KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

KUMASI.

COLLEGE OF SCIENCE

DEPARTMENT OF CHEMISTRY

COMPARATIVE STUDIES ON THE *IN VITRO* ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF METHANOLIC AND HYDRO-ETHANOLIC PLANT EXTRACTS FROM FIVE MEDICINAL PLANT PARTS OF GHANA

BY

DOVI, EVANS

B.Ed. (Science)

A THESIS SUBMITTED TO THE DEPARTMENT OF CHEMISTRY, COLLEGE OF SCIENCE AND THE SCHOOL OF GRADUATE STUDIES, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF

MASTER OF PHILOSOPHY (ANALYTICAL CHEMISTRY)

JUNE, 2013

DECLARATION

I hereby declare that this submission is my own work towards the M.Sc. and that, to the best of my knowledge; it contains no material previously published by another person nor material which has been accepted for the award of any degree of the University, except where due acknowledgement has been made in the text.

Evans Dovi (PG 3907009) …… ……
(Student Name & ID) Signature Date

Certified by:

Dr. Sylvester K. Twumasi …… ……
(Supervisor) Signature Date

Certified by:

Dr. R. B. Voegborlo …… ……
(Head of Department) Signature Date

DEDICATION

This work is dedicated to all mothers.

ACKNOWLEDGEMENT

I give all praise, glory and thanks to the Almighty God who has brought me this far; it is just by His grace, favour and boundless mercies that this work has been done. My sincerest thanks goes to my Supervisors, Dr. Sylvester K. Twumasi and Ms. Mercy Badu who have being with me throughout this work, without their guidance, direction and advice, this work would not have been done. To other lecturers of the Department of Chemistry, who gave various advice and directions I say, thank you and may God richly bless you. I am also indebted to Mr M.A. Arkoh (Theoretical and Applied Biology Department, KNUST) for the identification and authentification of the plant parts. This work would not have been accomplished without also the help of technicians of Chemistry and Biochemistry Departments especially, Mrs. Humu Osman. I am also very much grateful to Mr. Nicholas Nkansah and Emma (all of the Faculty of Pharmacy), for their assistance when I was performing the antimicrobial test. To all other individuals who have helped me in diverse ways, I say thank you very much and may your blessings be to the pinnacle. Thank you all!

ABSTRACT

Studies show that consumption of plant products has the potential to inhibit the damaging activities of free radicals in the human body. Five medicinal plant parts of Ghana namely: the seed of *Okuobaka aubrevillei*, leaves of *Annona muricata*, leaves of *Theobroma cacao*, the false stem and root *Cymbopogon citratus* were assessed for their antioxidant properties and antimicrobial properties. The experimental results indicate that hydro-ethanol is an effective solvent for extracting the phytoconstituents of the medicinal plants. The total antioxidant capacity (TAC) and total phenol content (TPC) in the methanol extracts (METE) and hydro-ethanol extracts (HETE) from the selected medicinal plants within the measured concentration range (0.1 - 3.0 mg/ml) decreased in the order *T. cacao* > *A. muricata* > root of *C. citratus* > false stem of *C. citratus* > *O. okuobaka.* A high and positive correlation was observed between TPC and TAC in both the METE and HETE from all the selected medicinal plants. The selected medicinal plant showed strong antioxidant properties with respect to their free radical scavenging activity and Fe^{3+} reduction ability with hydro-ethanol extracts indicating higher antioxidant potential compared with their respective methanol extracts. All extracts of the plants tested showed varying degree of antibacterial activities against the test bacterial species (*S. aureus, E. coli and B. substlis*). The hydro-ethanol extracts showed higher activity than the methanol extracts. However, the hydro - ethanol extract of the root of C. citratus showed no activity against *S. aureus, E. coli* at lower concentrations.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

1. INTRODUCTION

1.1 Background

Numerous physiological and biochemical processes in the human body may produce oxygen-centred free radicals and other reactive oxygen species as by products (Halliwell, 1994; Poulson *et al*.,1998). Overproduction of such free radicals can cause oxidative damage to biomolecules (e.g. lipids, proteins, DNA), eventually leading to many chronic diseases such as atherosclerosis, cancer, diabetes, aging and other degenerative diseases in humans (Halliwell, 1994; Poulson *et al*., 1998). Plants contain a wide variety of free radical scavenging molecules such as phenolic compounds (phenolic acid, flavonoids, guinnones, lignans, stilbenes, tannins), nitrogen compounds (alkaloids, amines), vitamins, terpenoids (including carotenoids) and some other endogenous metabolites which are rich in antioxidant (Larson,1988; Cotelle *et al*.,1996,). These antioxidants are known to protect cells against the damaging effects of reactive oxygen species (ROS) such as singlet oxygen superoxide and hydroxyl radicals (Halvorsen *et al*., 2002, Wu *et al*., 2004). The activity of antioxidant is mainly due to their redox properties, which allow them to act as reducing agent, hydrogen donators and singlet oxygen quencher (Rice-Evans *et al*., 1995) and have a metal chelating potential (Hopia,1999). Epidemiological studies have shown that many of these antioxidant compounds possess anti-inflammatory, anticancer, anti-mutagenic, antibacterial or antiviral effect (Cai *et al* 2004). In the past few years, phenolic compounds have attracted considerable attention due to their many potential health benefits (Duthie *et al*, 2000) such as their preventive role in the development of cancer and heart diseases (Serafini et al, 1998; Carbonneau et al,1998). Studies to date have also demonstrated that phytochemicals in common plants can have complementary and overlapping mechanisms of action, including scavenging of oxidative agents, stimulation of the immune system, regulation of gene

expression in cell proliferation and apoptosis, hormone metabolism, and antibacterial and antiviral effects (Waladkhani et al., 1998). Flavonoids and other plant phenolics are especially common in leaves, flowering tissues and woody parts such as stems and bark (Larson, 1998). These plant products are believed to have a low toxicity and great medicinal value (Newman *et al*., 2000). Therefore, promoting the use is becoming a more effective way of fighting chronic diseases. On the other hand, food spoilage is also of great concern to consumers and food manufacturers. In order to prolong the storage stability of foods, synthetic antioxidants are mainly used in industrial processing. But according to the toxicologist and nutritionists, the side effects of some synthetic antioxidant used in food process such as butylated hydroxyl toluene (BHT) and butylated hydroxyanisol (BHA) can show carcinogenic effects in living organisms (Ames 1983; Baardseth, 1989;Namiki, 1990).) From this point of view, governmental authorities and consumers are concerned about the safety of food and the potential effects of synthetic additives on health (Reische et al, 1998). Therefore, plants products known to possess potential natural agents for food preservation (Halendar *et al*., 1998) can be used in place of the synthetic additives. Extracts of fruits, herbs, vegetable and other plants materials rich in phenolics are of great interest in the food industry because they retard oxidative degradation of lipids; improve the quality and nutritional value of food (Loliger, 1991), and also due to their multiple biological effects including anti-microbial activity. The antimicrobial activities of plants extracts can form the basis of many applications, including raw and processed food preservation, pharmaceuticals etc. (Reynolds, 1996). Infectious diseases account for one half of all deaths in the tropical countries irrespective of efforts made in controlling the incidence of epidemic (Iwu, 1993). Therapy with several types of antibiotics used in the treatment (Hiramatsu *et al* 1997) is frequently accompanied by side effects and microbial resistance. Therefore, new and potent

antimicrobial agents, particularly antifungal and anti *staphylococcus aureus* agents are still needed and should be actively sought (SelitrenniKoff, 1992; Chambers, 1997).

In this project work, comparative studies on the in vitro biological activity on methanolic and hydro-ethanolic plant extracts from five indigenous medicinal plants of Ghana (IMPsG) were carried out for their antioxidant properties in different antioxidant property determination assays, and also their antimicrobial activities against common food-borne pathogens. The plants were selected based on the fact that, in Ghana, the aqueous extracts of *Cymbopogon citratus* (lemon grass), leaves of *Annona muricata* and the leaves of *Theobroma cacao* are used to treat fever, while the seed of *Okoubaka aubrevillei* is being used for the treatment of boils. About 80% of individuals from developing countries use traditional medicine, which involves compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency (Kirtikar and Basu, 1975).The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. Hence, studies involving the use of plants as therapeutic agents should be emphasized, especially those related to the control of antibiotic-resistant microbes.

1.2 Statement of Problem

It is an established fact that chemical compounds with unpaired electrons such as powerful oxidants and free radicals are capable when present in the body to damage lipids, proteins, DNA and consequently may bring about mutations (Ellnain-wojtaszek et al, 2003). Radical reactions have been implicated in the pathogenesis of chronic diseases that are life limiting such as cancer, hypertension, cardiac infarction etc. (Nia, *et al*. 2004). Free radicals can be produced in the body tissues by biochemical processes and through environmental exposures, such as cigarette smoke, air pollution, and ultraviolet radiation from the sun (Verhagen *et al*,

2006). The use of natural antioxidants as food additive is of great importance in terms of reducing oxidative stress that is thought to cause damage to biological molecules (Bektas et al, 2005). Therefore, the active promotion and use of medicinal plants through investigation are important to uplift the status of our traditional medicinal plants.

1.3 Aims and Objectives

This research work aims to compare the in vitro biological activity, with respect to antioxidant property and antimicrobial activity of the hydro-ethanolic and methanolic extracts from the false stem and root of *Cymbopogon citratus* (lemon grass), *Okoubaka aubrevellei* seeds, *Theobroma cacao* leaves and *Annona muricata* leaves.

Specific Objectives

1. To determine the photochemicals present in the root, and false stem of *Cymbopogon citratus* (lemon grass), *Okuobaka aubrevellei* seeds, leaves of *Theobroma cacao* and leaves of *Annona muricata* samples.
2. To determine the antioxidant property of the methanolic and hydro-ethanolic extracts from the root and false stem of *Cymbopogon citratus* (lemon grass), seeds of *Okuobaka aubrevillei*, *Theobroma cacao* leaves and the leaves of *Annona muricata* in different antioxidant property determination assay with respect to:

   a. Total phenolic content using tannic acid as a reference drug

   b. Total antioxidant capacity using ascorbic acid as a reference drug

   c. Reducing power using ascorbic acid as a reference drug

   d. DPPH scavenging activity using ascorbic as a reference drug

3. To evaluate the antimicrobial activity of the methanolic and hydro- ethanolic extracts from the root and false stem of *Cymbopogon citratus* (lemon grass), seeds of *Okuobaka aubrevillei*, *Theobroma cacao* leaves *and the* leaves of *Annona muricata* samples against common pathogens *Bacillus subtilis, Escherichia coli, and Staphylococcus aureus* (all bacteria) using tetracycline as a reference drug.

1.4 Justification of Specific Objectives

Antimicrobial activity and antioxidant capacity of the medicinal plants are of great value to consumers and food manufacturers. Therefore, identifying plants with antioxidant properties and antimicrobial activities is in order. In Ghana, the aqueous extracts from *Cymbopogon citratus* (lemon grass), leaves of *Annona muricata* and the leaves of *Theobroma cacao* are used to treat fever, while the seed of *Okoubaka aubrevillei* is being used for the treatment of boils. It is then quite laudable and beneficial that the medicinal effects of these plants are explored and possibly antioxidant and antimicrobial drug developed from them. The knowledge of the bioactivity of these medicinal plants may also provide an important data for consumers and industrialist to decide on which plants extracts to use in the preservation of food and treatment of microbial infections. The information on the bioactivity of these medicinal plants may also contribute to the usage of such medicinal plants with the resultant improvement of public health in Ghana. Consequently, this study was framed to investigate the antioxidants and antimicrobial effects of hydro – ethanolic and methanolic extracts of the selected medicinal plants.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Reactive Oxygen Species (ROS) Or Oxidants

Reactive oxygen species (ROS) are derived from the metabolism of molecular oxygen (Halliwell, 1999). ROS include superoxide anion radical ($O_2^{\bullet -}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and the highly reactive hydroxyl radical ($^{\bullet}OH$). The deleterious effects of oxygen are said to result from its metabolic reduction to these highly reactive and toxic species (Buechter, 1988). In living cells, the major sources of endogenous ROS are hydrogen peroxide and superoxide anion, which are generated as by-products of cellular metabolism such as mitochondrial respiration (Nohl *et al*. 2003) ROS are also generated by exogenous sources such as ionizing radiation, tobacco smoke, certain pollutants, organic solvents, and pesticides. Many diseases are linked to damage from ROS as a result of an imbalance between radical-generating and radical-scavenging systems, a condition called oxidative stress.

