea Causing Bacteria in Southwestern Nigeria. *African Journal of Biotechnology*, *4*, 682-684.

Aebi, H. E and Bergmeyer, H.U. (1983). *Methods in enzymatic analysis*. New York Academic press Incorporation, 3, 276-286.

Agnaniet, H., Arguillet, J., Bessieve, J. M. and Menut, C. (2005). Aromatic plant of tropical Central Africa. Part XL-VIL Chemical and Biological investigation of essential oil of Ocimum species from Gabon. *Journal of Essential Oil Research, 17*(14), 466-470.

Aguiyi, J. C., Obi, C. I., Gang, S. S. and Igweh, A. C. (2000). Hypoglycaemic Activity of *Ocimum gratissimum* in Rats. *Fitoterpia*, *71*(4), 444-446.

Ajayi, G. O., Olagunju, J. A., Ademuyiwa, O. and Martins, O. C. (2011). Gas Chromatography-Mass Spectrometry Analysis and Phytochemical Screening of Ethanolic Root Extract of *Plumbago Zeylanica*, Linn. *Journal of Medicinal Plant Research. 5*(9), 1756-1761.

Akinjogunla, O. J., Ekoi, O. H. and Odeyemi, A. T. (2011). Phytochemical Screening and *In-Vitro* Antibacterial Assessment of Aqueous Leaf Extracts of *Vernonia Amygdalina* (Asteraceae) and *Ocimum gratissimum (Lamiaceae*) on Moxifloxacin Resistant *Escherichia coli* Isolated From Clinical and Environmental Samples. *Nature and Science, 9*(7), 42-52.

Akinmoladun, A. C., Ibukun, E. O., Afor, E., Obuotor, E. M. and Farombi, E. O. (2007). “Phytochemical Constituent and Antioxidant Activity of Extract from the Leaves of *Ocimum gratissimum*”. *Scientific Research and Essay, 2*(5), 163-166.

Akinyemi, K. O., Mendie, U. E., Smith, S. T., Oyefolu, A. O. and Coker, A. O. (2004). Screening of Some Medical Plants for Anti-*Salmonella* Activity. *Journal of Herb Pharmocother, 5*(1), 45-60.

Alabi, D. A., Oyero, I. A. and Jimoh, A. N. A. (2005). Fungitoxic and Phytotoxic Effect of *Vernonia amygdalina (L), Bryophyllum pinnantus* Kurz, *Ocimum gratissimum* (Closium) *L. a*nd *Eucalyptna globules* (Caliptos) Labill Water Extracts on Cowpea And Cowpea Seedling Pathogens in Ago-Iwoye, South Western Nigeria. *World Journal of Agricultural Science, 1*, 70-5.

Alexander, R. R. and Grifiths, J. M. (1993a). *Haematocrit*. In Alexander R. R and Grifiths J. M. (Eds.), *Basic biochemical methods.* New York: John Wiley and sons, 186-187.

Alexander, R. R. and Grifiths, J. M. (1993b). *Haemoglobin determination by the cyanomethaemoglobin method.* In Alexander R. R and Grifiths J. M. (Eds.), *Basic biochemical methods.* New York: John Wiley and sons: 188-189.

Aliyu, A. B., Musa, A. M. and Oshaniyi, J. A. (2008). Phytochemical Analysis and Mineral Composition Analysis of Some Medicinal Plants of Northern Nigeria. *Nigerian Journal of Pharmaceutical Sciences, 7*(1), 119.

Allam, S. S. M. and Mohammed, H. M. A. (2002). Thermal Stability of Some Commercial Natural and Synthetic Antioxidants and Their Mixtures. *Journal of Food Lipid, 9*(4), 277-293.

Almeida, I., Alviano, D. S., Viera, D. P., Alves, P. B., Blank, A. F., Lopes, A. H., Alviano, C. S. and Rosa, M. S. (2007). Antigiardial Activity of *Ocimum basilicum* Essential Oil. *Journal of Parasitology Research, 101*(2), 443-452.

Amokaha, R. A., Ubwa, S.T., Otokpa, M. and Shenga, G. (2002). Phytochemical Screening of *Danta sramo* Seeds. *Journal of Chemical Society of Nigeria,* 27(1), 105-107.

Anonymous, 2002

Aprioku, J. S. and Obianime, A. W. (2008). Antioxidant Activity of the Aqueous Crude Extract of *Ocimum gratissimum Linn* Leaf on Basal and Cadmium-induced Serum Levels of Phosphatases in Male Guinea-pigs. *Journal of Applied Sciences and Environmental Management, 12*(4), 33 – 39.

Arhoghro, E. M., Ekpo, K. E. and Ibeh, G. O. (2009). Effect of Aqueous Extract of Scent Leaf (*Ocimum gratissimum*) On Carbon Tetrachloride (CCl4) Induced Liver Damage in Albino Wister Rats. *African Journal of Pharmacy and Pharmacology, 3*(11), 562-567.

Asuquo, O. R., Edet, A. G., Mesembe, O. and Atanghwo, J. (2010). Ethanolic Extracts of *Vernonia amygdalina* and *Ocimum gratissimum* Enhance Testicular Improvement in Diabetic Wistar Rats. *The Internet Journal of Alternative Medicine, 8*(2), 25-32.

Badarinath, A. V., Mallikarjuna, R. K., Madhu, S. C., Ramkanth, S., Rajan, T. V. S. and Gnanaprakash, K. (2010). A Review on *In*-vitro Antioxidant Methods: Comparisions, Correlations and Considerations International. *Journal of PharmTech Research, 2*(2), 1276-1285.

Badary, O. A., Taha, R. A., Gamal-el-din, A. M. and Abdel-Wahab, M. H. (2003). Thymoquinone is a Potent Superoxide Anion Scavenger. *Drug and Chemical Toxicology*, *26*(2), 87–98.

Bahorun, T., Soobrattee, M. A., Luximon-Ramma, V. and Aruoma, O. I. (2006). Free Radicals and Antioxidants in Cardiovascular Health and Disease. *Internet Journal Medical Update, 1,* 1-17

Bailey, L. H. (1924). *Manual of cultivated plants*. New York: MacMillan. 101-103

Banchio, E., Xie, X., Zhang, H. and Pare, P. W. (2009). Soil Bacteria Elevate Essential Oil Accumulation and Emissions in Sweet Basil. *Journal of Agriculture and Food Chemistry, 57*(2), 653–657.

Bannister, J. V., Bannister, W. H. and Rotilio, G. (1987). Aspects of Structure, Functions and Application of Superoxide Dismutase. *Critical Review in Biochemistry, 22*, 111-180.

Baratta, M. T., Dorman, H. J. D., Deans, S. G., Figueiredo, A. C., Barroso, J. G. and Ruberto, G. (1998). Antimicrobial and Antioxidant Properties of Some Commercial Essential Oil. *Flavour and Fragrance Journal*, *13,* 235-234.

Begum, J., Yusuf, M., Chowdhury, U. and Wahab, M. A. (1993). Studies on Essential Oils for Their Antibacterial and Antifungal Properties. Part 1. Preliminary Screening of 35 Essential Oils. *Journal of Scientific Industrial Research, 28,* 25-33.

Behera, B. C., Verma, N., Sonone, A. and Makhija, U. (2006). Determination of antioxidate potential of lichen Usnea ghattensis in vitro. *LWT-Food Science Technology, 39,* 80-85

Bekele, A.J., Obeng-Ofori, D. and Hassanali, A. (1996). Evaluation of *Ocimum suave* (Wild) as a Source of Repellents, Toxicants and Protectants in Storage Against Three Stored Product Insect Pests. *International Journal of Pest Management, 42,* 139-142.

Benzie, I. F. F. and Strian, J. J. (1999). Ferric Reducing/ Antioxidant Power Activity of Biological Fluids and Modified Version of Simultaneous Measurement of Total Antioxidant Power and Ascorbic Acid Concentration. *Methods in Biochemistry, 299,* 15-27.

Benzie, I. F. F. (2003). Evolution of Dietary Antioxidants. *Comparative Biochemistry and Physiology, 136*, 113-126.

Bhasin, M. (2012). Ocimum- Taxonomy, Medicinal Potentialities and Economic Value of Essential Oil. *Journal of Biosphere, 1*, 48-50.

Bhat, K. G. (2003). *Flora of Udupi*. India: Indian Naturalist.

Bhuiyan, M. A. R., Hoque, M. Z. and Hossain, S. J. (2009). Free Radical Scavenging Activities of *Zizyphus mauritiana World Journal of Agricultural Sciences, 5*(3), 318-322.

Brand-Williams, W., Cuvelier, M. E. and Berset, C. (1995). Use of a Free Radical Method to Evaluate Antioxidant Activity. *LWT-Food Science and Technology, 28,* 25-30.

Buck, D. F. and Edwards, M. K. (1997). Antioxidants to Prolong Shelf Life. *Food Technology International*, 29–33.

Bunrathep, S., Palanuvej, C. and Ruangrungsi, N. (2007). Chemical Compositions and Antioxidative Activities of Essential Oils from Four *Ocimum* species Endemic to Thailand. *Journal of Health Research, 3,* 201-206.

Cakmak, Y. S., Aktumsek, A. and Duran, A. (2012). Studies on Antioxidant Activity, Volatile Compound and Fatty Acid Composition of Different Parts of *Glycyrrhiza Echinata L. Excli Journal, 11,* 178-187.

Caliskan, O., Odabas, M. S. and Cirak, C., (2009). The Modeling of the Relation Among the Temperature and Light Intensity of Growth in *Ocimum basilicum L. Journal of Medicinal Plants Research, 3*(11), 967–979.

Chan, E. W. C., Lim, Y. Y., Wong, S. K., Lim, K. K., Tan, S. P., Lianto, F. S. and Yong, M. Y. (2009). Effect of Different Drying Methods on the Antioxidant Properties of Leaves and Tea of Ginger Species. *Food Chemistry. 113,* 166–172.

Chang, X., Alderson, P. G. and Wright, C. J. (2009). Variation in the Essential Oils in Different Leaves of Basil (*Ocimum basilicum L.*) at Day Time. The *Open Horticulture Journal, 2*, 13-16.

Chatha, S. A. S., Anwar, F., Manzoor, M. and Bajwa, J. (2006). Evaluation Of The Antioxidant Activity of Rice Bran Extracts Using Different Antioxidant Assays. *Grassas y Aceites, 57*, 328–335.

Chelikani, P., Fita, I. and Loewen, P. C. (2004). Diversity Of Structures And Properties Among Catalases. *Cell Molecular Life Science, 61*, 192-208.

Cheung, L. M., Cheung, P. C. K. and Ooi, V. E. C. (2003). Antioxidant and Total Phenolics of Edible Mushroom Extracts. *Food Chemistry, 81*, 249-255.

Chiang, L. C., Cheng, P. W., Chiang, W. and Lin, C. C. (2005). Antiviral Activity of Extracts and Selected Pure Constituents of *Ocimum Basilicum*. *Clinical and Experimental Pharmacology and Physiology, 32,* 811-816.

Choudhury, G. B., Behera, M., Jena, P. K. And Tripathy, S. K. (2011). Pharmacognostical and Phytochemical Investigation of Various Tulsi Plants Available In South Eastern Odisha. *International Journal of Research in Pharmaceutical and Biomedical Sciences, 2*(2), 605-610.

Chu, Y., Sun, J., Wu X. and Liu, R. H. (2002). Antioxidant and Antipoliferative Activity of Common Vegetables. *Journal of Agricultural and Food Chemistry, 50,* 6910-6916.

Chung, K. T., Wong, T. Y., Huang, Y. W. and Lin, Y. (1998). Tannins and Human Health: A Review. *Critical Reviews in Food Science and Nutrition, 38,* 421–464.