2.1.1General and Toxicity of Selected Oxidants in Biological System

Many biochemical reactions vital to normal aerobic metabolism of human and microbial cells require the transfer of four electrons to molecular oxygen to form H_2O. Under most circumstances, this transfer occurs simultaneously without the formation of other intermediates. However, molecular oxygen does have the capacity to undergo sequential univalent reduction to form other oxygen intermediates with different toxicities prior to the generation of H_2O.The addition of one electron to O_2 yields the superoxide radical ($^{\cdot}O_2^-$) which at physiologic pH rapidly reduces itself to form the divalent oxygen reduction product, hydrogen peroxide (H_2O_2). Trivalent oxygen reduction in vitro occurs via the reaction of $H_2O_2$with $^{\cdot}O_2^-$ produce the hydroxyl radical ($^{\cdot}OH$). However, at physiologic pH, this reaction

is of little biologic importance unless a transition metal catalyst (e.g., Fe^{3+}) is present to enhance the reaction rate, yielding $^{\cdot}OH$ via the Haber-Weiss reaction. Besides $^{\cdot}OH$ formation, experimentally induced interactions between H_2O_2 and iron chelates may also lead to the production of the reactive iron peroxocomplex and ferryl ion. However, their role in human and microbial physiology is largely unknown. Although most investigations have focused on $^{\cdot}OH$ formation via the Haber-Weiss mechanism, evidence also exists for the formation of $^{\cdot}OH$ from $^{\cdot}O_2^-$- mediated reduction of hypochlorous acid (HOCl). A potent oxidant in itself, HOCl is generated by the interaction of H_2O_2 with phagocyte-derived peroxidases.

Myelo (eosinophil) peroxidase..... $H_2O_2 + HX \longrightarrow HOX + H_2O$

Nitric oxide synthase..... L- Arginine $\longrightarrow$ L- citrulline + $NO^{\cdot}$

Peroxynitrite formation/Decomposition..... $NO^{\cdot} + {}^{\cdot}O_2^- \longrightarrow ONOO^-$

$ONOO^- + H^+ \longrightarrow ONOOH$

$ONOOH \longrightarrow {}^{\bullet}OH + NO^{\bullet}$

Superoxide ($^{\cdot}O_2^-$)

A superoxide, also known by the obsolete name hyperoxide, is a compound that possesses the superoxide anion with the chemical formula O_2^-. It is important as the product of the one-electron reduction of dioxygen O_2, which occurs widely in nature. With one unpaired electron, the superoxide ion is a free radical, and, like dioxygen, it is paramagnetic. Superoxide anion is a reduced form of molecular oxygen created by receiving one electron. Superoxide anion is an initial free radical formed from mitochondrial electron transport systems. Mitochondrial generate energy using four electron chain reactions, reducing oxygen to water. Some of the electrons escaping from the chain reaction of mitochondria directly react with oxygen and form superoxide anions (Harman 2000).

$$O_2 \xrightarrow[H^+]{e^-} HO_2 \xrightarrow[H^+]{e^-} H_2O_2 \xrightarrow[H^+]{e^-} {}^{\bullet}OH \xrightarrow[H^+]{e^-} H_2O$$

The superoxide anion plays an important role in the formation of other reactive oxygen species such as hydrogen peroxide, hydroxyl radical, or singlet oxygen

($2^{\cdot}O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$) in living systems (Stief 2003). The superoxide anion can react with nitric oxide ($NO\cdot$) and form peroxynitrite ($ONOO^-$), which can generate toxic compounds such as hydroxyl radical and nitric dioxide ($ONOO^- + H^+ \rightarrow \cdot OH + \cdot NO_2$) (Halliwell 1997). Superoxide is so toxic that intracellular levels above 1nM are lethal. The biological toxicity of superoxide has capacity to inactivate iron-sulphur cluster containing enzymes (which are critical in a wide variety of metabolic pathways), thereby liberating free iron in the cell, which can undergo Fenton chemistry and generate the highly reactive hydroxyl radical. In its HO_2 form (hydroperoxyl radical,can initiate lipid peroxidation of polyunsaturated fatty acids. It also reacts with carbonyl compounds and halogenated carbons to create toxic peroxy radicals. Superoxide can also form tyrosine peroxides as a result of reaction with enzymes containing tyrosyl radicals (such as ribonucleotide reductase) and can also oxidize hemoglobin (forming the non-oxygen carrying met-hemoglobin), and possibly other low-potential heme proteins and thiols. As such, superoxide is one of the main causes of oxidative stress. In phagocytes, superoxide is produced in large quantities by the enzyme NADPH oxidase for use in oxygen-dependent killing mechanisms of invading pathogens. Mutations in the gene coding for the NADPH oxidase cause an immunodeficiency syndrome called chronic granulomatous disease, characterized by extreme susceptibility to infection.

Hydrogen Peroxide (H_2O_2)

Hydrogen peroxide can be generated through a dismutation reaction from superoxide anion by superoxide dismutase. Enzymes such as amino acid oxidase and xanthine oxidase also produce hydrogen peroxide from superoxide anion. Hydrogen peroxide is highly diffusible and crosses the plasma membrane easily. Hydrogen peroxide is the least reactive molecule among reactive oxygen species and is stable under physiological pH and temperature in the

absence of metal ions. Hydrogen peroxide is a weak oxidizing and reducing agent and is thus regarded as being poorly reactive. Hydrogen peroxide can generate the hydroxyl radical in the presence of metal ions and superoxide anion ($\cdot O_2^- + H_2O_2 \rightarrow \cdot OH + OH^- + O_2$) (Halliwell, 1997). Hydrogen peroxide can produce singlet oxygen through reaction with superoxide anion or with HOCl or chloroamines in living systems (Stief 2000, 2003). Hydrogen peroxide can degrade certain heme proteins, such as hemoglobin, to release iron ions.

Hydroxyl Radical ($^{\cdot}OH$)

Hydroxyl radical is the most reactive free radical and can be formed from superoxide anion and hydrogen peroxide in the presence of metal ions such as copper or iron.

$$\bullet O_2^- + H_2O_2 \longrightarrow \bullet OH + OH^- + O_2$$

Hydroxyl radicals have the highest 1-electron reduction potential (2310 mV) and can react with everything in living organisms at the 2nd-order rate constants of 10^9 to 10^{10}/M/s (Korycka-Dahl and Richardson 1978). In general, aromatic compounds or compounds with carbon–carbon multiple bonds undergo addition reactions with hydroxyl radicals, resulting in the hydroxylated free radicals. In saturated compounds, a hydroxyl radical abstracts a hydrogen atom from the weakest C–H bond to yield a free radical (Korycka-Dahl and Richardson 1978). The resulting radicals can react with oxygen and generate other free radicals. Hydroxyl radicals react with lipid, polypeptides, proteins, and DNA, especially thiamine and guanosine (Ashok and Ali 1999).

Peroxynitrite

Reaction of NO and superoxide anion can generate peroxynitrite

($^{\bullet}O_2^- + NO\bullet \rightarrow OONO^-$). Peroxynitrite is a cytotoxic species and causes tissue injury and oxidizes low-density lipoprotein (LDL) (Halliwell 1997). Peroxynitrite appears to be an

important tissue-damaging species generated at the sites of inflammation (Papas 1999) and has been shown to be involved in various neurodegenerative disorders and several kidney diseases (Knight 1999). Peroxynitrite (OONO–) can cause direct protein oxidation and DNA base oxidation and modification acting as a "hydroxyl radical- like" oxidant (McVean and others 1999). The significance of peroxynitrite as a biological oxidant comes from its high diffusibility across cell membranes. Nitrotyrosine, which can be formed from peroxynitrite-mediated reactions with amino acids, has been found in age-associated tissues. (Knight 1999).

Nitric Oxide ($NO^{\cdot}$**)**

Nitric oxide ($NO^{\cdot}$) is a free radical with a single unpaired electron. Nitric oxide, known as the 'endothelium-derived relaxing factor', is biosynthesized endogenously from L-arginine, oxygen and NADPH by various nitric oxide synthase (Fang *et al*,2000; Stryer, 1995). Nitric oxide itself is not very reactive free radical, but the over production of $NO^{\cdot}$ in ischemia reperfusion causes neurodegenerative and chronic inflammatory disease such as rheumatoid arthritis and inflammatory bowel disease. Nitric oxide is a vasodilator resulting from the breakdown of arginine to citrulline, in a reaction catalysed by a family of NADPH-dependent enzymes called nitric oxide synthases. , the formation of nitric oxide in mitochondria may have important consequences because this compound binds to haem groups from cytochromes (in particular cytochrome oxidase) and inhibits respiration. This may, in turn, stimulate $O_2^{-}\bullet$ formation (for example from Complex I) (Poderoso *et al.* 1996), which in turn may react with more nitric oxide forming peroxynitrite, an oxidant capable of inhibiting important enzymes and affecting mitochondrial integrity (Cassina & Radi, 1996). Nitric oxide, exposed in human blood plasma, can deplete the concentration of ascorbic acid and uric acid, and initiate lipid peroxidation (Halliwell 1996).

2.1.2 Oxidative Stress

Oxidative stress as the state in which the level of toxic reactive oxygen intermediates (ROI) overcomes the endogenous antioxidant defenses of its host. (Bulger and Helton 1998; Oufnac, 2006) Oxidative stress represents an imbalance between the production and manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of tissues can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. Some reactive oxidative species can even act as messengers through a phenomenon called redox signaling. In humans, oxidative stress is involved in many diseases. Examples include Sickle Cell Disease (Amer, *et al.*2006) atherosclerosis, Parkinson's disease, heart failure, myocardial infarction, Alzheimer's disease, Schizophrenia, Bipolar disorder, fragile X syndrome (De Diego-Otero, *et al* 2006) and chronic fatigue syndrome, but short-term oxidative stress may also be important in prevention of aging by induction of a process named mitohormesis.(Gems, *et al.* 2008). Reactive oxygen species can be beneficial, as they are used by the immune system as a way to attack and kill pathogens.

2.1.3 Antioxidants and Free Radicals

Numerous antioxidants are plant-based and play a fundamental role in protecting plants that are open to the elements such as sunlight and severe oxygen stress (Oufnac, 2006). It has been suggested that antioxidants may amend cellular oxidative status and prevent biologically significant molecules such as DNA, proteins, and membrane lipids from oxidative damage and as a result lessen the risk of several chronic diseases including cancer and cardiovascular disease (Zhou and others 2004; Oufnac,2006). The core dietary antioxidants are vitamins A, C and E, carotenoids, terpenes, and polyphenols, including flavonoids (Stanner and others

2004; Oufnac, 2006). A sufficient ingestion of natural antioxidants in food is therefore of great consequence for the defense of macromolecules against oxidative damage (Halliwell and Gutteridge 1999; Oufnac, 2006 and Wallace 1997). According to Stratil *et al* (2006), the cells most frequently damaged by oxidative stress are unsaturated fatty acids in lipids, cholesterol, different functional polypeptides and proteins, and nucleic acids. Mechanisms of antioxidants consist of free radical quenching, transition metal chelating, reducing peroxide, and simulation of in vivo antioxidative enzyme activities (Zhou and Liangli 2004; Oufnac, 2006). In living systems, the antioxidants may elevate the levels of endogenous defenses. The action of antioxidants in foods and biological systems is reliant on the systems' composition, interfacial phenomena, and partitioning properties of the antioxidants between lipid and aqueous phases (Diaz-Reinoso and others 2006; Oufnac, 2006). Bulger and Helton (1998) define oxidative stress as the state in which the level of toxic reactive oxygen intermediates (ROI) overcomes the endogenous antioxidant defenses of its host. This results in a surplus of free radicals, which can react with cellular lipids, proteins, and nucleic acids leading to damage and eventual organ dysfunction. Lipids are the most exposed biomolecules to a free radical attack. Free radicals play a vital role in a number of biological processes, some of which are necessary for life, such as the intracellular killing of bacteria by neutrophil granulocytes. Free radicals have also been implicated in certain cell signaling processes. The peroxyl radical is the most common free radical in human biology, but the hydroxyl radical, singlet oxygen, superoxide radical, and reactive nitrogen species all are present in biological systems (Wu and others 2004; Oufnac, 2006). They result from molecular oxygen under reducing conditions, because of their reactivity; these same free radicals can have a role in unwanted side reactions causing cell damage. A variety of aging symptoms such as atherosclerosis are credited to free radical-induced oxidation of numerous chemicals. Since free radicals are essential for life, the body has a number of mechanisms to reduce free

radical induced damage and to restore damage that does occur, such as the enzymes, superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase (Campos 2003; Oufnac, 2006 and Pinzino 1999).

2.2. Some Natural Antioxidants

Most of the natural antioxidants are obtained from plants. Examples are Vitamin E, Vitamin C, beta carotene and tannins.

2.2.1 Alpha Tocopherol (Vitamin E)

Vitamin E is found naturally in some foods, added to others, and available as a dietary supplement. "Vitamin E" is the collective name for a group of fat-soluble compounds with distinctive antioxidant activities (Traber, et al.2006). Naturally occurring vitamin E exists in eight chemical forms (alpha-, beta-, gamma-, and delta-tocopherol and alpha-, beta-, gamma-, and delta-tocotrienol) that have varying levels of biological activity.

Alpha- (or α-) tocopherol is the only form that is recognized to meet human requirements. Vitamin E is a fat-soluble antioxidant that stops the production of ROS formed when fat undergoes oxidation (Traber ,et al.2006). Vitamin E might help prevent or delay the chronic diseases associated with free radicals. Alpha-tocopherol inhibits the activity of protein kinase C, an enzyme involved in cell proliferation and differentiation in smooth muscle cells, platelets, and monocytes. Vitamin-E replete endothelial cells lining the interior surface of blood vessels are better able to resist blood-cell components adhering to this surface. Vitamin E also increases the expression of enzymes that suppress arachidonic acid metabolism, thereby increasing the release of prostacyclin from the endothelium, which, in turn, dilates blood vessels and inhibits platelet aggregation. Vitamin E is being tested as a treatment for many chronic diseases for the elderly, including Alzheimer's, macular degeneration,

osteoarthritis, and prostate enlargement. And it is thought that this powerful antioxidant may help keep some types of cancer from developing.

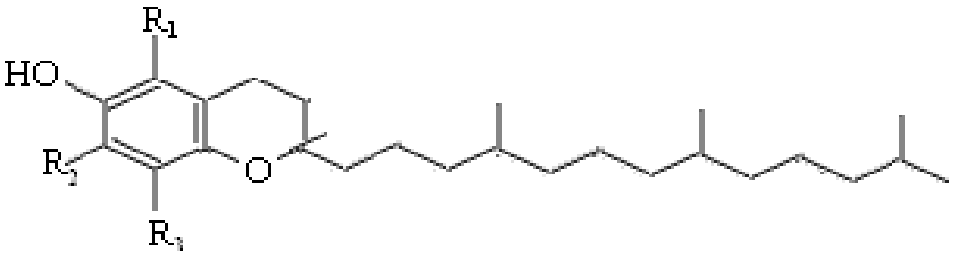


Figure 2.1: Structure of alpha-Tocopherol
(modified from vita-dose.com/structure-of- vitamin-E)

2.2.2 Ascorbic Acid (Vitamin C)

Vitamin C is a water-soluble, antioxidant vitamin which performs numerous physiological functions in the human body. These functions include the synthesis of collagen (a protein that gives structure to bones, cartilage, muscle, and blood vessels.), carnitine, and neurotransmitters; catabolism of tyrosine; and the metabolism of microsome (Gropper, et al. 2004). Vitamin C also aids in the absorption of iron, and helps maintain capillaries, bones, and teeth. Vitamin C is a natural antihistamine. It both prevents histamine release and increases the detoxification of histamine. A study found that taking two grams of vitamin C daily lowered blood histamine levels 38 percent in healthy adults in just one week. (Martinez del Rio, 1997). In humans, vitamin C is also very essential to a healthy diet as well as being a highly effective antioxidant, acting to lessen oxidative stress. (Bánhegyi, and Mandl, 2001)

Ascorbic acid (reduced form) Dehydroascorbic acid (oxidized form)

Figure 2.2: (Structure of Ascorbic acid from en.wikipedia.org/wiki/Vitamin C)

2.2.3 Carotenoids

Carotenoids have been demonstrated to act as antioxidants *in vitro* systems, in cellular systems, and in animal systems (Krinsky, 1993; Palozza and Krinsky, 1992) .Epidemiological studies show that a high intake of /3-carotene as well as other carotenoids had been found to decreased risk of some cancers, cardiovascular disease, and other diseases (Ziegler, 1989; Gaziano and Hennekens, 1993; Mayne, 1996). Evidence supports the fact that lipid peroxidation or oxidative stress is the underlying mechanism in such diseases and investigators have stressed the potential usefulness of antioxidant nutrients, including carotenoids (Jones *et al.*, 1995), in prevention and treatment of such diseases (Halliwell *et al.*, 1991; Omaye and Zhang, 1997). Carotenoids function to protect organisms against excess radiation, either by quenching dangerous excited species such as singlet oxygen, or primarily through energy transfer reactions (Frank and Cogdell, 1996).

The polyene chain is the characteristic most responsible for the chemical reactivity of carotenoids toward oxidizing agents and free radical (Jones *et al.*, 1995). Some forms of carotenoids (figure 2.3) present in plants are;

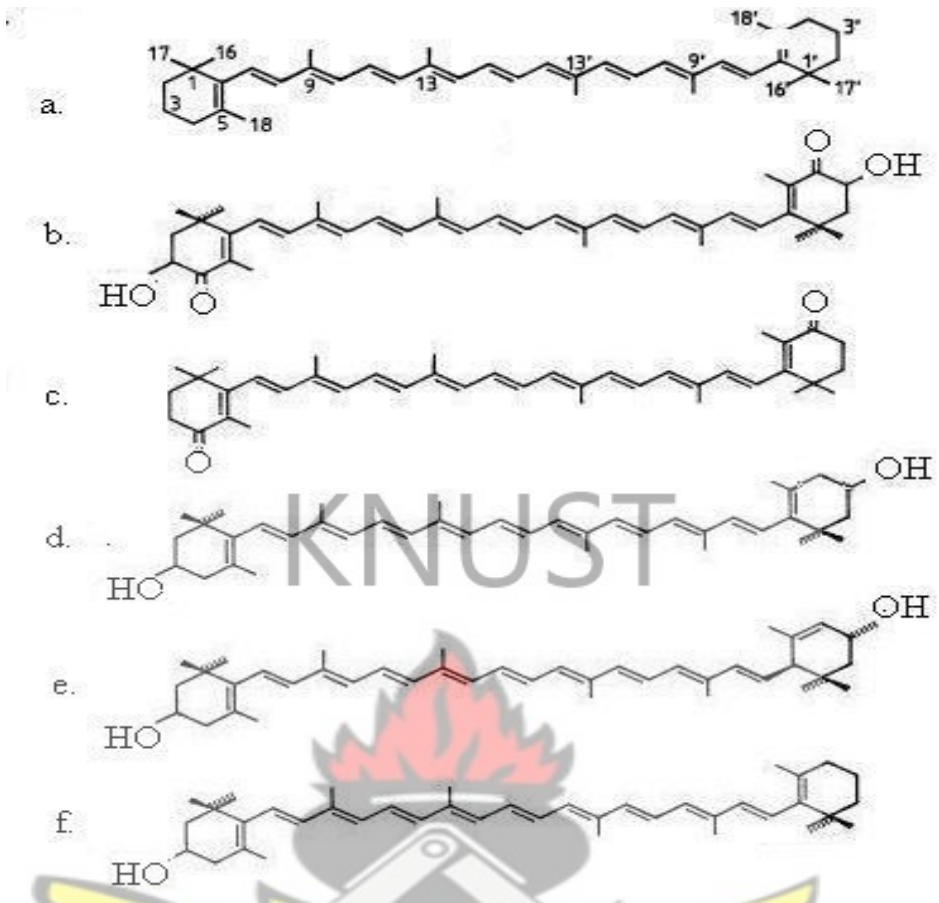


Figure 2.3 **:** Chemical structures of carotenoids: (a)-β-Carotene, (b)- astaxanthin, (c)-canthaxanthin, (d)- zeaxanthin, (e)-lutein, (f) –cryptoxanthin (modified from Straub.com)

2.3 Reaction Mechanism of Antioxidants against Free Radicals

Antioxidants can deactivate radicals by two major mechanisms: Hydrogen Atom Transfer (HAT) and Single Electron Transfer (SET). The end result is the same, regardless of mechanism, but kinetics and potential for side reactions differ. HAT-based methods measure the classical ability of an antioxidant to quench free radicals by hydrogen donation. Relative reactivity in HAT methods is determined by the bond

dissociation energy (BDE) of the H-donating group in the potential antioxidant, dominating for compounds with BDE of ~ -10 kcal/mol and ionization potential (IP) of < -36 kcal/mol (Wright *et al* 2001).

$$X^{\bullet} + AH \rightarrow XH + A^{\bullet}$$

HAT reactions are solvent and pH independent and are usually quite rapid. The presence of reducing agents, including metals, is a complication in HAT assays and can lead to erroneously high apparent reactivity.(Wright *et al* 2001; Prior and others 2005). On the other hand, SET-based methods detect the ability of a potential antioxidant to transfer one electron to reduce any compound, including metals, carbonyls, and radicals (Wright *et al* 2001)

$$X^{\bullet} + AH \longrightarrow X^{-} + AH^{\bullet+} \quad \text{(1)}$$

$$AH^{\bullet+} \xleftrightarrow{H_20} A^{\bullet} + H_3O^{+} \quad \text{(2)}$$

$$X^{-} + H_3O^{+} \longrightarrow XH + H_2O \quad \text{(3)}$$

$$M(III) + AH \longrightarrow AH^{+} + M(II) \quad \text{(4)}$$

SET reactions are usually slow and can require long times to reach completion, so antioxidant capacity calculations are based on percent decrease in product rather than kinetics. (Sartor, *et al* 1999). When a free radical gains the electron from an antioxidant it no longer needs to attack the cell and the chain reaction of oxidation is broken (Dekkers, *et al* 1996). After donating an electron an antioxidant becomes a free radical by definition. Antioxidants in this state are not harmful because they have the ability to accommodate the change in electrons without becoming reactive. There are methods utilizing both HAT and SET mechanisms. The TEAC and DPPH assays are usually classified as SET reactions, however these two indicator radicals in fact may be neutralized either by direct reduction via electron transfers or by radical quenching via HAT (Jimenez and others 2004

2.4 Antioxidant Capacity Assays

Most natural antioxidants are multifunctional in complex heterogeneous foods; their activity cannot be assessed by any one method (Frankel and Meyer 2000; Oufnac, 2006 and Sanchez-Moreno 2002). No single assay will accurately reflect all of the radical foundations or all

antioxidants in a mixed or complex system, and it must be appreciated at the outset that there are no simple universal methods by which antioxidant capacity can be measured accurately and quantitatively (Prior and others 2005; Oufnac, 2006)

2.4.1 DPPH (2, 2-Diphenyl-1-picrylhydrazyl) Assay

DPPH• radical is one of a few stable and commercially available organic nitrogen radicals bearing no similarity to the highly reactive and transient peroxyl radicals involved in various oxidative reactions in vivo (Huang, 2005 and Wu, 2004).