Cimanga, K., Kambu, K., Tona, L., Apers, S., Bruyne, T., Hermans, N., Totté, J., Pieters, L. and Vlietinck, A. J. (2002). Correlation Between Chemical Composition and Antibacterial Activity of Essential Oils of Some Aromatic Medicinal Plants Growing in the Democratic Republic Of Congo. *Journal of Ethnopharmacology, 79,* 213-220.

Colman, R. W. and Schmaier, A. H. (1997). Contact System: A Vascular Biology Modulator with Anticoagulant, Profibrinolytic, Antiadhesive and Proinflammatory Attributes. *Blood*, 90, 3819-3843.

Corpuz, M. J. A. T., Osi, M. O. and Santiago, L. A. (2013). Free radical scavenging activity of *Sargassum siliquosum* J. G. Agardh. *International Food Research Journal 20*(1): 291-297.

Cristiana, M., Murbach, F., Marcia, O. M. and Mirtes, C. (2006). Effects of Seasonal Variation on The Central Nervous System Activity of *Ocimum gratissimum* L. Essential Oil. *Journal of Ethnopharmacology, 105,* 161-6.

Dalziel, J. M. (1993). Plants Description. *Useful Plants of West Tropical Africa, 3,* 431-433.

Darrah, H. H. (1980). *The cultivated basils*. Buckeye Printing: Independence, MO.

Debella, A., Makonnen, E., Abebe, D., Teka, F. and Kidanemariam, A. T. (2003). Pain Management in Mice Using the Aqueous and Ethanol Extracts of Four Medicinal Plants. *East Africa Medical Journal, 80,* 435-439.

Dekkers, J. C., Dormen, N., Van, L. J. and Kemper, H. C. (1996).The Role of Antioxidants, Vitamins and Enzymes in the Prevention of Exercise Induced Muscle Damage. *Sports Medicine, 21*(3), 213-228

Demo, A., Petrakis, C, H., Kefalas, P. and Boskou, D. (1998). Nutrients Antioxidant in Some Herbs and Mediterranean Plant Leaves. *Food Research International, 32,* 351-354.

Devi, J. A. I. And Muthu, A. K. (2014). Gas Chromatography-Mass Spectrometry Analysis of Bioactive Constituents in the Ethanolic Extract of Saccharum Spontaneum Linn. *International Journal of Pharmacy and Pharmaceutical Sciences, 6*(2), 755-759.

Devlieghere, F., Vermeire, L. and Debevere, J. (2004). New Preservation Technologies: Possibilities And Limitations. *International Dairy Journal, 14,* 273-285.

Dhawan, B. N., Patnaik, G. K., Rastogi, R. P., Singh, K. K. and Tandon, J. S. (1977). Screening of Indian Plants for Biological Activity. *Indian Journal of Experimental Biology, 15*(3), 208-219.

Donaldson, M. S. (2004). Nutrition and Cancer: A Review of the Evidence for an Anti-Cancer Diet. *Nutrition Journal, 3,* 19-25.

Droge, W. (2002). Free Radicals in the Physiological Control of Cell Function Review. *Physiology Review, 82,* 47-95.

Dubey, N. K., Tiwari, T. N., Mandin, D., Andriamboavonjy, H. and Chaumont, J. P. (2000). Antifungal Properties of *Ocimum gratissimum* Essential Oil (Ethyl Cinnamate Chemotype). *Fitoterapia, 71,* 567-569.

Duh, P. D. (1998). Antioxidant Activity of Burdock (*Arctium lappa Linné*): It's Scavenging Effect on Free-Radical and Active Oxygen. *Journal of American Oil Chemists' Society, 75,* 455-461.

Effraim, K. D., Jacks, T. W. and Sodipo, O. A. (2003). Histopathological Studies on the Toxicity of *Ocimum gratissimum* Leave Extract on Some Organs of Rabbit. *African Journal of Biomedical Research, 6,* 21-5.

Egusie, U. G., Adelaiye, A. B., Ibu, J. O. and Egesie, O. J. (2006): Safety and Hypoglycaemic Properties of Aqueous Leaf Extract of *Ocimum gratissimum* in Streptozotocin Induced Diabetic Rats. *Nigerian Journal of Physiological Sciences, 21*(1-2), 31-35

Esmon, C. T. (2001). Role of Coagulation Inhibitors in Inflammation. *Thrombosis and Haemostasis, 86,* 51-56.

Fabbro, S. D. and Nazzi, F. (2008). Repellent effect of sweet basil compounds on *Ixodes ricinus* ticks. *Experimental and Applied Acarology, 45,* 219–228.

Farag, R. S., El-Baroty, G. S. and Basuny, A. (2003). Safety Evaluation of Olive Phenolic Compounds As Natural Antioxidants. *International Journal of Food Science and Nutrition, 54,* 159–174.

Fichtl, R. and Adi, A. (1994). *Honeybee flora of Ethiopia*. Magraf Verlag, 97985 Weikersheim, Germany.

Fraga, C. G., Leibovitz, B. E. & Toppel, A. L. (1988). Lipid Peroxidation Measured as TBARS in Tissue Slices: Characterization and Comparison with Homogenates and Microsomes. *Free Radical, Biology and Medicine, 4,* 155-161

Garcia, L. D., Sandra, P. T., Crespo, M. L. and Leticia F. (1998). Study Farmacognóstico of *Ocimum gratissimum* L. (*Cimarrón Oregano*). *Cuban Journal of Medicinal Plant Review, 3(1*), 31-36.

Gayathri, B., Manjula, N., Vinaykuma, K. S., Lakshmi, B. S. and Balakrishnan, A. (2007). Pure Compound from *Boswellia serrata* Extract Exhibits Anti-Inflammatory Property in Human PBMCs and Mouse Macrophages Through Inhibition of TNF-α, IL-1β,NO and MAPkinases. *International Journal of Immunopharmacology, 7,* 473–482.

Gege-Adebayo, G. I., Igbokwe, V. U., Shafe, M. O., Akintayo, C. O. and Mbaka, D. I. (2013). Anti-Ulcer Effect of *Ocimum gratissimum* on Indomethacin Induced Ulcer And Percentage Of Superoxide Dismutase On Wistar Rats. *Journal of Medicine and Medical Sciences, 4*(1), 8-12.

Genestra, M. (2007). Oxyl Radicals, Redox-Sensitive Signalling Cascades and Antioxidants. *Review of Cellular Signalling*, *19*, 1807-1819.

Gershenzon, J., McConkey, M. E. and Croteau, R. B. (2000). Regulation of Monoterpene Accumulation in Leaves of Peppermint. *Plant Physiology, 122,* 205–213.

Goldfarb, A. H. (1999). Nutritional Antioxidants as Therapeutic and Preventive Modalities in Exercise- Induced Muscle Damage. *Canadian Journal of Applied Physiology*, *24,* 249-266.

Goodman, D.W.S. (1980). Vitamin A Metabolism. Federation Proceedings. *Federation of American Societies for Experimental Biology, 39,* 2716-2722.

Grayer, R. J., Kite, G. C., Veitch, N. C., Eckert, M. R., Marin, P. D., Senanayake, P. And Paton, A. J. (2002). "Leaf Flavonoid Glycosides as Chemosystemic Characters in *Ocimum" Biochemical Systematics and Ecology, 30,* 327-342.

Grice, H. C. (1986). Safety Evaluation of Butylated Hydroxytoluene (BHT) in the Liver, Lung and Gastrointestinal Tract. *Food Chemistry and Toxicology, 24,* 1127-1130.

Gulcin, I., Topal, F., Cakmakci, R., Bilsel, M., Goren, A. C. and Erdogan, U. (2011). Pomological Features, Nutritional Quality, Polyphenol Content Analysis and Antioxidant Properties Of Domesticated and Three Wild Ecotype Forms of Raspberries (*Rubus Ideus L.). Journal of Food Science*, 76, C585-C593.

Gupta, M., Mazumdar, U. K., Sivakumar, T., Vamis, M. L. M., Larkis, S., Sunbathkumar, R. and Mainkadan, L. (2003). Antioxidant and Anti-inflammatory activities of *Acalypha fruticasa*. *Nigerian Journal of Natural Products and Medicine, 7,* 25-29.

Gutteridge, M. C. (1993). Biological Origin of Free Radicals and Mechanisms of Antioxidant Protection. *Chemico-Biological Interactions, 91,* 133-140.

Guyton, A. C. and Hall, J. C. (2000). *Textbook of Medical Physiology*. 10th ed. W. B. Saunders Company Philadelphia. 200-215.

Halliwell, B. (1994). Free Radicals and Antioxidants: A Personal View. *Nutrition Review, 52*, 253-265.

Halliwell, B. (2007). Biochemistry of Oxidative Stress. *Biochemical Society Transasactions*, *35*, 1147-50.

Halliwell, H. (1994). Free Radicals, Antioxidants and Human Disease: Curiosity, Cause or Consequence? *Lancet, 344,* 721-724.

Hanneken, A., Lin, F. F., Johnson, J. and Maher, P. (2006). Flavonoids Protect Human Retinal Pigment Epithelial Cells from Oxidative-Stress-Induced Death. *Investigative Ophthalmology and Visual Science*, *47*, 3164-3177.

Harbone, J. B. (1973). *Phytochemical Methods*. London: Chapman and Hall, Ltd. 49-188.

Harmening, D. M. (1997). *Clinical Hematology and Fundamentals of Hemostasis*. 3rd ed. Philadelphia: 481-508.

Harvey, A. (2000). Strategies for Discovering Drugs from Previously Unexplored Natural Products. *Drug Discovery Today, 5*, 294-300.

Hassan, K. A., Deogratius, O., Nyafuono, J. F., Francis, O. and Engeu, O. P. (2011). Wound Healing Potential of the Ethanolic Extracts of *Bidens pilosa* and *Ocimum Suave*. *African Journal of Pharmacy and Pharmacology 5*, 132-136.

Hassanali, A., Lwande, W., Ole-Sitayo, N., Moreka, L., Nokoe, S. and Chapya, A. (1990). Weevil Repellent Constituents of *Ocimum Suave* Leaves and *Eugenia caryophyllata* Cloves Used as Grain Protectants in Parts of Eastern Africa. *Discovery and Innovation, 2*(2), 91-95.

Hippeli, S. and Elstner, F. E. (1999). Transition Metal Ion Catalyzed Oxygen Activation During Pathogenic Processes. *FEBS Letters, 443, 1-7.*

Hirose, M., Ito, T., Takahashi, S., Ozaki, M., Ogiso, T., Nihro, Y., Miki, T. and Shirai, T. (1998). Prevention of Synthetic Phenolic Antioxidants Of *2-Amino-3,8-Dimethylimidazo[4,5-F]Quinoxaline(Melqx)-* Or Activated Melqx- Induced Mutagenesis And Melqx-Induced Rat Hepatocarcinogenesis, And Role Of Antioxidant Activity In The Prevention Of Carcinogenesis. *European Journal for Cancer Prevention, 7,* 233–241.

Hirose, M., Takhashi, S., Ogawa, K., Futakuchi, M., Shirai, T., Shibutani, M., Uneyama, C., Toyoda, K. and Iwata, H. (1999) Chemoprevention of Heterocyclic

Amine-Inducedcarcinogeneses by Phenolic Compounds in Rats. *Cancer Letters, 143*, 173–178.

Ho, Y. S., Magnenat, J. L., Gargano, M. and Cao, J. (1998). The Nature of Antioxidant Defense Mechanisms: A Lesson from Transgenic Studies. *Environmental Health Perspectives, 106*(5), 1219-1228.

Holetz, F. B., Ueda-Nakamura, T., Filho, B. P. D., Cortez, D. A. G., Morgado-Diaz, J. A. and Nakamura, C. V. (2003). Effect of Essential Oil of Ocimum Gratissimum on the Trypanosomatid *Herpetomonas Samuelpessoai*. *Acta Protonzoological, 42*, 269-276

Hussain H. H., Babic G., Durst T., Wright J. S., Flueraru M., Chichirau A. and Chepelev L. L. (2003). Development of Novel Antioxidants: Design, Synthesis and Reactivity. *Journal of Organic Chemistry, 68*(18), 7023–7032.