Principle

This assay is based on the measurement of the reducing ability of antioxidants toward DPPH•. The reducing ability can be evaluated by electron spin resonance or by measuring the decrease of its absorbance. The measurement of the loss of DPPH• colour at 517 nm following the reaction with test compounds is what the antioxidant assays are based on (Prior, 2005). Thus, when DPPH• radical accepts an electron or hydrogen in the presence of a suitable free radical scavenger or antioxidant compound (reducing agent), it changes to its reduced DPPH form (figure 2.4). The changes in colour from deep violet to light yellow (Residual DPPH) is then measured at 517 nm on a UV/visible light spectrophotometer. The absorbance decreases with increasing free radical scavenging ability of the antioxidant compound. The % DPPH scavenging effect or % Radical Scavenging Acitivity (% RSA) of the antioxidant compound is calculated using the equation below:

$$\%\ RSA = \frac{Absorbance\ (Control) - Absorbance\ (extract\ or\ drug)}{Absorbance\ (Control)} \times 100$$

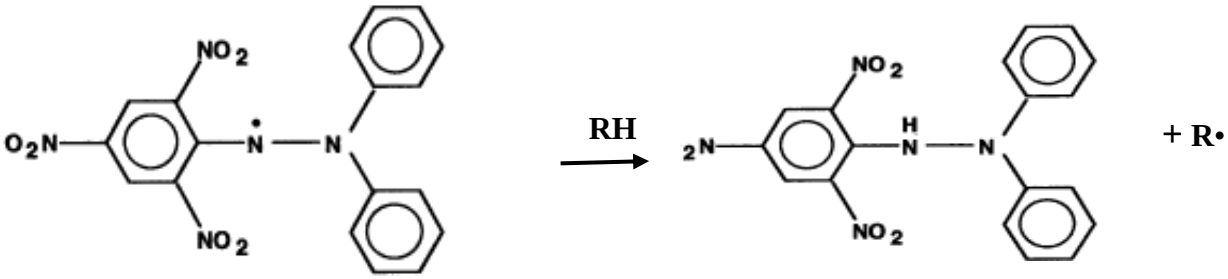


Figure 2.4: Diphenylpicrylhydrazyl (free radical) Diphenylpicrylhydrazine (nonradical)

Using the DPPH assay has its advantages. It is simple, rapid and needs only a UV-Visible spectrophotometer to carry out. However, it has many disadvantages. Because DPPH is both a radical probe and an oxidant, the assay is not a competitive reaction. DPPH color can be lost due to radical reaction (HAT) or reduction (SET) as well as unrelated reactions, and steric accessibility is a major factor for determining results of the reaction (Prior, 2005).

2.4.2 Folin-Ciocalteu or Total Phenolics Assay

The Folin-Ciocalteu (F-C) method has for many years been used as a means to determine total phenolics in natural products (Prior, 2005). The reaction that takes place is an oxidation/reduction one and because this reaction is the basic mechanism, F-C can also be considered an antioxidant capacity method. This assay has many variations.Different reagent concentrations and timing of additions and incubation are frequently varied (Prior, 2005). Also, many studies show the recommended reference standard (gallic acid) being replaced with tannic acid equivalents, caffeic equivalents, vanillic acid equivalents and catechin equivalents, among others. Phenolic compounds can be found in flavonoides, phenolic acids, hydroxycinnamic acid derivatives and lignans.

Principle

Phosphomolybdate-phosphotungstate salts of Folin-Ciocalteu's reagent are reduced by phenolic compounds in alkaline medium giving a blue colouration. The intensity of the blue colouration is then quantified spectrophotometrically at 760 nm. Absorbance increases with increasing phenol content.

2.4.3 Reducing Power Assay

Principle

The assay depends upon the ability of a test compound to reduce Fe^{3+} to Fe^{2+}. The resultant Fe^{2+} then reacts with ferric cyanide ion to form a Prussian blue Iron ferric cyanide complex with maximum absorbance at 700 nm. The greater the reducing power, the greater the intensity of the blue complex and the higher the absorbance (Oyaizu, 1986).

The equations of the reactions are as follows:

Fe^{3+} to Fe^{2+} ……(1)

$K_3 Fe(CN)_6 (aq) + Fe^{2+} (aq) \longrightarrow K Fe[Fe(CN)_6] + 2K^+ (aq)$ …… (2)

2.4.4 Total Antioxidant Capacity (TAC) Assay

The TAC Assay is based on the reduction of Molybdate (VI) to Molybdate (V) by the antioxidant compound and subsequent formation of green Phosphate/Molybdate (V) complex at acid pH. The greater the antioxidant capacity, the greater the intensity of the green colour and the higher the absorbance (Prieto *et al.*, 1999).

2.5 OVERVIEW OF THE SELECTED MEDICINAL PLANTS

The occurrence, description, agronomy and health benefits of the selected indigenous medicinal plants are reviewed below

2.5.1 *Annona muricata*

Family: Annonnaceae

Common Names: Brazilian pawpaw, soursop, prickly custard apple, Soursapi

Occurrence

A. muricata trees grew natively in the Caribbean and Central America but are now widely cultivated and in some areas, escaping and living on their own in tropical climates throughout the world.

Figure 2.5 Picture of *Annona muricata*

(en.wikipedia.org/wiki/*Annona muricata*)

Description

Annona muricata is a small, upright, evergreen that can grow to about 4 metres (13 ft) tall and cannot stand frost. The young branches are hairy. Leaves are oblong to oval, 8 centimetres (3.1 in) to 16 centimetres (6.3 in) long and 3 centimetres (1.2 in) to 7 centimetres (2.8 in) wide. Glossy dark green with no hairs above, paler and minutely hairy to no hairs below.The leaf stalks are 4 millimetres (0.16 in) to 13 millimetres (0.51 in) long and without hairs.Flower stalks (peduncles) are 2 millimetres (0.079 in) to 5 millimetres (0.20 in) long and woody. They appear opposite from the leaves or as an extra from near the leaf stalk, each with one or two flowers, occasionally a third. Stalks for the individual flowers (pedicels) are stout and woody, minutely hairy to hairless and 15 millimetres (0.59 in) to 20 millimetres (0.79 in) with small bractlets nearer to the base which are densely hairy. Petals are thick and yellowish. Outer petals meet at the edges without overlapping and are broadly ovate, 2.8 centimetres (1.1 in) to 3.3 centimetres (1.3 in) by 2.1 centimetres (0.83 in) to 2.5 centimetres (0.98 in), tapering to a point with a heart shaped base. Evenly thick, covered with long, slender, soft hairs externally and matted finely with soft hairs within. Inner petals are oval shaped and overlap. 2.5 centimetres (0.98 in) to 2.8 centimetres (1.1 in) by 2 centimetres (0.79 in). Sharply angled and tapering at the base. Margins are comparatively thin, with fine matted soft hairs on both sides. The receptacle is conical and hairy. Stamens 4.5 millimetres (0.18 in) long and narrowly wedge-shaped. The connective-tip terminates abruptly and anther hollows are unequal. Sepals are quite thick and do not overlap. Carpels are linear and basally growing from one base. The ovaries are covered with dense reddish brown hairs, 1-ovuled, style short and stigma truncate. Dark green, prickly (or bristled) fruits are egg-shaped and can be up to 30 centimetres (12 in) long, with a moderately firm texture. Flesh is juicy, acid, whitish and aromatic.

Agronomy

Annona muricata L. proliferates better in warm and humid climates. It grows in well-drained soils. Optimal soils are sandy, slimy, clayey, and sandstone. This plant species develops well in a slightly acid soil-pH (5.5 to 6.5). *Annona muricata* L. tolerates poor soils. This tree is frequently cultivated by its edible fruits in low forests in the tropics around the whole world. It flowers during the whole year.

Nutrition and Health Benefits

Annona muricata has been used as folkloric herbal medicine in many regions thought the world. It is considered to be antispasmodic, sudorific and emetic. A decoction (boiling in water) of *Annona muricata* leaves is used to kill bedbugs and head lice. To reduce fever, a decoction of leaves can be taken internally or the leaves added to bathing water also has the same effect. The crushed fresh leaves are also applied on skin eruptions for faster healing. A poultice of young *Annona muricata* leaves is applied on the skin to alleviate rheumatism and other skin infections like eczema. Applied during the healing of wounds, this can result in less or no skin scars. The decoction can also be used as a wet compress on swollen feet and other inflammations. . Several studies by different researchers demonstrated that the leaves have antispasmodic, anticonvulsant, vasodilator, smooth muscle relaxant, and cardio-depressant activities in animals. (Hasrat, et al.1997). The leaves are also used for diabetes and leaf tea as a sedative, heart tonic and for liver problems and nervine for heart conditions, coughs, grippe, difficult childbirth, asthma, asthenia, hypertension. The crushed seeds are used as a vermifuge and anthelmintic against internal and external parasites, head lice, and worms (Bories, et al. 1991). Seeds contain approximately 45% of yellow-colored, non-secant oil, which is irritant venom that can cause a severe ocular inflammation. Petroleum ether seed extracts and chloroform seed extracts are toxic for black scarab larvae that live in carpets. The oil extracted from seeds kill head louses. The oil has also been traditionally used as

venom in order to capture fishes. In the Caribbean, a tincture prepared with pulverized seeds and golden rum is used as a strong emetic (Bories, et al. 1991). Roots are used as sedative, antispasmodic, hypotensive and nervine (to tone up nerves and stimulate its action). Roots are drunk as tea. The roots are also used against diabetes. They are also used as vermifuge (Bories, et al. 1991). The stem bark is used against diabetes, as well as sedative and antispasmodic. In Guyana, native tribes prepare a bark tea and use it as sedative and heart tonic. The bark is also used as sedative and nervine (it is, to tone up and stimulate nerves), for heart diseases, cold, flu, childbirth difficulties, asthma, asthenia, hypertension, and against parasites. The juice obtained from the fruit is used as diuretic and as a remedy for haematura (presence of blood in urine) and urithritis. Drunk on empty stomach, this juice is believed to alleviate liver diseases and leprosy. Eaten as fruit or drunk as juice, the fruits ared in order to combat worms, reduced fever, increase milk production by women afterbirth (lactagogue), and as astringent against diarrhea and dysentery. Ripe fruits are rich in vitamin B1, B2 and C and in phosphorus.

2.5.2 *Cymbopogon citratus*

Family: Poaceae

Common Names: lemongrass, barbed wire grass, silky heads, citronella grass, fever grass, Hierba Luisa or Gavati Chaha

Occurrence

Lemongrass is native to India and the nearby island of Sri Lanka. It is found growing naturally in tropical grasslands. It is also extensively cultivated throughout tropical Asia. The herb is most popular in Thailand, Vietnam, Malaysia, Cambodia and Indonesia and as far as African and American continents for its culinary and medicinal uses.

Figure 2.6 (Picture of *Cymbopogon citratus*)

(http//theida.com/essential-oils/lemongrass-Cymbopogon-citratus)

Description

Cymbopogon citratus is a tall, aromatic, perennial grass with culms (stems) up to 2 m tall. Its linear leaves are up to 1 m long and 2 cm wide and taper towards the sheath. They are smooth and hairless, white on the upper surface and green beneath. The ligules (appendage between the leaf sheaf and blade) are less than 2 mm long, and are rounded or truncate (ending abruptly as if cut off). The inflorescence is a loose, nodding panicle, about 60 cm long and reddish to russet in colour. The pedicels (stalks of the spikelets) are tinged with purple.

Agronomy

It is easily grown in average, medium, well-drained soils in full sun. Tolerates light shade, but prefers full sun. Tolerates a wide range of soils, but is best in organically rich loams with good drainage.