Hussain, A. I., Anwar, F., Sherazi, S. T. H., Przybylski, R. (2008). Chemical Composition, Antioxidant and Microbial Activities of Basil (*Ocimum basilicum*) Essential Oils Depends on Seasonal Variations. *Food Chemistry, 91, 131-137*

Ijeh, I. I., Omodamiro, O. D. and Nwanna, I. J. (2005). Antimicrobial Effects of Aqueous and Ethanolic Fractions of Two Spices, *Ocimum gratissimum* and *Xylopia aethiopica. African Journal of Biotechnology, 4,* 953-6.

Illoh, H. C. and Awojide, A. O. (2000). Systematic Folliar Anatomy of Four Species of *Bauhinia* Linn Occurring in Nigeria. *Plant Science Resources Communication, 1*(1), 13-22.

Ilori, M.O., Sheteolu, A. O., Omonigbehin, E. A. and Adeneye A.A. (1996). Antidiarrhoeal Activities of *Ocimum gratissimum (Lamiaceae). Journal of Diarrhoeal Disease Research, 14,* 283-286.

Imlay, J. A. and Linn, S. (1998). DNA Damage and Oxygen Radical Toxicity. *Science, 240,* 1302-1309.

Iqbal, S., Bhanger, M. I. and Anwar, F. (2005). Antioxidant Properties and Components of Some Commercially Available Varieties of Rice Bran in Pakistan. *Food Chemistry, 93,* 265–272.

Iwu, E. C. (1993). Ethnobotanical Importance of Some Nigerian Plants. *Journal of Ethnomedicine, 55,* 893-906.

Jain, R. and Yadav, N. D. (2012). Phytochemical Constituents and Antioxidant Activity of Various Fractions of Guazuma Tomentosa Root Heartwood. *International Journal of Phytomedicine, 4,* 304-309.

Janine De Aquino, L., Xisto, S. P., Orionaida De Fatima, L. F., Realino, De Paula, J., Pedro, H. F., Hasimoto, De Suza, L. K., Aline De Aquino, L. and Maria De Rosario, R. S. (2005). Antifungal Activity from Ocimum Gratissimum L. Towards Cryptococus Neoformans. *Memorias do Instituto Oswaldo Cruz, 1*(100), 55-58.

Javanmardi, A., Khalighi, A., Kashi, A., Bais, H. P., Vivanco, J. M., (2002). Chemical Characterization of Basil (*Ocimum Basilicum L.)* Found in Local Accessions and Used in Traditional Medicines in Iran. *Journal of Agriculture and Food Chemistry, 50,* 5878−5883

Jayaprakasha, G. K., Singh, R. P. and Sakariah, K. K. (2001). Antioxidant Activity of Grape Seed (*Vitis vinifera*) Extracts on Peroxidation Models *In Vitro*. *Food chemistry, 73,* 285-290.

Jesberger, J. A. and Richardson, J. S. (1991). Oxygen Free Radicals in Brain Dysfunction. *International Journal of Neuroscience, 57,* 1-17.

Johnson, I. T. (2001). *Antioxidants and Antitumor Properties*. Cambridge: Woodhead Publishing Limited. 100-123.

Juntachote, T. and Berghofer, E. (2005). Antioxidative, Properties and Stability of Ethanolic Extracts of Holy Basil and Galangal. *Food Chemistry, 92,* 193-202.

Kahl, R. (1984). Synthetic Antioxidants: Biochemical Actions And Interference With Radiation, Toxic Compounds, Chemical Mutagens And Chemical Carcinogens. *Toxicology, 33,* 185-228.

Kalita, J. and Khan, M. L. (2013). Commercial Potentialities of Essential Oil of *Ocimum* Members Growing in North East India. *International Journal of Pharmacy and Life Sciences*, *4*(4), 2559-2567.

Karlsson, J. (1997). *Introduction to Nutraology and Radical Formation. In: Antioxidants and Exercise.* Illinois: Human Kinetics Press. 1-143.

Kashyap, C. P., Ranjeet, K., Vikrant, A. and Vipin, K. (2011).Therapeutic Potency of *Ocimum Kilimandscharicum Guerke* - A Review. *Global Journal of Pharmacology*. *5*(3), 191-200.

Katsube, T., Tsurunaga, Y., Sugiyama, M., Furuno, T. and Yamasaki, Y. (2009). Effect of Air-Drying Temperature on Antioxidant Capacity and Stability of Polyphenolic Compounds in Mulberry (*Morus alba L.*) Leaves. *Food Chemistry, 113,* 964–969.

Kaurinovic, B., Popovic, M., Vlaisavljevic, S. and Trivic, S. (2011). Antioxidant Capacity of *Ocimum basilicum L*. and *Origanum vulgare L*. Extracts. *Molecules, 16,* 7401-7414.

Kim, H. M., Han, S. B., Chang, W. I., Hyun, B. H., Oh, G. T., Ahn, C. J. and Cha, Y. N. (1996). Selective Suppression of *In Vitro* T-Dependent Humoral Immunity by Synthetic Food Additives Antioxidants. *Journal of Toxicological Sciences, 21*(1), 41–45.

Kitts, D. D. (1997). An Evaluation of the Multiple Effects of the Antioxidant Vitamins. *Trends Food Science and Technology, 8,* 198-203.

Kitula, R. A. (2007). Use Of Medicinal Plants For Human Health In Udzungwa Mountains Forests: A Case Study Of New Dabaga Ulongambi Forest Reserve, Tanzania. *Journal of Ethnobiology and Ethnomedicine, 3*(7), 1-4.

Klein, B. P. and Perry, A. K. (1982). Ascorbic Acid and Vitamin A Activity in Selected Vegetables from Different Geographical Areas of The United States. *Journal of Food Sciences, 47,* 941-945.

Klimankova, E., Holadova, K., Hajslova J., Cajka, T., Poustka, J. and Koudela, M. (2008). Aroma profiles of five basil *(Ocimum basilicum L.*) Cultivars grown under conventional and organic conditions. *Food Chemistry, 107,* 464–472

Kokwaro, J. A (1976). *Medicinal Plants of East Africa.* Kenya Literature Bureau, Nairobi. P 384.

Kong, A. N., Yu, R., Lei, W., Mandlekar, S., Tan, T. H. And Ucker, D. S. (1998). Differential Activation of MAPK and ICE/ Ced-3 Proteasein Chemical Induced Apopyosis: The Role of Oxidative Stress in the Regulation of Mitogen-Activatedprotein Kinases (Mapks) Leading to Gene Expression and Survival or Activation of Caspasses Leading to Apoptosis. *Restoration Neurology and Neuroscience, 12,* 63–70.

Kothari, S. K., Bhattacharya, A. K. And Ramesh,, S. (2004). Essential Oil Yield and Quality of Methyl Eugenol Rich *Ocimum tenuiflorum* L.f. (syn. *O. santum* L) Grown In South India As Influenced By Method Of Harvest. *Journal of Chromatography a, 1054,* 67-72.

Kpoviessi, B. G. H., Ladekana, E. Y, Kpoviessia, D. S. S., Gbaguidi, F., Yehouenou, F., Quetin-Leclercq, J., Figueredoe, G., Moudachirou, M. and Accrombessia, G. C. (2012). Chemical Variation of Essential Oil Constituents of *Ocimum gratissimum* L. from Benin and Impact on Antimicrobial Properties and Toxicity against Artemiasalina Leach. *Chemistry and Biodiversity, 9,* 139-150.

Kubmarawa. D., Khan, M. E., Punah, A. M and Hassan, M. (2009) Phytochemical and Antimicrobial Screening of *Ficus platyphylia* Against Human/Animal Pathogens. *The Pacific Journal of Science and Technology, 10*(1), 382-386.

Kumar, P. P., Kumarave, L S. and Lalitha, C. (2010). Screening of Antioxidant Activity, Total Phenolics and GC-MS Study of *Vitex negundo*. *African Journal of Biochemistry Research, 4*(7), 191-195.

Kumar, S., Kumar, D. and Prakash, O. (2008). Evaluation of Antioxidant Potential, Phenolic and Flavonoid Contents of *Hibiscus tiliaceus* Flowers. *Electronic Journal of environmental, agriculture and food chemistry, 7*(4), 2863-2871.

Kweka, E. J., Mwang'onde, B. J., Mahande, M. J., Mosha, F. W., Kimaro, E. E., Msangi, S., Lyaruu, L. and Mahande, A.M. (2008). Evaluation of *Ocimum Suave* Essential Oil Against Anthropophilic Malaria Vector *Anopheles gambiae* S.S after Ten Years of Storage. *Tanzania Journal of Health Research, 11*(2), 94-96.

Kweka, E.J., Mosha, F., Lowassa, A., Mahande, A., Kitau, J., Matowo, J., Massenga, C., Tenu, F., Feston, F., Lyatuu, E., Mboya, M., Mndeme, R., Chuwa, G. and Temu, E. A. (2008). Ethnobotanical Study of Some of Mosquito Repellent Plants in North-Eastern Tanzania. *Malaria Journal, 7,* 1-9.

Ladipo, M. K., Doherty, V. F. and Kanife, U. C. (2010). Phytochemical Screening and Antibacterial Investigation of The Extract of *Ocimum gratissimum* (Scent Leaf) on Selected *Enterobacteriaceae. Production Agriculture and Technology, 6*(2), 75-84

Lai, P. K. and Roy, J. (2004). Antimicrobial and Chemopreventive Properties of Herbs and Spices. *Current Medicinal Chemistry, 11*(11), 1451-1460.

Lanigan, R. S. and Yamarik, T. A. (2002). Final Report on the Safety Assessment of BHT (1). *International Journal of Toxicology, 21,* 19–94.

Laskar, S. and Majumdar, S. G. (1998). Variation of Major Constituents of Essential Oil of Leaves of *Ocimum santum linn. Journal of Indian Chemical Society, 65,* 301-302

Lawrence, B. M. (1993*). Labiatae* oils; mother nature's chemical factory. Illinois: Allured Publishing. 188–206.

Leal, P. F., Chaves, F. C. M., Ming, L. C., Petenate, A. J. and Meireles, M. A. A. (2006). Global yields, Chemical Compositions and Antioxidant Activities of Clove Basil (*Ocimum gratissimum* L.) Extracts Obtained By Supercritical Fluid Extraction. *Journal of Food Process Engineering, 29*(5), 547–559.

Lessof, M. H. (1992). Reaction to Food Additives. *Journal of Royal Society of Medicine, 85,* 513–515.

Lewinsohn, E., Ziv-Raz, I., Dudai, N., Tadmor, Y., Lastochkin, E., Larkov, O., Chaimovitsh, D., Ravid, U., Putievsky, E., Pichersky, E. and Shoham, Y. (2000). Biosynthesis of estragole and methyl-eugenol in sweet basil (*Ocimum basilicum* L). Developmental and chemotypic association of allylphenol O-methyltransferase activities. *Plant Science, 160,* 27-35.

Lewis, S. M. and Decie, W. M. (2002*). Practical Haematology*. Philadelphia Lippincott: Elsevier Science Press. 13(3), 340-412.

Lewis, S. M., Bain, B. J. and Bates, I. (2006). *Dacie and Lewis Practical Haematology*. Philadelphia: Churchill Livingstone.

Li, Y. and Schellhorn, H. E. (2007). New Developments and Novel Therapeutic Perspectives for Vitamin C. *Critical Review in Nutrition, 137,* 2171-2184.

Li, Y., Seacat, A., Kuppusamy, P., Zweier, J. L., Yager, J.D. and Trush, M. A. (2002). Copper Redox Dependent Activation of 2-Tert-Butylhydroquinone: Formation of Reactive Oxygen Species and Induction of Oxidative DNA Damage in Isolated DNA and Cultured Rat Hepatocytes. *Mutation Research, 518,* 123–133.

Lim, K. T., Llu, C. and Kitts, D. D. (2002). Antioxidant Activity of a *Rhus vemiciflu*a Stokes In Ethanol Extract. *Food and Chemical Toxicology, 39,* 229-239.

Lim, Y. Y., Limt, T. and Tee, J. J. (2007). Antioxidant Properties of Several Tropical Fruits: A Comparative Study. *Food Chemistry. 103,* 1003-1008.

Lopez, P., Sanchez, K., Batlle, R. and Nerin, C. (2005). Solid and Vapour Phase Anti-Microbial Activities of Six Essential Oils Susceptibility of Selected Food Borne Bacterial and Fungal Strains. *Journal of Agric Food Chemistry, 17(*53), 6939-6946.

Luize, P. S., Tiuman, T. S. and Morello, L. G. (2005). Effects of Medicinal Plant Extracts on Growth of *Leishmania amazonensis* (L.) and *Trypanosoma cruzi*. Brazilian *Journal of Pharmarceutical Science,. 41,* 85-95.

Makonnen, E., Debella, A., Abebe, D. and Teka, F. (2003a). Analgesic Properties of Some Ethiopian Medicinal Plants in Different Models of Nociception in Mice. *Phytotherapy Research, 17,* 1108-1112.

Makonnen, E., Debella, A., Zerihun, L., Abebe, D. and Teka, F. (2003b). Antipyretic Properties of the Aqueous and Ethanol Extracts of the Leaves of *Ocimum suave* and *Ocimum lamiifolium* In Mice. *Journal of Ethnopharmacology, 88,* 85-91.

Mann, A. (2012). Phytochemical Constituents and Antimicrobial and Grain Protectant Activities of Clove Basil (*Ocimum gratissimum* L.) Grown in Nigeria. *International journal of plant research, 2*(1), 51-58

Maria, G., Zinaida, B., Elvira, G., Spac, A., Ganea, A. and Botnarenco, P. (2012). Differences in Essential Oil Content and Chemical Composition of New *Ocimum basilicum* Genotypes in Relation to Some Quantitative Characters. *Journal of Medicinal Plants Research, 6*(8), 1447-1454

Marotti, M., Piccaglia, R. and Giovanelli, E. (1996). Differences in Essential Oil Composition of Basil (*Ocimum basilicum* L.) Italian Cultivars Related to Morphological Characteristics. *Journal of Agricultural Food Chemistry, 44,* 3926–3929.

Martin, J. P., Dailey, M. and Sugarman, E. (1987). Negative and Positive Assays of Superoxide Dismutase Based on Heamatoxylin Autoxidation, *Archive of Biochemistry and Biophysics,* 255, 329-336.

Martin, K. W. and Ernst, E. (2004). Herbal Medicines for Treatment of Fungal Infections: A Systematic Review of Controlled Clinical Trials. *Mycoses, 47,*87-92.

Marwat, K. S., Khan, A. M., Akbari, H. A., Shoaib, M. and Shah, A. M. (2011). Interpretation and Medicinal Potential of Ar-Rehan (*Ocimum basilicum* L)-A Review. *American-Eurasian Journal of agriculture and Environmental Sciences, 10*(4), 478-484.

Masresha, B., Makonnen, E. and Debella, A. (2012). *In Vivo* Anti-Inflammatory Activities of *Ocimum suave* in Mice. *Journal of Ethnopharmacology, 142*(1), 201-205.

Matasyoh, L. G., Josphat, C. M, Francis, N. W., Miriam, G. K., Anne, W. T. M. and Titus, K. M. (2007). Chemical Composition and Antimicrobial Activity of the Essential Oil of *Ocimum gratissimum* L. Growing in Eastern Kenya. *African Journal of Biotechnology, 6,* 760-765.

Matekaire, T. and Bwakur, T. M. (2004). Ethnoveterinary Medicine: A Potential Alternative to Orthodox Animal Health Delivery in Zimbabwe. International *Journal of Applied Research in Veterinary medicine, 2*(4), 269-273.

Matill, H. A (1947). Antioxidants. *Annual Review of Biochemistry,16,* 177-192.

Mbata, T. I. and Saikia, A. (2007). Antibacterial Activity of Essential Oil from *Ocimum gratissimum* on Listeria Monocytogenes. *International Journal of Food Safety, 7,* 15-19.

McClain, R. M. and Jochen, B. J. (2003). Summary of Safety Studies Conducted with Synthetic Lycopene. *Regulatory Toxicology and Pharmacology, 37,* 274-285.

Miyauchi, M., Nakamura, H., Furukawa, F., Son, H. Y., Nishikawa, A. and Hirose, M. (2002). Promoting Effects of Combined Antioxidants And Sodium Nitrite Treatment On Fore Stomach Carcinogenesis In Rats After Initiation With N-Methyl- N′-Nitro-N-Nitrosoguanidine. *Cancer Letters, 178,* 19–24.

Momen, F. M. and Amer, S. A. A. (2003). Influence of the Sweet Basil, (*Ocimum basilicum* L.) on Some Predacious Mites of the Family *Phytoseiidae* (Acari: Phytoseiidae). *Acta Phytopathologica et Entomologica Hungarica, 38*(1-2), 137-143.

Morales, M. R. and Simon, J. E. (1996). *New Basil Selections with Compact Inflorescences for the Ornamental Market.* Arlington: ASHS Press, 543-546.

Morton, L.W., Caccetta, R. A., Puddey, I. B. and Croft, K. D. (2000). Chemistry and Biological Effects of Dietary Phenolic Compounds: Relevance to Cardiovascular Disease. *Clinical and Experimental Pharmacology and Physiology, 27,* 152-159.

Muhammed, A., Tanko, Y., Okasha, M. A., Magaji, R. A. and Yaro, A. H. (2007). Effects of Aqueous Leaves Extract of *Ocimum gratissimum* on Blood Glucose Levels of Streptozocin Induced Diabetic Wistar Rats. *African Journal of Biotechnology, 6*(18), 2087-2090.

Mwangi, E. N., Hassanali, A., Essuman, S., Myandat, E., Moreka, L. and Kimondo, M. (1995). Repellent and Acaricidal Properties of *Ocimum suave* against *Rhipicephalus appendiculatus* ticks. *Experimental and Applied Acarology, 19,* 11–18.

Nadkarni, K. M. and Nadkarni, A. K. (1999). *Indian Materia Medica*. India: Popular Prakashan Pvt Ltd. 1:481-484.

Naidu, A. S. (2000). *Natural Food Anti-Microbial System*. Boca Raton: CRC Press.

Nakamura, C. V., Ueda-Nakamura, T., Bando, E., Melo, A. F. N., Cortez, D. A. G. and Filho, B. P. D. (1999). Antibacterial Activity of *Ocimum gratissimum* L. Essential Oil. *Memorias Do Instituto Oswaldo Cruz. 94*, 675-678.

Narwal, S., Rana, A. C., Tiwari, V., Gangwani, S. and Sharma, R. (2011). Review on Chemical Constituents and Pharmacological Action of *Ocimum kilimandscharicum*. *Indo Global Journal of Pharmaceutical Sciences,* 1(4), 287-293.

Natarajan, V., Krithica, N., Madhan, B. and Sehgal, P. K. (2012). Preparation and Properties of Tannic Acid Crosslinked Collagen Scaffold and Its Application in Wound Healing. *Journal of Biomedical Materials Research, Part B*, 1-8.

Ngassoum, M. B., Essia-Ngang, J. J., Tatsadjieu, L. N., Jirovetz, L., Buchbauer, G. and Adjoudji, O. (2003). Antimicrobial Study of Essential Oils of *Ocimum gratissimum* Leaves and *Zanthoxylum xanthoxyloides* Fruits from Cameroon. *Fitoterapia, 74*(3), 284-287.

Niki, E. (1996). *α-Tocopherol*. New York: Marcel Dekker. 3-25.

Njoku, C. J. and Asuzu, I.U. (1998). The anthelmintic effects of the leaf extract of *Ocimum gratissimum* (L.). *Phytomedicine, 5*(6), 485-488.

Nwanjo, H. U. and Oze, G. O. (2007). Hypolipidaemic and Antioxidant properties of *Ocimum gratissimum* on Diabetic rats. *Plant Products Research Journal, 11,* 1-4.

Nweze, E. I. and Eze, E. E. (2009). Justification for the Use of *Ocimum gratisimum* L. in Herbal Medicine and Its Interaction with Disc Antibiotics. *Complementary and Alternative Medicine, 9*, 37-42.

Nweze, E. I., Okafor, J. I. and Njoku, O. (2004). Antimicrobial Activities of Methanolic Extracts of *Trema guineensis* (Schumm and Thorn) and *Morinda lucida* Benth Used in Nigeria Herbal Medicinal Practice. *Journal of Biological Research and Biotechnology, 2*(1), 36-39.

Nwinyi, O. C., Chinedu, N. S., Ajani, O. O., Ikpo, C. O. and Ogunniran, K. O. (2009). Antibacterial Effects of Extracts of *Ocimum gratissimum* and *Piper guineense* on *Escherichia coli* and *Staphylococcus aureus*. *African Journal of Food Science, 3*(1), 022-025.

Nwosu, M. O. and Okafor, J. I. (1995). Preliminary Studies of the Antifungal Activities of Some Medicinal Plants Against Basidiobolus And Some Other Pathogenic Fungi. *Mycoses, 38,* 191-195.

Obadoni, B. O. and Ochuko, P. O. (2001). Phytochemical Studies and Comparative Efficacy of the Crude Extract of Homostatic Plants in Edo and Delta State of Nigeria. *Global Journal of Pure and Applied Sciences, 8b,* 203-208.

Odalo, J. O., Omolo, M. O., Malebo, H., Angira, J., Njeru, P. M., Ndiege, I. O. and Hassanali, A., (2005). Repellency of Essential Oils of Some Plants from the Kenyan Coast Against *Anopheles gambiae*. *Acta Tropica, 95*, 210-218.

Offiah, V. N. and Chikwendu, U. A. (1999). Antidiarrhoeal Effects of *Ocimum gratissimum* Leaf Extract in Experimental Animals. *Journal of Ethnopharmacology, 68*(1-3), 327-330.

Ogedegbe, H. O. (2002). An Overview of Hemostasis. *Laboratory medicine, 33*(12), 948-953.

Oguanobi, N. I., Chijioke, C. P. and Ghasi, S. I. (2012). Effects of Aqueous Leaf Extract of *Ocimum gratissimum* on Oral Glucose Tolerance Test In Type-2 Model Diabetic Rats. *African Journal of Pharmacy and Pharmacology, 6*(9), 630-635.

Ojianwuna, C. C., Edafemakor, A. G. and Iloh, A. C. (2011). Toxicity of *Ocimum suave* (wild basil) Leaf Oil on Adult Housefly (*Musca domestica*). *International Research Journal of Agricultural Science and Soil Science, 1*(10), 417-420.

Ojo, O. D., Adebayo, O.S., Olaleye, O. and Orkpeh, U. (2012). Basil (*Ocimum basilicum*) Genetic Variability and Viral Disease Assessment in Nigeria. *Asian Journal of Agricultural Sciences, 4(*1), 1-4.

Okafor, J. I., Nweze, E. I. and Njoku, O. U. (2001). Antibacterial Activities of the Methanolic Extracts of *Zapotica portericensis* Benth and *Cissus Quadrangularis* Linn. *Nigerian Journal of Pure and Applied Sciences, 1*(2).

Okubo, T., Yokoyama, Y., Kano, K. and Kano, I. (2003). Cell Death Induced by the Phenolic Antioxidant Tert-Butylhydroquinone and Its Metabolite Tert-Butylquinone in Human Monocytic Leukemia U937 Cells. *Food and Chemical Toxicology, 41*(5), 679–688.

Oladele, A. T., Mojisola, C. C. and Elufioye, T. A. (2012). Ethnobotanical Survey of Plants Used as Memory Enhancer and Antiaging in Ondo State, Nigeria. *International Journal of Pharmaceutics, 2*(1), 26-32.