Nutrition and Health Benefits

The culms (stems or stalk) of lemon grass are widely used in teas and other beverages, herbal medicines, and to flavour southeast Asian cuisine, particularly fish stews and sauces. Its leaves are used to make tea which can relieve stomach and gut problems. It can also act as an antidepressant and as a mood enhancer. In the folk medicine of Brazil it is believed to have anxiolytic, hypnotic and anticonvulsant properties (Blanco *et al.* 2009; Rodrigues and Carlini, 2006).

2.5.3 *Okoubaka aubrevillei*

Family: Santalaceae

Common Names: **Ghana** *Akan-Asante* odii ,*Fante* duyin **Ivory Coast** *Anyi* oku baka (death tree) **Liberia** *Mano* yai yili **Sierra Leone** *Kono* yɔua *Mende* yuwe,

Occurrence

Okoubaka is a genus of tall forest tree native to west and central tropical Africa. Its name is from the Anyin language meaning a tree with allelopathic properties, or a tree that causes the death of surrounding vegetation (Hallé, 1987). *Okoubaka aubrevillei* is usually found in forest on rocky hills. It is usually solitary but there are reports from Côte d'Ivoire and Ghana that it is found in pure stands

Description

Monoecious, deciduous, medium-sized tree up to 30 m tall; bole cylindrical, straight, up to 80 cm in diameter; bark surface coarse, greyish brown to reddish brown; branches horizontal, branchlets slightly grooved, densely hairy. Leaves alternate to almost opposite, simple and entire; stipules absent; petiole 3–15 mm long, 2-ribbed; blade ovate to oblong, 7.5–15 cm ×

3.5–6 cm, base rounded to slightly cordate, apex acuminate, dark green, with 3–5 pairs of lateral veins, densely hairy when young. Inflorescence a panicle on older branches, up to 20 cm long. Flowers unisexual, 5-merous, regular, sessile, green; male flowers with triangular petals, c. 2.5 mm × 2.5 mm, short-hairy, stamens c. 0.5 mm long, disk cup-shaped, lobed, ovary with abortive ovules, style c. 1 mm long, stigma 4-pointed; female flowers slightly larger than male flowers, stamens sterile, disk cup-shaped, prominently lobed, hairy on upper margin, ovary superior, 4-celled, style c. 1 mm long, stigma 4-lobed. Fruit an ellipsoid drupe up to 9 cm long, glabrous, green turning yellow, 1-seeded. Seed ellipsoid, 7 cm × 4.5 cm, longitudinally ridged. Seedling with hypogeal germination.

Nutrition and Health Benefits

In West Africa *Okoubaka aubrevillei* bark is widely used as a medicine to treat skin problems, including those caused by syphilis and leprosy by washing with, or bathing in a macerate or infusion of the bark in water. External application of bark preparations is also used to counteract poisoning. Bark macerate is drunk to cure tachycardia and is taken as a vapour bath or as nose drops to cure oedema. In a compress it is used to disperse haematomas. It is also used for stomach upsets caused by poisoning and to boost the system in cases of tiredness, depression and allergies in the western world.

2.5.4 *Theobroma cacao*

Family: Sterculiaceae

Common Names: Chocolate, Cacao, Cocoa

Occurrence

Native to South America, probably on the equatorial slopes of the Andes; now cultivated pan tropically, especially in West Africa.

Figure 2.7a: Picture of *T. cacao* plant (http://en.wikipedia.org/wiki/file. Cacao)

Figure 2.7b: Picture of *T. cacao* flower (http://en.wikipedia.org/wiki/Theobroma cacao)

Description

Theobroma cacao also cacao tree and cocoa tree, is a small (4–8 m (13–26 ft) tall) evergreen tree in the family Sterculiaceae (alternatively Malvaceae), native to the deep tropical region of the Americas. Cacao leaves are alternate, entire, unlobed, 10–40 cm (3.9–16 in) long and 5–20 cm (2.0–7.9 in) broad. The flowers are produced in clusters directly on the trunk and older branches; they are small, 1–2 cm (0.39–0.79 in) diameter, with pink calyx. The fruit, called a cacao pod, is ovoid, 15–30 cm (5.9–12 in) long and 8–10 cm (3.1–3.9 in) wide, ripening yellow to orange, and weighs about 500 g (1.1 lb) when ripe. The pod contains 20 to 60 seeds, usually called "beans", embedded in a white pulp.

Agronomy

Ranging from Subtropical Dry to Wet through Tropical Very Dry to Wet Forest Life Zones, cacao is reported to tolerate annual precipitation of 4.8 to 42.9 dm annual temperature of 18.0 to 28.5°C and pH of 4.3 to 8.7 (Duke, 1978). The plant requires uniformly high temperatures with recommended mean of 26.6°C. Trees are wind-intolerant and therefore are often planted on hillsides for wind protection and good drainage. Being drought-intolerant, cacao thrives in climates with high humidity and rainfall. Plants are shade-tolerant, and thrive in rich, organic, well-drained, moist, deep soils. Shallow laterite soils are said not to be suitable. Maximum temperature of 33.5°C and minimum 13°C, with diurnal temperature variation between 33.5 and 18°C suggested (Reed, 1976).

Nutrition and Health Benefits

Cocoa seeds are used for cocoa products such as cocoa liquor, cocoa butter , cocoa cake and cocoa powder and chocolate. Roasted and pounded seeds could be used to treat eczema, dry skin by applying it to the affected areas as poultice after a warm compress. Root decoction is reported to be used as emmenagogue (promotes or stimulates menstrual flow), ecbolic (promotes labor by stimulating uterine contractions), antiseptic, diuretic, and infusions of the leaves are used cardiotonic, cough, fever, malaria, nephrosis (Duke and Wain, 1981). Cocoa butter (oil of Theobroma) is an excellent emollient for use to prevent chapped lips and hands. A tea made from the seed shells act as an effective diuretic. Cocoa is high in magnesium which is known to increase the overall vigour of the heart muscles and decreases blood coagulation which lower blood pressure and helps the heart pump more effectively, reducing the likelihood of blood clots and stroke.

2.6 Antimicrobial Activity

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi or protozoans, as well as destroying viruses. Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (microbistatic).

Disinfectants are anti-microbial substances used on non-living objects. However, the future effectiveness of antimicrobial therapy is somewhat in doubt. Microorganisms, especially bacteria, are becoming resistant to most antimicrobial agents. Bacteria found in hospitals appear to be especially resilient, and are causing increasing difficulty for the sickest patients-those in the hospital to recover quickly. Currently, bacterial resistance is combated by the discovery of new drugs. However, microorganisms are becoming resistant more quickly than new drugs are being found. Thus, future research in antimicrobial therapy may focus on finding how to overcome resistance to antimicrobials, or how to treat infections with alternative means.

2.6.1 Antibiotics

Antibiotics are generally used to treat bacterial infections. Since 1980, the introduction of new antimicrobial agents for clinical use has declined. Paralleled to this there has been an alarming increase in bacterial resistance to existing antibiotic agents. Antibiotics are among the most commonly used drugs. However, antibiotics are also among the drugs commonly misused by physicians, e.g. usage of antibiotic agents in viral respiratory track infection. The inevitable consequence of widespread and injudicious use of antibiotics has been the emergence of antibiotic-resistant pathogens, resulting in the emergence of a serious threat to global public health.

2.6.2 Antiviral

Antiviral drugs are a class of medication used chiefly for treating viral infections. Like antibiotics, specific antivirals are used for specific viruses. They are relatively harmless to the host, and therefore, can be used to treat infections. Antiviral drugs work by inhibiting the virus ether before it enters the cell, stopping it from reproducing, or in some cases preventing it from exiting the cell. However, like antibiotics, viruses may evolve to resist antiviral drugs.

2.6.3 Antifungal

An antifungal drug is a medication used to treat fungal infections such as athlete's foot, ringworm, candidiasis (thrush) among other diseases. Antifungals work by exploiting differences between mammalian and fungal cells to kill off the fungal organism without dangerous effects on the host.

2.6.4 Non-pharmaceutical antimicrobial

A wide range of chemical and natural compounds are used as antimicrobials. Organic acids such as lactic acid, citric acid and their salts are widely used as antimicrobials in food products. Traditional healers have long used plants to prevent or cure infectious disease. Large numbers of plant products have been shown to inhibit the growth of pathogenic microorganisms. A number of these agents appear to have structures and modes of action that are distinct from those of the antibiotics in current use, suggesting that cross-resistance with agents already in use may be minimal.(Morrison, 2009)

2.6.5 Gram-Positive Bacteria and Gram-Negative Bacteria

Gram-positive bacteria are bacteria that are able to retain the violet stain because of the high amount of peptidoglycan in the cell wall. The presence of teichoic acids (and lipoteichoic acids) in their cell walls act as chelating agents. Examples are *Staphylococcus aureus, Bacillus subtilis* and *Streptococcus suis*.

Gram-negative bacteria are those bacteria that cannot retain the crystal violet stain but which take up the counter stain (safranin or funchsin) and appearing red or pink. Examples of Gram-negative bacteria are *Escherichia coli, Pseudomonas aeruginosa, K. Pneumonia* and *S. typhimurium.* (Morrison, 2009)

2.6.6 Properties of the selected Bacterial strain

This section reviewed the nature, pathogenesis and antibiotic resistance of the selected bacterial strains.

2.6.6.1 *Bacillus subtilis*

Fig.2.8 Micrograph of stained *Bacillus subtilis* (w.w.w.shop.arravit.com)

Bacillus subtilis, also known as the hay bacillus or grass bacillus is a gram-positive, catalase-positive bacterium commonly found in soils. It is rod-shaped and has the ability to form a tough, protective endospore which allows it to tolerate extreme environmental conditions such as heat, acid, and salt (Nakano and Zuber, 1998; Morrison, 2009).

Pathogenesis

Bacillus subtilis is not considered a human pathogen; however, it may contaminate food and but rarely causes food poisoning. *Bacillus subtilis* spores can survive the extreme heating that is often used to cook food and it is responsible for causing *ropiness* (a sticky, stringy consistency caused by bacterial production of long-chain polysaccharides) in spoiled bread dough (Nakano and Zuber, 1998).

2.6.6.2 Escherichia coli

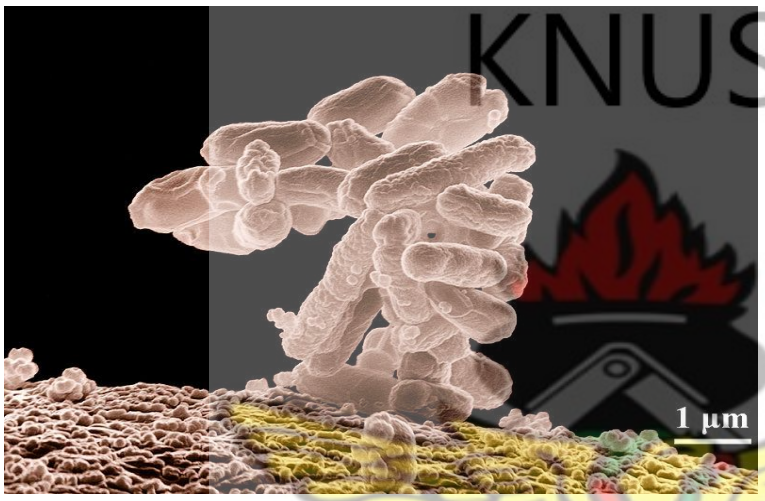


Fig.2.9 Electron micrograph of a cluster of *E. coli* bacteria(en.wikipedia.org/wiki/E.coli)

Escherichia coli is a gram negative, facultative anaerobic and non-sporulating bacterium commonly found in the lower intestine of warm-blooded organisms (endotherms). Its cells are typically rod-shaped. *Escherichia coli* also has the ability to survive to survive for brief periods outside the body (Vogt *et al*., 2005; Morrison, 2009).