Oliver, B. (1980). *Medicinal plants in Nigeria*. Ibadan: Nigerian College of Arts, Science and Technology. 90-94.

Omale, J., Olajide, J. E. and Okafor, P. N. (2008). Comparative Evaluation of Antioxidant Capacity and Cytotoxicity of Two Nigerian *Ocimum* Species. *International Journal of Chemical Sciences, 6*(4), 1742-1751.

Omidbaigia, R., Mirzaeea, M., Hassanib, M. E. and Sedghi, M. (2010). Induction and Identification of Polyploidy in Basil (*Ocimum basilicum* L.) Medicinal Plant by Colchicine Treatment. *Moghad International Journal of Plant Production, 4*(2), 87-98.

Omolo, M. O., Okinyo, D., Ndiege, I. O., Lwande, W. and Hassanali, A. (2004). Repellency of Essential Oils of Some Kenyan Plants Against *Anopheles Gambiae*. *Phytochemistry, 65*, 2797-2802.

Omori, E. O., Calistus, O., Mbugua, P. K. and Okemo, P. O. (2012). Ethnobotanical Identification and Anti-microbial Evaluation of Some AntiDiarrhoreal Plants Used by the Samburu Community, Kenya. *Malaysian Journal of Microbiology, 8*(2), 68-74

Omotosho, A. E. Eseyin, O. O. and Sulaiman, M. (2014): Phytochemical Analysis of *Cnidoscolus aconitifolius (Euphorbiaceae*) Leaf with Spectrometric Techniques. *Nigerian Journal of Pharmaceutical and Applied Science Research, 3*(1), 38-49.

Onajobi, F. D. (1986). Smooth Muscle Contracting Lipid-Soluble Principles in Chromatographic Fractions of *Ocimum gratissimum*. *Journal of Ethnopharmacology, 18*(1), 3-11.

Onaolapo, A. Y. and Onaolapo, O. J. (2012). *Ocimum gratissimum* Linn Causes Dose Dependent Hepatotoxicity in Streptozotocin-Induced Diabetic Wistar Rats. *Macedonian Journal of Medical Sciences, 5*(1), 17-25.

Orafidiya, L. O., Agbani, E. O., Oyedele, A. O., Babalola, O. O., and Onayemi, O. (2002). Preliminary Clinical Tests on Topical Preparations of *Ocimum gratissimum* Linn Leaf Essential Oil for the Treatment of *Acne vulgaris*. *Clinical and Drug Investigation, 22,* 313-319.

Orafidiya, L. O., Agbani, E. O., Oyedele, A. O., Babalola, O. O., and Onayemi. O. (2004a). Effect of Aloe Vera Gel on the Anti Acne Properties of the Essential Oil of *Ocimum gratissimum* Linn Leaf. A Preliminary Clinical Investigation. *International Journal of Aromatherapy, 14,* 15-21.

Orafidiya, L. O., Oyedele, A. O., Shittu, A. O. and Elujoba, A. A. (2001). The Formulation of an Effective Topical Antibacterial Product Containing *Ocimum gratissimum* Leaf Essential Oil. *International Journal of Pharmaceutics, 224*, 177-183.

Pacher, P., Beckman, J. S. and Liaudet, L. (2007). Nitric Oxide and Peroxynitrite in Health and Disease. *Physiological Review, 87,* 315-424.

Paton, A. (1992). A Synopsis of *Ocimum L. (Labiatae) in Africa*. Kew Bul. 47: 403–435

Percival, M. (1998). Antioxidants. *Clinical Nutrition Insights, 31,* 1-4.

Pessoa, L. M., Morais, S. M., Bevilaqua, C. M. L. and Luciano, J. H. S. (2002). Antihelmintic Activity of Essential Oil of *Ocimum gratissimum* Linn and Eugenol Agains*t Haemoachus contortus. Veterinary Parasitology, 109,* 59-63.

Pham-Huy, L. A., He, H. and Pham-Huy, C. (2008). *Free Radicals, Antioxidants in Disease and Health. International journal of Biomedical science, 4*(2), 89-96.

Phippen, W. B. and Simon, J. E. (1998). Anthocyanins in Basil (*Ocimum basilicum L.*) *Journal of Agriculture and Food Chemistry, 46,* 1734–1738.

Prabhu, K. S., Lobo, R., Shirwaikar, A. A. and Shirwaikar, A. (2009). *Ocimum gratissimum*: A Review of Its Chemical, Pharmacological and Ethnomedicinal Properties. *The Open Complementary Medicine Journal, 1,* 1-15.

Prakash P. and Gupta N. (2005). Therapeutic Uses of *Ocimum sanctum* Linn (Tulsi) With a Note on Eugenol and Its Pharmacological Actions: A Short Review. *Indian journal of physiology and pharmacology, 49*(2), 125-131.

Prasannabalaji, N., Muralitharan, G., Sivanandan, R. N., Kumaran, S. and Pugazhvendan, S. R. (2012). Antibacterial Activities of Some Indian Traditional Plant Extracts. *Asian Pacific Journal of Tropical Disease,* S291-S295

Pratt, D. E. (1996). *Natural Antioxidants Not Exploited Commercially*. *New York:* John Wiley & Sons, Inc., 3: 524–545.

Pravst, I., Mitek, K. and Mitek, J. (2010). Coenzyme Q10 Contents in Foods and Fortification Strategies. *Critical Reviews in Food Science and Nutrition, 50,* 269-280.

Przedborski, S. and Jackson-Lewwis, V. (1998). Experimental Developents in Movements Disorders: Update on Proposed Free Radical Mechanisms. *Current Opinion in Neurology, 11,* 335-339.

Rabelo, M., Souza, E. P., Soares, P. M., Miranda, A. V., Matos, F. J. and Criddle, D. N. (2003). Anti-Nociceptive Properties of the Essential Oil of *Ocimum gratissimum L*. (Labiatae) in mice. *Brazilian Journal of Medical and Biological Research, 36*(4), 521-524.

Rajeswari, G., Murugan, M. and Mohan, V. R. (2012). GC-MS Analysis of Bioactive Components of *Hugonia mystax* L. (Linaceae). *Research Journal of Pharmaceutical, Biological And Chemical Sciences,* 3(4), 301-308.

Rajeswari, G., Murugan, M. and Mohan, V. R. (2013). GC-MS Analysis of Bioactive Components of *Hugonia mystax* L. Bark (Linaceae). *Journal of Pharmaceutical and Biomedical Sciences, 29*(29), 818-824.

Ramesh, B. and Satakopan, V. N. (2010). *In Vitro* Antioxidant Activities of *Ocimum Species: Ocimum basilicum* and *Ocimum sanctum*. *Journal of Cell and Tissue Research, 10*(1), 2145-2150.

Ramesh, C. K., Raghu, K. L., Jamuna, K. S., Govinden, S. J., Ranghoo-Sanmukhiya, V. M. and Vijay, A. B. R. (2011). Comparative Evaluation of Antioxidant Property in Methanol Extracts of Some Common Vegetables of India. *Annals of Biological Research, 2*(2), 86-94.

Rang, H., Dale, P. M. and Ritter, J. M. (1999). *Pharmacology*. 4th edition: Churchill Livingstone, UK, 589-600, 340-348.

Rangan, M. D. and Barceloux, D. G. (2009). Food Additives and Sensitivities. *Disease-A-Month, 55,* 92-311.

Ravikumar, V. R., Gopal, V. and Sudha, T. (2012). Analysis of Phytochemical Constituents of Stem Bark Extracts of Zanthoxylum Tetraspermum Wight & Arn.

Research Journal Of Pharmaceutical, Biological And Chemical Sciences, 3(4), 391-402.

Reische, D. W., Lillard, D. A. and Eitenmiller, R. R. (1998). *Food Lipids: Chemistry, Nutrition and Biotechnology.* New York: Marcel Dekker, Incorporation. 423–448.

Rice-Evans, C. A., Miller, N. J. and Paganga G. (1996). Structure-Antioxidant Activity Relationships of Flavonoids and Phenolic Acids. *Free Radical Biology and Medicine, 20*(7), 933-956.

Rice-Evans, C. and Miller, N. (1994). Total Antioxidant Status in Plasma and Body Fluids. *Methods in Enzymology, 234*(24), 279-293.

Rodak, B. F. (2002). Hematology, *Clinical Principles and Applications*. Philadelphia: W.B Saunders, 609-753.

Roge, A. B, Firke, S. N., Kawade, R. M., Sarje, S. K. and Vadvalkar, S. M. (2011). Brief Review on: Flash Chromatography. *International Journal of Pharmaceutical Science Research, 2*(8), 1930-1937.

Rohloff, J. (1999). Monoterpene Composition of Essential Oil from Peppermint (*Mentha X Piperita* L.) with Regard to Leaf Position Using Solid-Phase Micro-Extraction and Gas Chromatography/Mass Spectrometry Analysis. *Journal of Agriculuture and Food Chemistry, 47,* 3782–3786.

Runyoro, D., Ngassapa, O., Vagionas, K., Aligiannis, N., Graikou, K. and Chinou, I. (2010). Chemical Composition and Antimicrobial Activity of the Essential Oils of Four *Ocimum* Species Growing in Tanzania. *Food Chemistry 119,* 311-316.

Saito, M., Sakagami, H. and Fujisawa, S. (2003). Cytotoxicity and Apoptosis Induction by Butylated Hydroxyanisole (BHA) and Butylated Hydroxyltoluene (BHT). *Anticancer Research, 23,* 4693–4701.

Sale, J. F. and Maji, J. O. (2006). The Phytochemical and Antimicrobial Screening of Honey –Based (Ilesha Wonder Drug). Proceedings of the 1st National Conference of the Faculty of Natural Sciences, University of Abuja.

Sanchez-Morano, C., Larrauri, J. A. and Saura-Calixto, F. (1999). Free Radical Scavenging Capacity and Inhibition of Lipid Oxidation of Wine, Grape Juices And Related Polyphenolic Constituent. *Food Research International, 32,* 407-412.

Sanganuwan, A. S. (2010). Some Medicinal Plants of Arabian Penninsula. *Journal of Medinal Plants Research, 4*(9), 766-788.

Sarafian, T.A., Kouyoumjian, S., Tashkin, D. and Roth, M. D. (2002). Synergistic Cytotoxicity of 9-Tetrahydrocannabinol and Butylated Hydroanisole. *Toxicology Letters, 133,* 171–179.

Seung-Joo-Lee, K. U., Takayuki, S. and Kwang-Guen, L. (2004). Identification of Volatile Components in Basil (*Ocimum basilicum*) and Thyme Leaves (*Thymus vulgaris*) and Their Antioxidant Properties. *Food Chemistry, 91,* 131-137.

Seyoum, A., Kabiru, E. W., Lwande, W., Killeen, G. F., Hassanali, A. and Knols, B. G. (2002). Repellency of Live Potted Plants Against *Anopheles gambiae* from Human Baits in Semi-Field Experimental Huts. *American Journal of Tropical Medicine and Hygiene, 67,* 191-195.

Seyoum, A., Killeen, G. F., Kabiru, E. W., Knols, B. G. J. and Hassanali, A. (2003). Field Efficacy of Thermally Expelled or Live Potted Repellent Plants Against African Malaria Vectors in Western Kenya. *Tropical Medicine and International Health, 8,* 1005-1011.

Seyoum, A., Palsson, K., Kunga, S., Kabiru, E. W., Lwande, W., Killeen, G. F., Hassanali, A. and Knols, B. G. J. (2002). Traditional Use of Mosquito Repellent Plants in Western Kenya and Their Evaluation in Semi-Field Experimental Huts Against *Anopheles gambiae*: Ethnobotanical Studies and Application by Thermal Expulsion and Direct Burning. *Transaction of the Royal Society of Tropical medicine and Hygiene, 96,* 225-231.

Shahidi F., Janitha, P. K. and Wanasundara, P. D. (1992). Phenolic Antioxidants. *Crirical Reviews in Food Science and Nutrition, 32,* 67-103.

Shahidi, F. (2003). *Food Additive Data Book*. Oxford, U.K: Blackwell Science Ltd.

Shahidi, F. and Wanasundara, P. K. (1992). Phenolic Antioxidants. *Critical Reviews in Food Science and Nutrition, 32*, 67–103.

Shahidi, F. and Ho, C. (2005). Phenolics in Food and Natural Health Products: An Overview. *ACS Symposium Series, 909,* 1-8.

Shahidi, F. (2008). Antioxidants: Extraction, Identification, Application and Efficacy Measurement. *Electronic Journal of Environmental, Agricultural and Food Chemistry, 8*, 3325-3330.

Sharma, H. and Clark, C. (1998). *An excerpt from the medical textbook Contemporary Ayurveda*. Edinburgh. ISBN: 0 443 05594 7.

Sherwin, E. R., Brancn, A. L., Davidson, P. M. and Salmincn, S. (1990). *Food Additives.* New York: Marvel Dekker Incorporation, 139-193.

Sies, H. and Stahl, W. (1995). Vitamins C and E, β-Carotene and Other Carotenoids as Antioxidants. *American Journal of Clinical Nutrition, 62,* 13155-13215.

Siman, C. M. and Eriksson, U. J. (1996). Effect of Butylated Hydroxytoluene on A-Tocopherol in Liver and Fat Tissue of Rats. *Toxicology Letters, 87,* 103–108.

Singh, E., Sharma, S., Dwivedi, J. and Sharma, S. (2012). Diversified Potentials of *Ocimum sanctum* Linn (Tulsi): An Exhaustive Survey. *Journal of Natural Plant Resources, 2*(1), 39-48.

Singh, S. K., Anand, A., Verma, S. K., Siddiqui, M. A., Mathur, A. and Saklani, S. (2011). Analysis of Phytochemicals and Antioxidant Potential of *Ocimum kilimandscharicum* Linn. *International Journal of Current Pharmaceutical Research, 3*(2), 40-46.

Singleton, V. L., Orthofer, R. and Lamuela-Raventos, R. M. (1999). Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants By Means of Folin-Ciocalteu Reagent. *Methods in Enzymology*, 299, 152-178.

Sjodin, T., Westing, Y. H. and Apple, F. S. (1990). Biochemical Mechanisms for Oxygen Free Radical Formation During Exercise. *Sports Medicine, 10*, 236-254.

Slamenova, D., Horvathova, E., Robichova, S., Hrusovska, L., Gabelova, A., Keib, K., Jakubikova, J. and Sedlak, J. (2003). Molecular and Cellular Influences of Butylated Hydroxyanisole Chinese Hamster V79 Cells Treated With N-Methyl -N´-Ntrosoguanidine. Antimutagenesis of BHA. *Environmental and Molecular Mutagenesis, 41,* 28–36.

Sodipo, O. A., Abdulrahman, F. I., Alemika, T. E. and Gulani, I. A (2012). Chemical Composition and Biological Properties of the Petroleum Ether Extract of *Solanum macrocarpum* L. (Local Name: Gorongo). *British Journal of Pharmaceutical Research, 2*(2), 108-128.

Sofowora, E. A. (1982). *Medicinal Plants and Traditional Medicine in Africa*. Chichester: J Wiley. 256

Sofowora, L. A. (1993). Medicinal plants and traditional medicine in Africa. Ibadan: Spectrum Books Ltd. 55-71.

Stiene-Martin, E. A., Lotspeich-Steininger, C. A. and Koepke, J. A. (1998). *Clinical Hematology. Principles, Procedures, Correlations.* Philadelphia: Lippincott. 599-611.

Sulistiarini, D., Oyen, L. P. A. and Nguyen, X. D. (1999). *Ocimum gratissimum* L. In: Plant Resources of South-East Asia. No. 19: Essential oils Plants. Prosea Foundation, Bogor, Indonesia, 140-142.

Tabudlo, R. (1996). Phytochemical and pharmalogical screening of Hagonoi (*Chromoalena sps*.). University of Northern Philippines.

Takahashi, O. (1995). Hemorrhagic Toxicity Of A Large Dose Of Α-, Β-, Γ- And Δ Tocopherols, Ubiquinone, Β-Carotene, Retinol Acetate And Ascorbic Acid In The Rat. *Food and Chemical Toxicology, 33,* 121-128.

Tan, P. V, Nyasse, B., Dimo, T. and Mezui, C. (2002). Gastric Cytoprotective Anti-Ulcer Effects of the Leaf Methanol Extract of *Ocimum suave* (Lamiaceae) in Rats. *Journal of Ethnopharmacology, 82*(2-3), 69–74.

Tan, P. V., Mezui, C. and Boda, M. (2011). Anti-Helicobacter and Gastroduodenal Cytoprotective Actions of the Leaf Aqueous Extract of *Ocimum suave* (Lamiaceae). *Journal of Medicinal Plants Research, 5*(25), 5958-5966

Tan, P. V., Mezui, C., Enow-Orock, G. E. and Agbor, G. (2013). Antioxidant Capacity, Cytoprotection and Healing Actions of the Leaf Aqueous Extract of *Ocimum suave* in Rats Subjected to Chronic and Cold-Restraint Stress Ulcers. *Ulcers,* 1-9.

Tan, P. V., Mezui, C., Enow-Orock, G. E., Njikam, N., Dimo, T. and Bitolog, P. (2008). Teratogenic Effects, Acute and Sub-Chronic Toxicity of the Leaf Aqueous Extract of *Ocimum suave* Wild (Lamiaceae) in Rats. *Journal of Ethnopharmacology, 115,* 232-237.

Tania, U., Nakamura, R. R. and Mendonca, F. (2006). Antileishmanial Activity of Eugenol-Rich Essential Oil from *Ocimum gratissimum*. *Parasitology International, 55,* 99-105.

Tanko, Y., Dewu, M. A., Deguem, K. C., Mohammed, K. A. and Mohammed, A. (2012). Hypotensive Effect of Aqueous and Methanol Leaves Extracts of *Ocimum Basilicum* (Sweet Basil) on Normotensive Cats Blood Pressure. *Central European Journal of Experimental Biology, 1*(4), 118-125.

Tanko, Y., Magaji, G. M., Yerima, M., Magaji, R. A. and Mohammed, A. (2008). Anti-Nociceptive and Anti-Inflammatory Activities of Aqueous Leaves Extract of *Ocimum gratissimum* (Labiate) in Rodents. *African Journal of Traditional, Complementary and Alternative Medicines, 5*(2), 141-146.

Threlfall, E. J. (2002). Antimicrobial Drug Resistance In *Salmonella*: Problems And Perspectives In Food And Water-Borne Infections. *FEMS Microbiology Reviews, 26,* 141-148.

Trease, G. E and Evans, W. C. (2002). *Pharmacognosy*. London: WB Saunders, 406.

Turgeon, M. L. (1999). *Clinical Hematology, Theory and Procedures.* Philadelphia: Lippincott Williams and Wilkins. 398-422

Umar, A. Imam G., Yimin W., Kerim P., Tohti, I., Berke, B. amd Moore, N. (2010). Antihypertensive Effects of *Ocimum basilicum* L. (OBL) on Blood Pressure in Renovascular Hypertensive Rats. *Hypertension Research, 33*(7), 727-730.

Umar, I. A., Mohammed, A., Dawud, F. A., Kabir, A. M., Sai, J. V., Muhammad, F. S. and Okalor, M. E. (2012). The Hypolipidemic and Antioxidant Actions of Aqueous Extracts of *Ocimum Basilicum* and *Ocimum suave* in High Fat Fed Rats. *Journal of Medicinal Plants Research, 6*(18), 3501-3505

Umar, I. A., Mohammed, A., Dawud, F. A., Kyari, H. and Abdullahi, M. (2012). Anti-Diabetic Action of the Aqueous Extract of *Ocimum suave* in Alloxan-Induced Diabetic Rats. *African Journal of Biotechnology, 11*(38), 9243-9247.

Valenzuela, A., Sanhueza, J. and Nieto, S. (2003). Cholesterol Oxidation: Health Hazard and the Role of Antioxidants in Prevention. Biological Research. 36: 291–302.

Vani, S. R., Cheng, S. F. and Chuah, C. H. (2009). Comparative Study of Volatile Compounds from Genus *Ocimum*. *American Journal of Applied Sciences, 6*(3), 523-528.

Venkatachalam, T., Kumar, V. K., Selvi, P. K., Maske, A. O and Kumar, N. S. (2011). Physiochemical and Preliminary Phytochemical Studies on the *Lantana camara* (L.) Fruits. *International Journal of Pharmacy and Pharmaceutical Sciences*, *3*(1), 52-54.

Verhagen, H. Schilderman, P. A. E. L. and Kleinjans, J. C. S. (1991). Butylated Hydroanisole In Perspective. *Chemico-Biological Interations, 80,* 109–134.

Verhagen, H., Beckers, H. H. G., Comuth, P. A. W. V., Maas, L. M., Hoor, F., Henderson, P. T. And Kleinjans, J. C. S. (1989). Disposition of Single Oral Doses of Butylated Hydroxytoluene in Man and Rat. *Food Chemistry and Toxicology, 27,* 765–772.

Verma, S. and Kothiyal, P. (2012). Pharmacological Activities of Different Species of Tulsi. *International Journal of Biopharm & Phytochemical Research, 1*(1), 21-39.

Vertuani, S., Angusti, A. and Manfredini, S. (2004). The Antioxidants and Pro-Antioxidants Network: An Overview. *Current Pharmaceutical Design, 10(*14), 1677-1694.

Vilioglu, Y. S., Mazza, G., Gao, L. and Oomah, B. D. (1998). Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables and Grain Products. *Journal of Agriculture and Food Chemistry, 46,* 4113-4117.

Wagensteen, H., Samuelsen, B. A. and Malterude, E. K. (2004). Antioxidant Activity in Extracts from Coriander. *Food Chemistry, 88,* 293-297.

Wan, J., Wilcock, A. and Coventry, M. J. (1998): The Effect of Essential Oils of Basil on the Growth of *Aeromonas hydrophila* and *Pseudomonas fluorescens*. *Journal of Applied Microbiology, 84,* 152-8.

Wang, B., Zhang, W., Duan, X. and Li, X. (2009). *In-vitro* Antioxidant Activities of Extract and Semi-Purified Fractions of The Marine Red Algae, *Rhodomela confervoides* (*Rhodomelaceae*). *Food Chemistry, 113,* 1101-1105.

Whysner, J., Wang, C. X., Zang, E., Iatropoulos, M. J. and Williams, G. M. (1994). Dose Response of Promotion of Butylated Hydroxyanisole in Chemically Initiated Tumors of the Rat Fore Stomach. *Food Chemistry and Toxicology, 32,* 215–222.

Wichi, H. P. (1988). Enhanced Tumour Development By Butylated Hydroxyanisole (BHA) from the Prospective of Effect on Forestomach and Oesophageal Squamous Epithelium. *Food Chemistry and Toxicology,* 26, 717-723.

Willcox, J. K., Ash, S. L. and Catignani, G. L. (2004). Antioxidants and Prevention of Chronic Disease. Review. *Critical Review in Food Science and Nutrition, 44,* 275-295.

Williams, G, M., Iatropoulos, M. J. and Whysner, J. (1999). Safety Assessment of Butylated Hydroxyanisole and Butylated Hydroxyl Toluene as Antioxidant Food Additives. *Food Chemistry and Toxicology, 37*, 1027–1038.

Williamson, G., Day, A. J., Plumb, G. W. and Couteau, D. (2000). Human Metabolic Pathways of Dietary Flavonoids and Cinnamates. *Biochemical Society Transactions, 28,* 16-22.

Xie, F. and Hu, Y. (1995). *A Colour Atlas of Traditional Chinese Medicines with Text on Techniques of Their Cultivation.* China: Jin Dun Press. 377.