Pathogenesis

E. coli normally colonizes an infant's gastrointestinal tract within 40 hours of birth, arriving with good or water or with individuals handling the child. In the bowel, it adheres to the mucus of the large intestine. It is the primary facultative organism of the human gastrointestinal tract. Most *E. coli* strains are harmless, but some can cause serious food poisoning in humans (Vogt *et al*., 2005).

Antibiotic Resistance

E. coli strains are resistant to many antibiotics that are effective against gram-positive bacteria (Vogt *et* al., 2005).

2.6.6.3 Staphyloccocus aureus

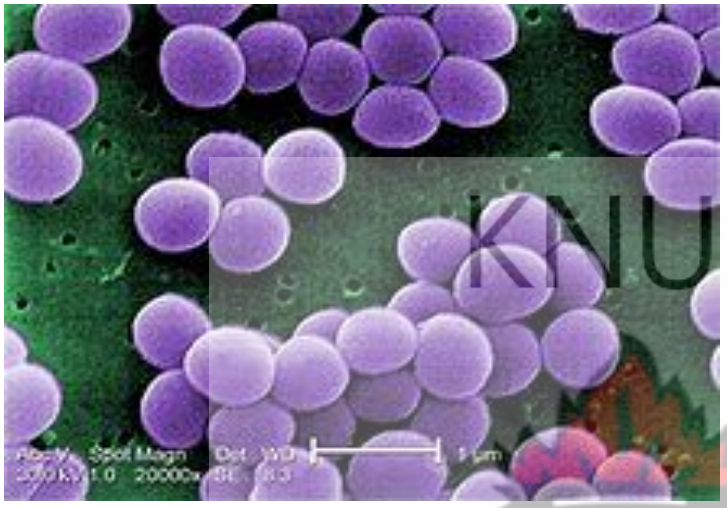


Fig.2.10 Scanning electron micrograph of *S. aureus*; false color added
(http://en.wikipedia.org/wiki/*Staphylococcus_aureus*)

Staphylococcus aureus, also known as golden staph is a spherical, gram-positive, facultatively anaerobic bacterium which appears as grape-like clusters when viewed through a microscope and has large, round, and golden-yellow colonies. It can survive for some hours on dry environmental surfaces (Kluytmans *et al*., 1999).

Pathogenesis

S. aureus is frequently found in the nose skin of a person. It can cause a range of illnesses from minor skin infections, such as pimples, boils, furuncles, carbuncles, scalded skin to life-threatening diseases such as pneumonia, meningitis and endocarditis among others (Kluytmans *et al*., 1999).

Antibiotic Resistance

It has a strong resistance to penincilin and requires a combination therapy with gentamicin to treat serious infections such as endocarditis (Kluytmans *et al*., 1999).

2.6.7 Antimicrobial Evaluation Method - Disc Diffusion Agar Method

Principle

The principle behind this technique is that, when a disc of paper saturated with a sample (or antibiotic) is placed on a nutrient agar previously inoculated with the test microorganism in a Petri disc, the disc imbibes moist content from the agar and creates a gradient. As a result, the sample diffuses radially outward through the agar until its concentration lowers. A clear zone is observed if the sample inhibits the growth of the test microorganism. The extent of inhibition is characteristic of the concentration, solubility, and diffusion rate of the sample. The broader the zone of microbial growth inhibition, the susceptible (less resistance) the test microorganism is (Mousumi Debnath, 2008).

CHAPTER THREE

3 MATERIALS AND METHODS

3.1Materials

3.1.1 Equipment

Soxhlet set-up (500 mL)

UV-visible spectrophotometer (LKB Biochrom, Cambridge, Cambridge, England, Model 4050)

Centrifuge (Sanyo MSE, MISTRAL 3000E, UK)

Incubator (Gallenkamp model IH, UK).

Rotary evaporator apparatus (BUCHI Rotavapor, R-144)

3.1.2 Chemical Reagents

The entire chemical reagents used were of analytical-reagent grade.

DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate) Sigma-Aldrich Chemie: Germany

Folin-Ciocalteu Phenol Reagent; Sigma-Aldrich Chemie: Steinheim, Germany

Ascobic acid; BDH: Poole, England

Anhydrous Sodium Carbonate; BDH: Poole, England

Antimony Trichloride Hydrate ; BDH: Poole, England

Sodium Acetate ; BDH: Poole, England

98% Acetic Acid (Sigma-Aldrich Chemie); Germany

Tannic Acid ; BDH: Poole, England

Sodium Acetate (Sigma-Aldrich Chemie),Germany

99% Methanol; BDH: (Sigma-Aldrich Chemie); Germany

98%Ethanol (Sigma-Aldrich Chemie) , Germany

98% Sulphuric acid (Sigma-Aldrich Chemie);

36% Hydrochloric acid (Sigma-Aldrich Chemie); Germany

Picric acid BDH: Poole, England

Ferric chloride BDH: Poole, England

Nutrient agar (Oxoid); England

Magnesium turnings,

Hydrogen peroxide (Sigma-Aldrich Chemie); Germany

Potassium hydroxide BDH: Poole, England

Fehling's solution BDH: Poole, England

Sodium hydroxide BDH: Poole, England

Chloroform (Sigma-Aldrich Chemie); Germany

3.1.3 Plant material

Seeds of *Okuobaka aubrevillei* were obtained from the Kumasi Central market. The false stem and roots of *Cymbopogon citratus* (Lemon grass) were obtained from Buokrom a suburb of Kumasi while the Leaves of *Annona muricata* and the leaves of *Theobroma cacao* were also collected from Adansi Dompoase in the Ashanti region. All plants and their parts were identified by Mr. M. A. Arkoh of Theoritical and Applied Biology Department of KNUST.

3.1.4 Plant Sample Preparation

The plant samples were washed with distilled water and air-dried at ambient temperature for 2 months after which the leaves and the seeds were ground into powdered form using a grinder, labeled and kept in refrigerator for future analyses.

3.2 Methods

Below is a description of the methods used for the study on the selected medicinal plant parts namely; extraction, screening for phytoconstituents, antioxidant capacity determination assays, and antibacterial activity assay.

3.2.1 Extraction

50 g of the air-dried powdered leaves of the vegetable samples were extracted with 500 ml Methanol (99 %), 500 ml distilled water and ethanol (98 %) mixture (1:1) for 10 hours using Soxhlet apparatus. The extracts were filtered and concentrated in a rotary evaporator apparatus (BUCHI Rotavapor, R-144) at approximately 60 °C. The extracts were kept in a refrigerator for future analyses. The percentage yields of the samples were calculated.

3.2.2 Screening for Phytoconstituents

The following secondary metabolites were screened using the method described in Trease and Evans' Pharmacognosy Book. The phytochemical screening were performed on the methanolic and hydro – ethanolic extracts of the following plants the seed of *Okuobaka aubrevillei*, the false stem and root of *Cymbopogon citratus*, leaves of *Annona muricata* and *Theobroma cacao*.

3.2.2.1 Test for saponins

1.0g of the plant extract was moistened with 10ml of distilled water and boiled over a water bath for 3mins. The hot content was filtered and the filtrate shaken vigorously. Persistent froth (foam) is indicative of saponins

3.2.2.2 Test for general glycosides

1.0g of the extract was put into two separate beakers and dried at 60^{o}C. To one beaker: 5ml of dil. H_2S0_4 was added, boiled, filtered and cooled. NaOH solution was added to the filtrate and heated with Fehling's solution (A and B) for 3mins. (Formation of reddish – brown precipitate indicates the presence of glycosides). To the other beaker: 5ml of distilled water was added, boiled for 3mins, filtrated and cooled. The filtrate was alkalinised and heated with Fehling's solution for 3mins. (Absence of precipitate indicates the presence of glycosides).

3.2.2.3 Test for flavonoids

Magnesium turnings were added to alcoholic solution of the plant extract. Conc. HCl was added dropwise afterwards. (Brick-red colouration indicates the presence of flavonoids).

3.2.2.4 Test for alkaloids

10 ml of 1% HCl was added to the sample or extract and left to stand for 3 minutes with occasional stirring. The acidified solution was filtered. Hager's regent (saturated aqueous solution of picric acid) was added to 2 ml of the filtrate. (Formation of yellow precipitate indicates the presence of alkaloids).

3.2.2.5 Test for Tannins

Test for tannins was carried out by adding 6ml of distilled water to 1.0g portion of the plant extract followed by 2ml ferric chloride solution. The content of the test tube was observed for reddish-black colour which is indicative of the presence of tannins.

3.2.2.6 Test for carotenoids

1.0 g of the extract was dissolved in 10 ml of chloroform in a test tube with vigorous shaking. The resulting mixture was filtered and 85 % sulphuric acid was added. A blue colour at the interface showed the presence of carotenoids.

3.2.2.7 Test for terpenoids

To 0.5 g of the extract was added 2 ml of chloroform. Concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

3.2.3 Evaluation of Antioxidant Potential

The antioxidant potential of the selected medicinal plants were evaluated using different antioxidant capacity determination assays.

3.2.3.1 DPPH radical scavenging assay

Radical scavenging activity of the extracts from the medicinal plants samples against stable DPPH• radical was determined spectrophotometrically. Radical scavenging activity of extracts was measured by slightly modified method of Govindarajan *et al.* (2003). The plant extract (0.1, 0.3, 1, 3 mg/ml in methanol) was compared with ascorbic acid (0.01, 0.03, 0.1, 0.3 mg/ml in methanol) as a reference free radical scavenger. 10 ml of the extract was centrifuged at 3000 rpm using a centrifuge (Sanyo MSE, MISTRAL 3000E, UK) for 10 minutes and the supernatant collected. The supernatant of the extract (1 ml) was added to 3 ml methanolic solution of DPPH (20 mg/l) in a test tube. The reaction mixture was kept at 25 ^{o}C for 1 hour in orbital shaker (BoroLabs, Aldermaston Berkshire, EC). The absorbance of the residual DPPH solution was determined at 517 nm in a UV-Visible spectrophotometer (LKB Biochrom, Cambridge, Cambridge, England, Model 4050). Methanol (1 ml) was added

to 3 ml DPPH solution and kept at 25 ^{o}C for 1 hour and used as control. Methanol was used as the blank. The measurements were done in triplicate. The results were expressed as % Radical Scavenging Activity against concentration and the IC_{50} determined using Ms Excel.

3.2.3.2 Total Phenolic Compounds Assay

The content of total phenolic compounds in the extracts from the medicinal plant samples (0.1, 0.3, 1 and 3 mg/ml) was quantitatively determined by colorimetric assay using Folin-Ciocalteu's (F-C) reagent (Singleton, 1977) with slight modifications. Tannic acid (0.01, 0.03, 0.1 and 0.3 mg/ml) was used as the reference drug. The plant extract (1 ml) was added to 1 ml of F-C reagent (diluted five folds in distilled water) in a test tube. The content of the test tube was then mixed and allowed to stand for five minutes at room temperature. 1 ml of 2 % sodium bicarbonate solution was added to the mixture. The reaction mixture was then incubated at 25^{o}C for 2 hours. The reaction mixture after the incubation period was centrifuged at 3000 rpm for 10 minutes to get a clear supernatant. The absorbance of the supernatant was then measured at 760 nm using the UV-Visible spectrophotometer (LKB Biochrom, Cambridge, Cambridge, England, Model 4050). Distilled water (1 ml) was added to 1 ml F-C reagent (diluted five folds in distilled water) processed in the same way as done for the test extracts and reference drug. The measurements were done in triplicate. The content of total phenolic compounds was expressed as Tannic Acid Equivalents (TAE mg/ml) using Ms excel.