Xiu-Qin, L., Chao, J., Yan-Yan, S., Min-Li, Y. and Xiao-Gang, C. (2009). Analysis of Synthetic Antioxidants and Preservatives in Edible Vegetable Oil by HPLC/TOF-MS. *Food Chemistry, 113*, 692–700.

Yamaguchi, T., Takamura, H., Matoba, T. and Terao, J. (1998). HPLC Method for Evaluation of Free Radical Scavenging Activity of Activity of Foods Using 2,2-Diphenyl-1-Picrylhydrazyl. *Bioscience, Biotechnology and Biochemistry, 62,* 1201-1204.

Yao, L. H., Jiang, Y. M., Shi, J., Tomas-Barberan, F. A., Datta, N., Singanuson, R. and Chen, S. S. (2004). Flavonoids in Food and Their Health Benefits. *Plant Foods for Human Nutrition, (59)*, 113-122.

Yen, G. C., Shao, C. H., Chen, C. J. and Duh, P. D. (1997). Effect of Antioxidant and Cholesterol on Smoke Points of Oils. *Lebensmittel-Wissenschaft Und Technologie, 30,* 648–652.

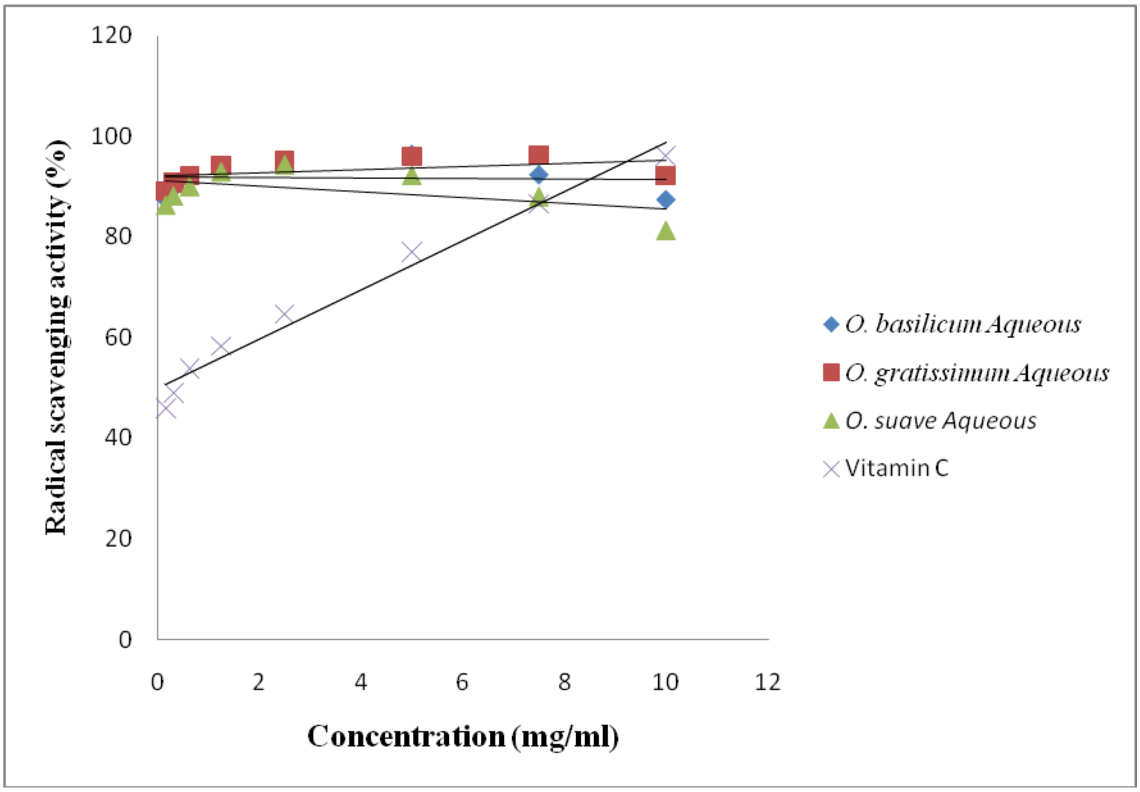
Yoshiyuki, M., Nobukazu, T., Hisaaki, Y., Takayoshi, K., Megumi, O., Hirokazu, S., Horie, T., Norikazu, A., Masakazu, Y., Akio, M., Shonen, Y. and Kengo, S. (1996). Fatty Acids Selectively Inhibit Eukaryotic DNA Polymerase Activities *In Vitro*. *Biochimica Et Biophysica Acta-Gene Structure And Expression, 1308*(3), 256-262.

Young, I. and Woodside, J. (2001). Antioxidants in Health and Disease. *Journal of Clinical Pathology, 54,* 176–186.

Yu, B. P. (1994). Cellular Defences Against Damage from Reactive Oxygen Species. *Physiological Reviews, 74,* 139-162.

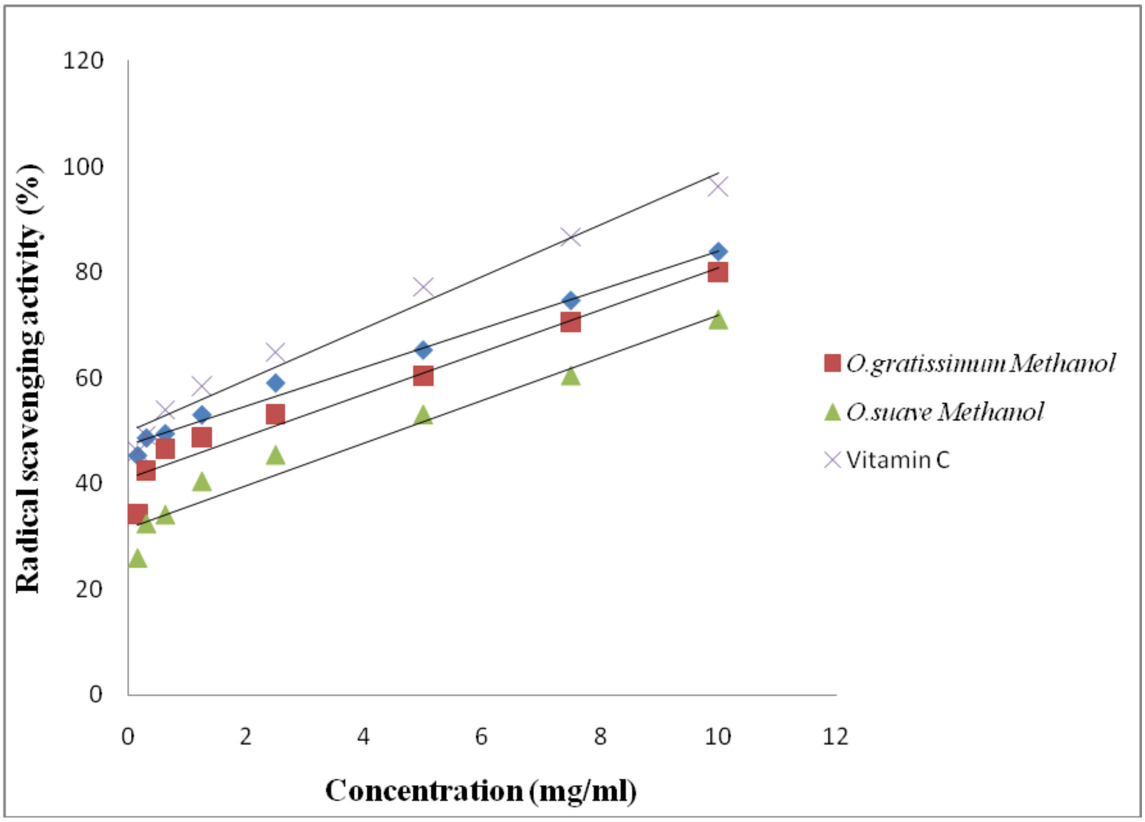
Zamocky, M. and Koller, F. (1999): Understanding the Structure and Functions of Catalases: Clue from the Molecular Evolution and *In-vitro* Mutagenesis. *Progress in Biophysics and Molecular Biology, 72*, 19-66.

APPENDIX 1.0



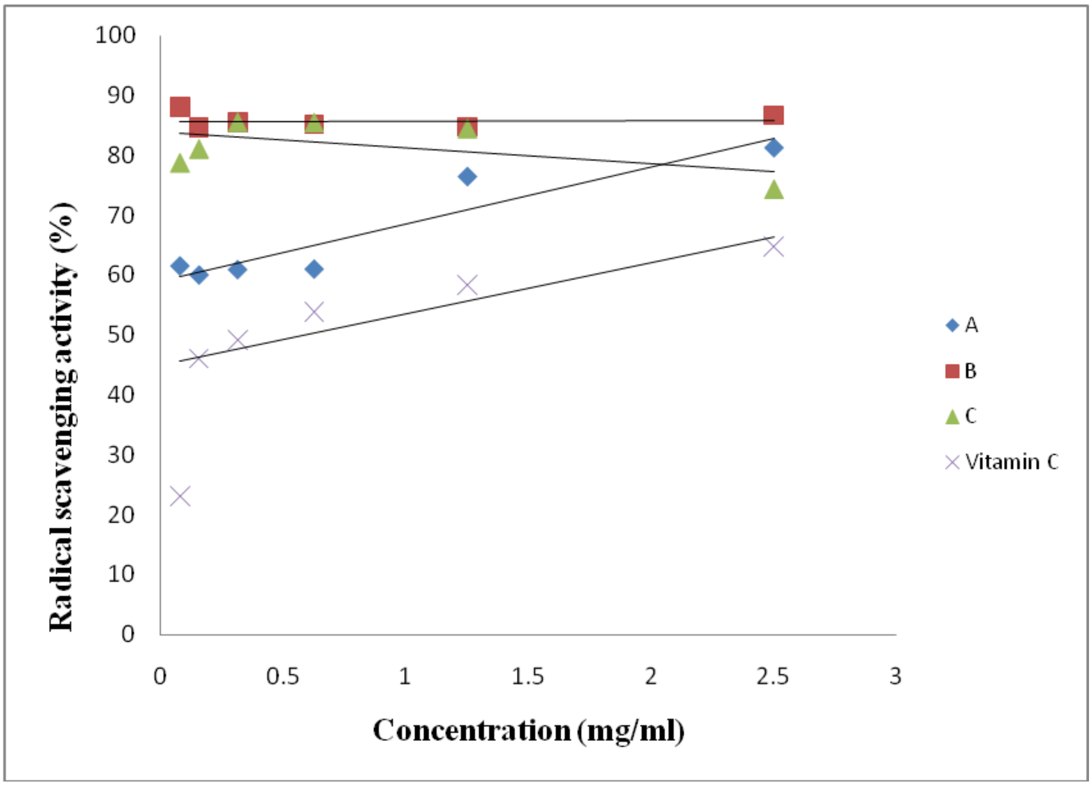
DPPH radical scavenging activity of Aqueous Exracts of Ocimum species

APPENDIX 2.0



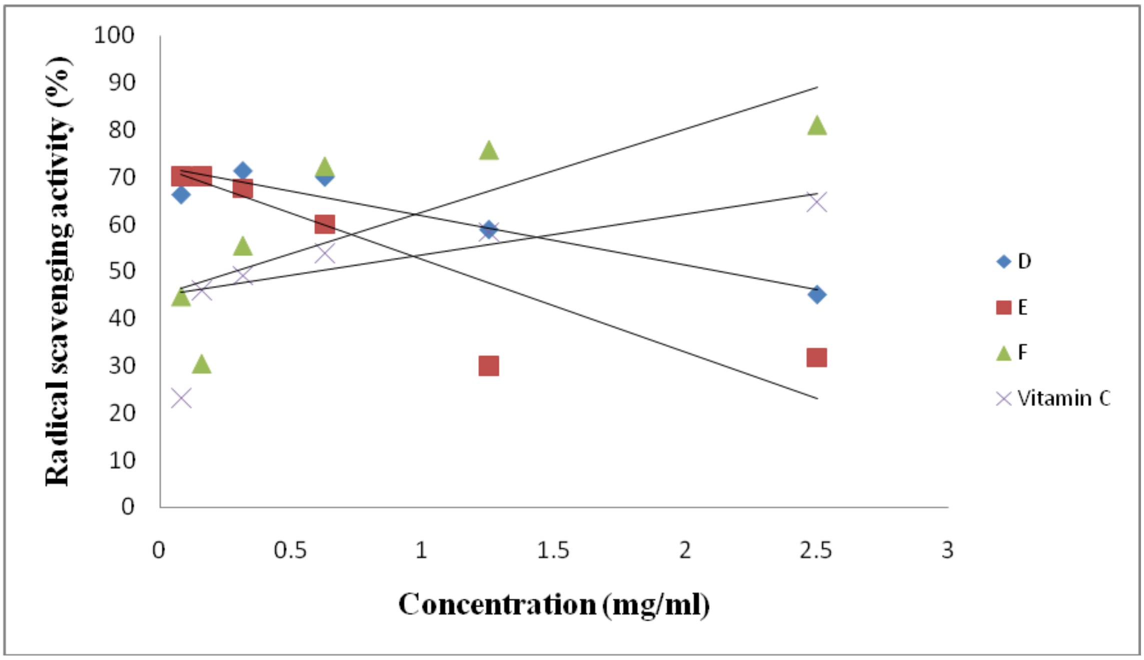
DPPH radical scavenging activity of Methanol Exracts of Ocimum species

APPENDIX 3.0



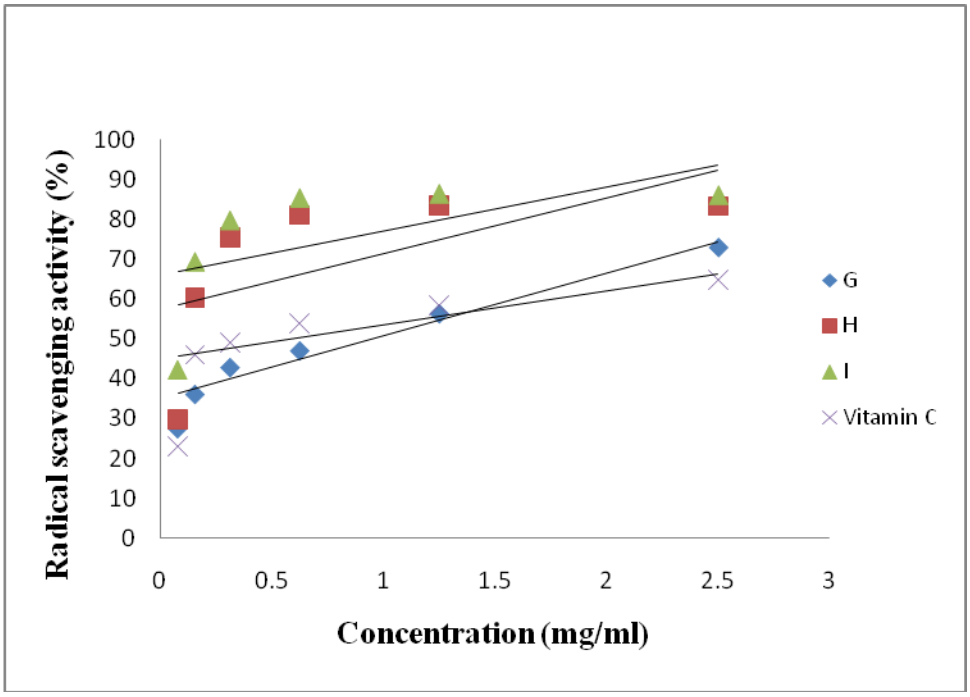
DPPH radical scavenging activity of column fractions A, B and C of Methanol Exracts of Ocimum basilicum

APPENDIX 4.0



DPPH radical scavenging activity of column fractions D, E and F of Methanol Exracts of Ocimum basilicum

APPENDIX 5.0



DPPH radical scavenging activity of column fractions G, H and I of Methanol Exracts of Ocimum basilicum

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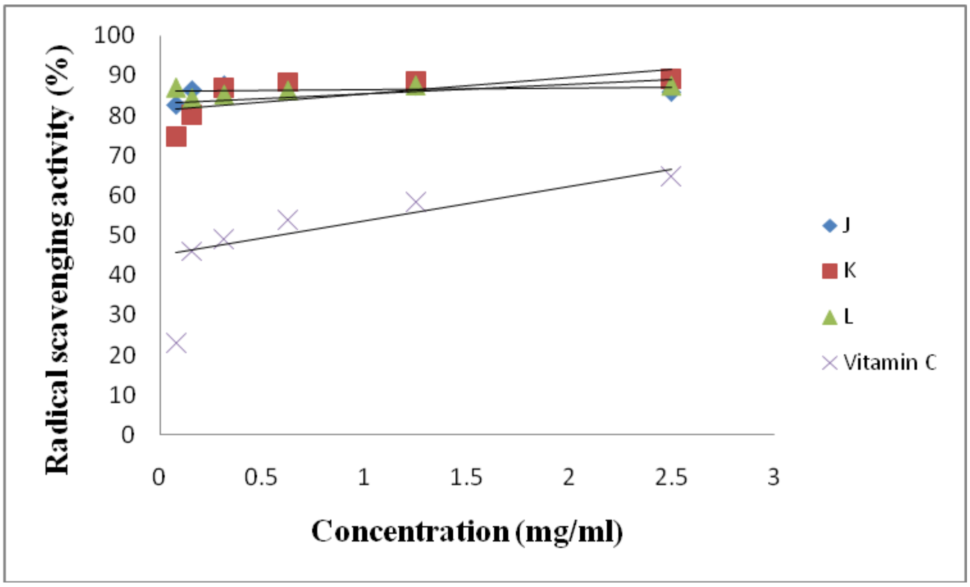
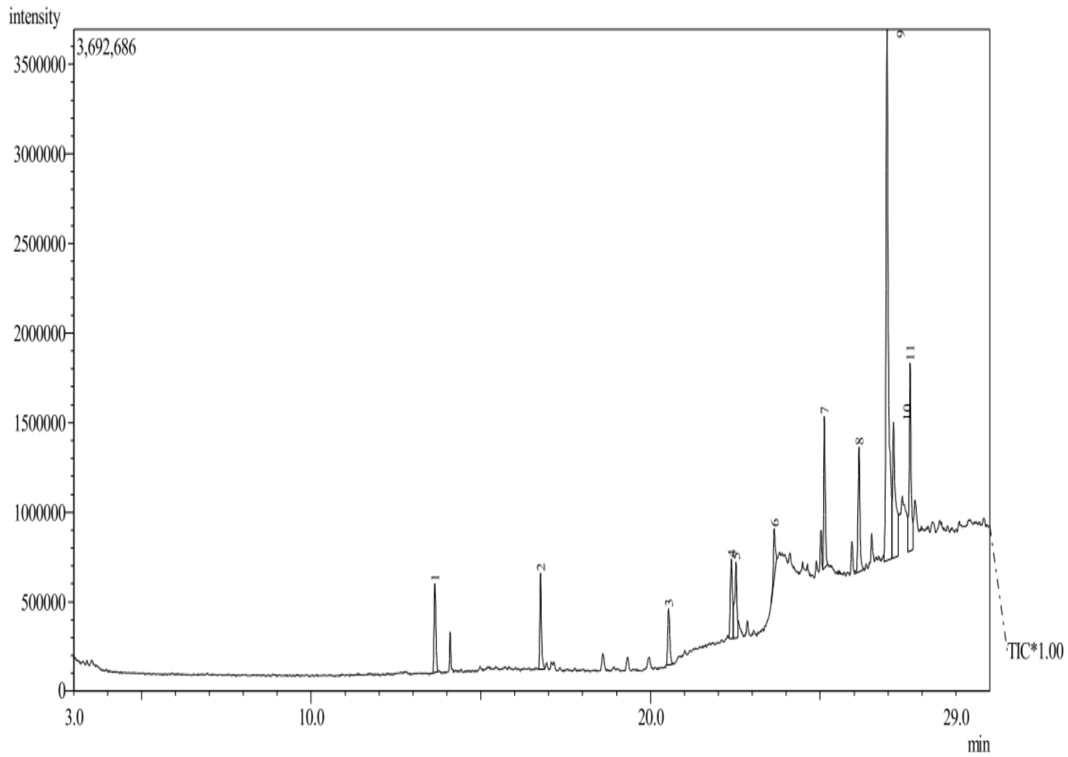


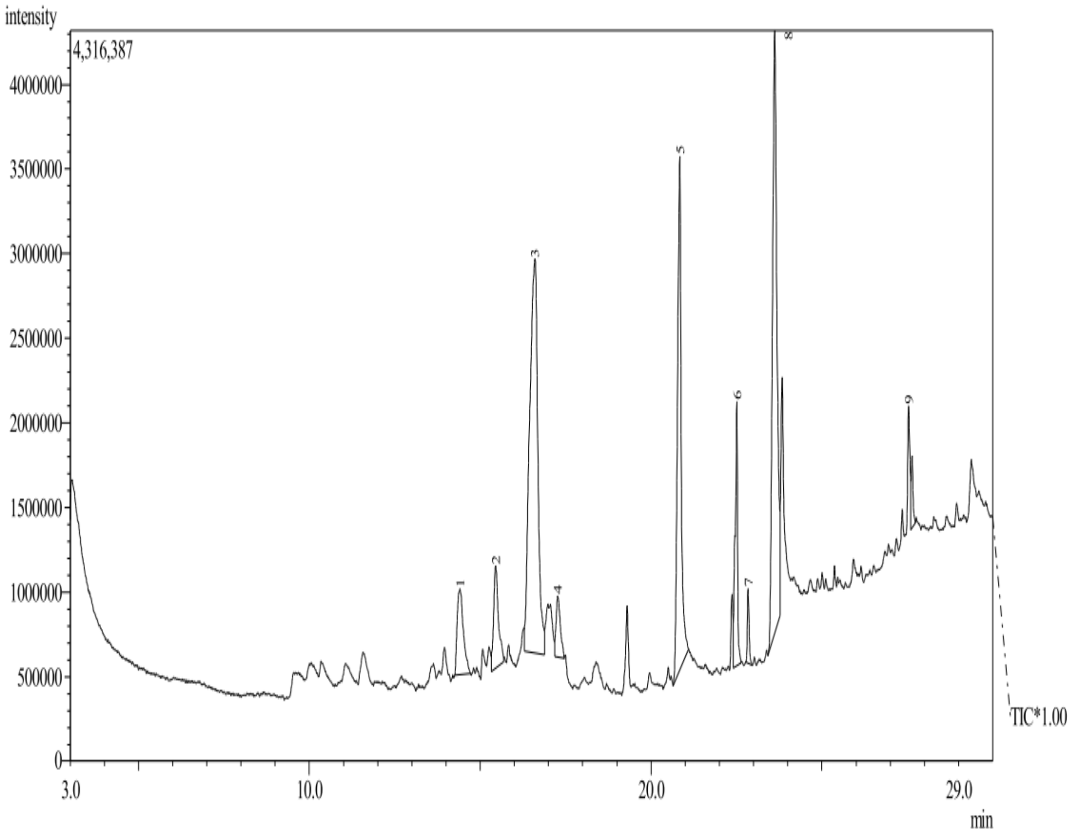
DPPH radical scavenging activity of column fractions J, K and L of Methanol Exracts of Ocimum basilicum

APPENDIX 7.0



CHROMATOGRAM OF FRACTION F, SUB-FRACTION 15 OF *Ocimum basilicum*

APPENDIX 8.0



Chromatogram of Fraction F, Sub-Fraction 15 of *Ocimum basilicum*