3.2.3.3 Reducing Potential Assay

The reducing potential of the extracts (0.1, 0.3, 1 and 3 mg/ml in methanol) was determined using the method described by Oyaizu (1986), with slight modifications using ascorbic acid (0.01, 0.03, 0.1 and 0.3 mg/ml) as a reference antioxidant drug. The extract/drug (1 ml) was mixed with 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1 % potassium

ferric cyanide solution in a test tube. The mixture was incubated at 50 ^{o}C for 20 minutes. Following this, 1.5 ml of 10 % Trichloroacetic acid solution was added to the incubated mixture and centrifuged at 3000 rpm for 10 minutes using the centrifuge (Sanyo MSE, MISTRAL 3000E, UK). 2.5 ml of the supernatant was mixed with 2.5 ml distilled water and 0.5 ml of 0.1 % ferric chloride solution [$FeCl_{3(aq)}$] in a test tube. The absorbance was then measured at 700 nm using the UV-Visible spectrophotometer (LKB Biochrom, Cambridge, Cambridge, England, Model 4050). Distilled water was used in place of the test drug/extract and used as the blank. The absorbance measurements were done in triplicates. Data was presented as concentration-absorbance curves using Ms excel.

3.2.3.4. Total Antioxidant Capacity (TAC) Assay

The total antioxidant capacity was evaluated using the method described by Prieto *et al*. (1999). Ascorbic acid (0.01, 0.03, 0.1 and 0.3 mg/ml) was used as the standard antioxidant drug. 3 ml of the extract/standard drug (0.1, 0.3, 1 and 3 mg/ml) was placed in a test tube. 0.3 ml of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM Ammonium molybdate) was then added and the resulting mixture was incubated at 95^{o}C for 90 minutes. After the mixture has cooled to room temperature, the absorbance of each solution was measured in triplicates using the UV-Visible spectrophotometer (LKB Biochrom, Cambridge, England, and Model 4050) at 695 nm against a blank. The total antioxidant capacity was expressed as Ascorbic Acid Equivalents (AAE mg/ml) using Ms excel.

3.2.4 Evaluation of Antimicrobial Activity

The antimicrobial activity of the extracts from the plant samples was evaluated using the agar diffusion method as described by Mousumi Debnath (2008). Three pathogenic bacterial strains were tested against methanolic and hydro – ethanolic extracts of the false stem and root of *Cymbopogon citratus* (lemon grass), leaves of *Annona muricata* and the leaves of *Theobroma cacao,* the seed of *Okoubaka aubrevillei*. Certified strains of bacterial viz. *E. coli*, *B. subtilis* and *S. aureus* were obtained from the Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi-Ghana. Susceptibility tests were performed on the extracts to ascertain their activity against *E. coli*, *B. subtilis* and *S. aureus* with higher concentrations of the extracts. (200 mg/ml, using methanol as solvent). For the determination of minimum inhibitory concentration, four different concentrations were prepared (100, 50, 25 and 12.5mg/ml) from the methanolic and hydro - ethanolic extracts of the selected plants. 20 ml of the agar nutrient (in boiling tubes) was sterilized in an autoclave at a pressure of 15 lb/in^2 for 20 minutes. The content of the boiling tube was poured into a Petri dish and allowed to set. The prepared agar nutrient broth was inoculated with each of the pathogenic strain on sterilized disc plates and grown for 24 hours at 37 ^{o}C. Wells were made on the cultured disc plates with a sterile cork borer of 4 mm diameter and impregnated with the different extracts and the disc plates were incubated at 37°C for 24 h. The zones of inhibition were measured at the end of the incubation period. Microbial growth was determined by measuring in duplicates the diameter of the zone of inhibition and the mean values were calculated.

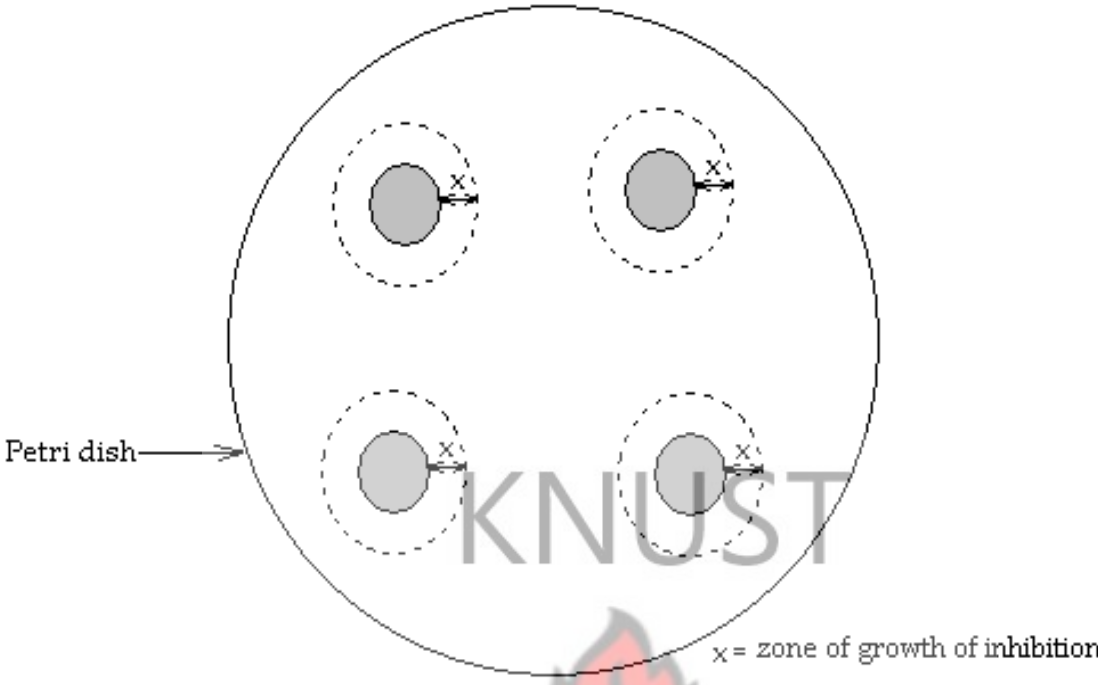


Figure: 2.11 Measurement of zone of growth of inhibition

3.3 Statistical analysis

The experimental results were expressed as mean of the three replicates. Percentage radical scavenging activity (%RSA) was plotted against the sample extract concentration and power regression curve was established in order to calculate IC_{50} (µg/ml), which is the amount of sample required to decrease the absorbance of the free radical by 50%. The results were processed using Microsoft Excel.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

This chapter appraises the findings, analyses and discusses the results obtained from the experiments as described in the previous chapter. In the following subsections, the results obtained for the percentage yield, phytochemical screening, antibacterial tests, and antioxidant properties of the plant extracts of are presented and discussed.

4.1 PERCENTAGE YIELD

Table 4.1 Methanolic and hydro-ethanolic extraction yields for the selected medicinal plants

Selected Medicinal Plants	Yield (%, W/W)	
	Methanolic Extracts	Hydro- Ethanolic Extracts
False stem of *Cymbopogon citratus*	4.2	6.0
Root of *Cymbopogon citratus*	6.5	7.1
Leaves of *Theobroma cacao*	6.9	7.5
Leaves of *Anonna muricata*	8.0	7.7
Seed of *Okuabaka aubrevillei*	5.3	6.9

4.2 RESULTS FROM PHYTOCHEMICAL SCREENING

The phytochemical screenings performed on the plants extracts have been summarised in the table below.

Table 4.2**:** summary of inferences from phytochemical screening

Phytochemicals	**INFFERENCE**									
	Hydro–ethanolic Extract					**Methanolic Extract**				
	AP	**LS**	**LR**	**CO**	**OA**	**AP**	**LS**	**LR**	**CO**	**OA**
Saponins	+	-	-	+	+	+	-	-	+	-
Terpenoids	-	+	+	+	-	-	+	+	+	-
Flavonoids	+	+	+	+	-	-	+	+	+	-
Tannins	+	+	+	+	-	+	-	-	+	-
Carotenoids	+	-	-	+	-	+	-	-	+	-
General Glycosides	+	-	+	+	-	-	+	-	-	-
Alkaloids	+	-	-	-	+	+	-	-	-	+

NB: (+) and (-) indicate presence and absence respectively.
AP= *Anonna muricata* LS= false stem *Cymbopogon citratus*
LR= Root of *Cymbopogon citratus*
CO= Leaves of *Theobroma cacao* *OA*= Seed of *Okuabaka aubrevillei*

4.3 ANTIOXIDANT AND FREE RADICAL SCAVENGING EFFECT

4.3.1 Total phenol assay

The phenol content of tannic acid (0.01 - 0.3 mg mL^{-1}) increased with increasing concentration (r^2 =0.9960) (Fig 2.12) The plant extracts (0.1 - 3 mg mL^{-1}) also showed a concentration dependent increase in phenolic content expressed as tannic acid equivalents (Fig.2.13)

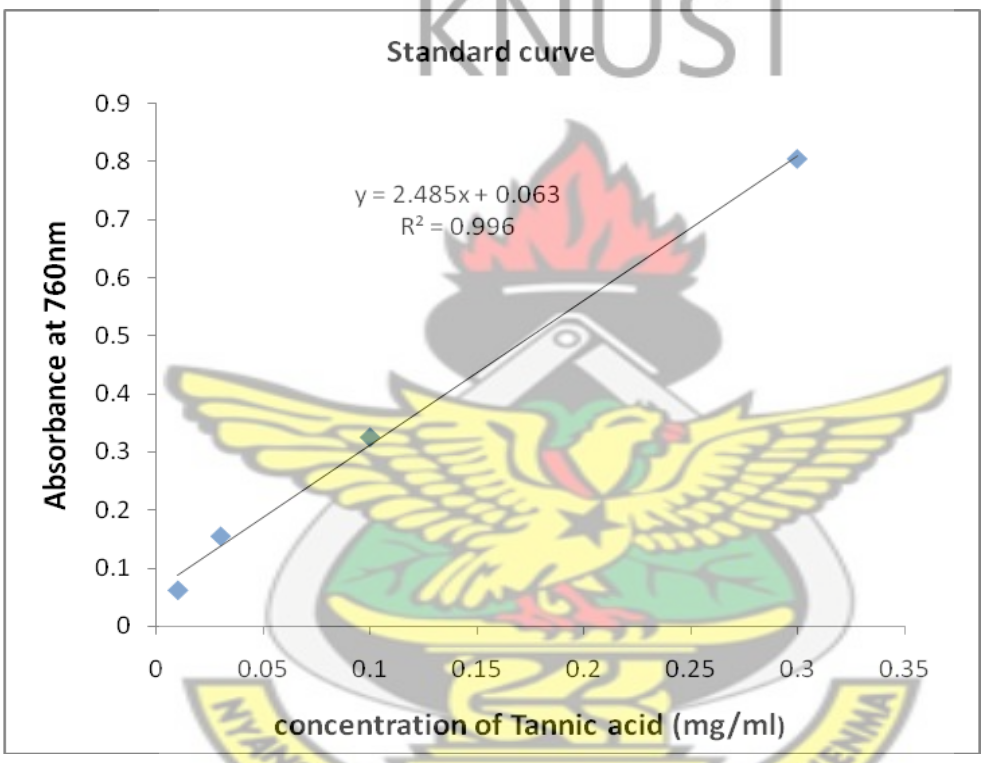


Figure 2.12: A relationship between the absorbance and concentration of tannic acid

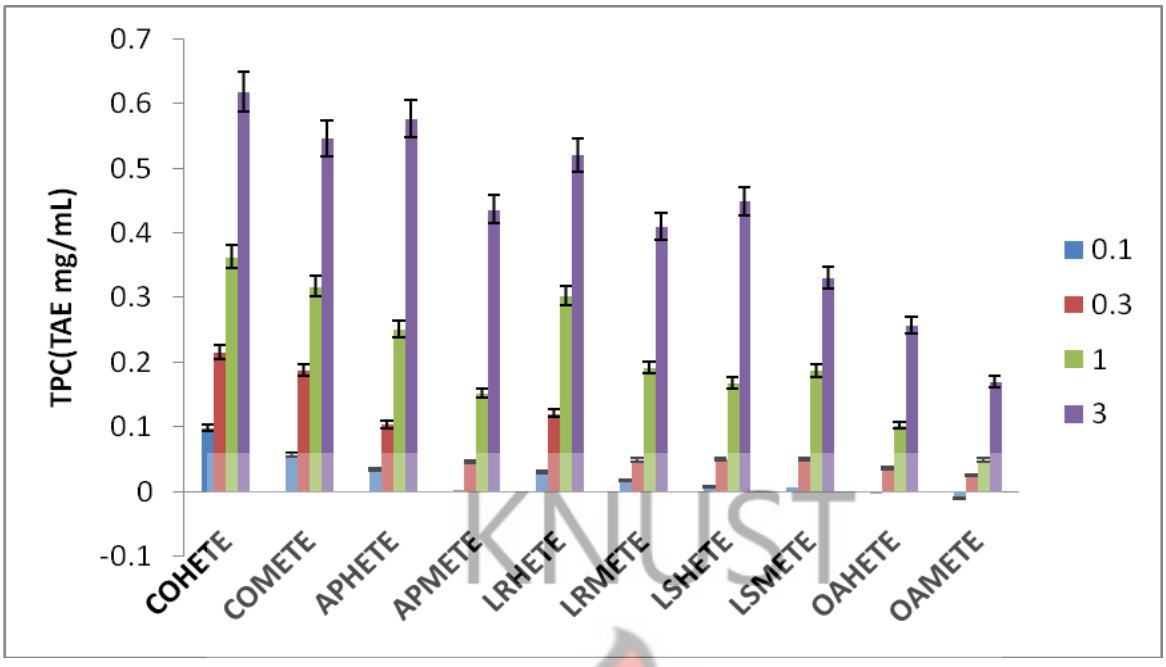


Figure: 2.13 A graph showing TPC (TAE mg/ml) at 0.1 – 3.0 mg/mL of methanolic (METE) and hydro - ethanolic extracts (HETE) from the selected plants, CO, leaves *Theobroma cacao*; LR, Root of *Cymbopogon citratus ;* LS, false stem of *Cymbopogon citratus*; AP, *Annona muricata; OA,* seed of *Okoubaka aubrevillei*]

4.3.2 Total antioxidant capacity

The total antioxidant capacity of ascorbic acid (0.01 - 0.3 mg mL^{-1}) increased with increasing concentration (r^2 =0.999) (Fig.2.14). The plant extracts (0.3 - 3 mg mL^{-1}) also showed an increase in total antioxidant capacity expressed as ascorbic acid equivalent (Fig.2.15)

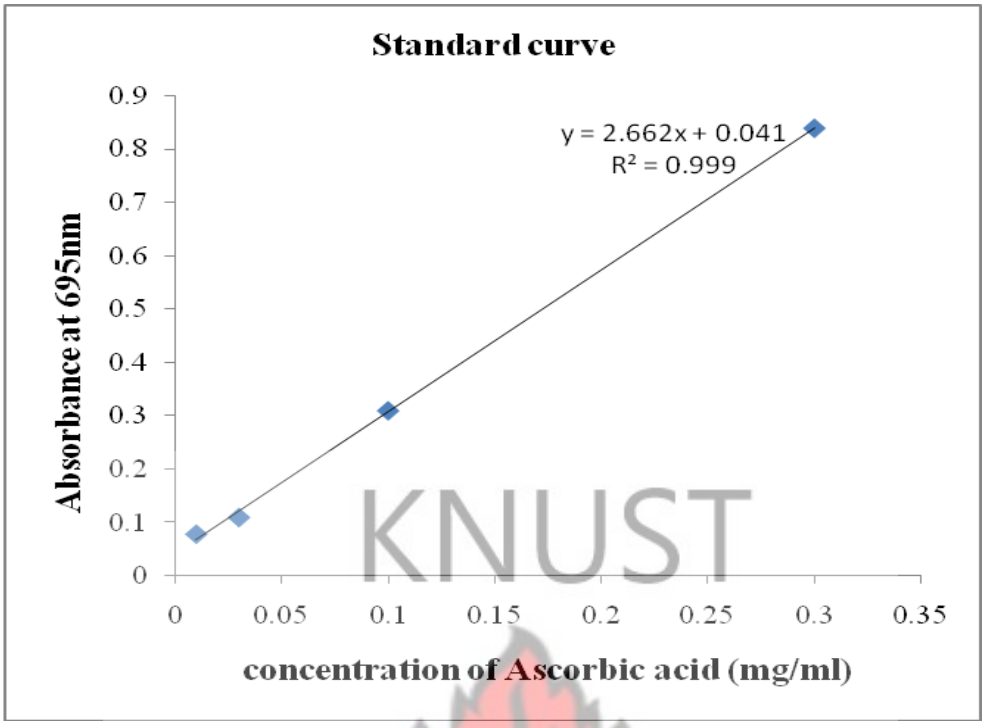


Figure 2.14: A relationship between the absorbance and concentration of ascorbic acid

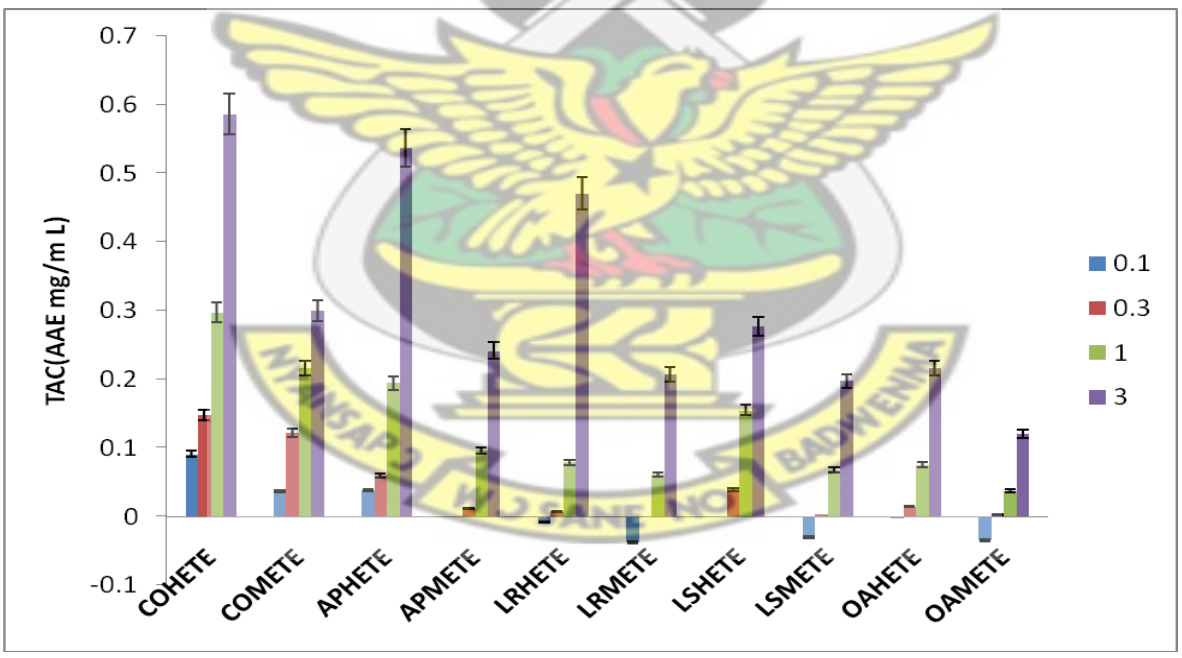


Figure: 2.15. A graph showing TAC (AAE mg/ml) at 0.1 – 3.0 mg/mL of methanolic (METE) and hydro - ethanolic extracts (HETE) from the selected plants, CO, leaves *Theobroma cacao*; LR, Root of *Cymbopogon citratus ;* LS, false stem of *Cymbopogon citratus*; AP, *Annona muricata; OA*, seed of *Okoubaka aubrevillei*]

4.3.3 Total Phenol Content (TPC) and Total Antioxidant Capacity (TAC)

The results of the total antioxidant capacity (expressed as AAE) and total phenol content (expressed as TAE) of the extracts from the selected medicinal plant are shown in Figures 2.11 and 2.13. The TAC and TPC in the methanolic extracts (METE) and hydro-ethanolic extracts (HETE) from the selected medicinal plants within the measured concentration range (0.1 - 3.0 mg/mL) decreased in the order *T. cacao* > *A. muricata* > root of *C. citratus* > false stem of *C. citratus* > *O. okuobaka* as shown in figures 2.11 and 2.13. From Table 4.3, the highest ascorbic acid equivalent was measured for *T.cacao* 0.2996 and 0.5866 mg AAE mL^{-1} on the METE and HETE, respectively, while *O.aubrevillei* showed the least AAE, 0.1202 and 0.2164 mg/ml on its METE and HETE, respectively. Phenolic antioxidants are potent free radical terminators and this is thought to be due to the ability to donate hydrogen to free radicals and their presence is a good marker of potential antioxidant activity. The high potential of phenolic compounds to scavenge free radicals may be explained by their phenolic hydroxyl groups. Detection of phenols in the selected plants extract was a preliminary evidence of its possible antioxidant activity. The total phenol was assayed based on the reduction of phosphomolybdate-phosphotungstate salts to form a blue complex that is detected quantitatively at 700 nm. The total phenolic content of the extract expressed as the tannic acid equivalents increased concentration dependently. Total antioxidant capacity is defined as a measure of the ability of substances extracted from food or herbal matrix to delay oxidation process in a controlled system (Miller and Rice-Evans, 1997). Diets with high vitamin C (ascorbic acid) content are reported to have the potential of improving the pulmonary function and reduce the risk of cancer (Block and Menkes, 1989; Pellegrini et al., 1998). Vitamin C is also reported to have neutralizing effect on hydrogen peroxide, hydroxyl and superoxide radicals (Pellegrini et al., 1998). The significant levels of ascorbic acid equivalent of the extracts from the selected medicinal plant suggest their antiradical potential. With respect to TAE, *T.cacao* had the highest phenol content (0.5462 and 0.6196 mg/mL) for the METE and HETE respectively

while *O.aubrevillei* had the least phenol content (0.1707 and 0.2573 mg/ml) for the METE and HETE respectively (as shown in table 4.3). Studies carried out by Manach et al. (2004), Rice-Evans et al. (1996) and Morrison and Twumasi (2010) on dietary medicinal plants and edible leafy vegetables showed that phenolic compounds possess a special ability to inhibit oxidative stress since phenolic compounds readily undergo electron-donation reactions with reactive oxygen species. Dykes and Rooney (2007), Morrison and Twumasi (2010) also reported the ability of phenolic compounds to boost the immune system. The activity of antioxidants has been attributed by various mechanisms, among which some of them are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity, and radical scavenging (Diplock, 1997). The experimental results therefore suggest that the selected medicinal plant possess antioxidant ability and can be used as supplements.

4.3.4 Correlation between TPC and TAC

A positive correlation was observed between TPC and TAC in both the METE and HETE from all the selected medicinal plants as shown in appendix 3.0. The positive correlation between the TPC and TAC suggests that the phyto-constituents responsible for the total phenol content may also be responsible for the total antioxidant capacity of the plant extracts. Moreover, the TAE in all the selected medicinal plants were higher than their respective AAE. This observation was consistent with the findings of Morrision and Twumasi. (2010) as shown in the table 4.3 below

Table 4.3 AAE and TAE of METE and HETE from the selected medicinal plants (3.0 mg/mL) expressed as Mean.

Methanol extract (METE)	**TPC expressed as TAE (mg/mL)**	**TAC expressed as AAE (mg/mL)**
Leaves of *T. cacao*	0.5462	0.2996
Leaves of *A. muricata*	0.4369	0.2414
Root of *C. citratus*	0.4109	0.2066
False stem of *C. citratus*	0.3309	0.1975
Seed of *O. aubrevillei*	0.1707	0.1202
Hydro-ethanol extract (HETE)		
Leaves of *T. cacao*	0.6196	0.5866
Leaves of *A. muricata*	0.5772	0.5368
Root of *C. citratus*	0.5212	0.4709
False stem of *C. citratus*	0.4449	0.2774
Seed of *O. aubrevillei*	0.2573	0.2164

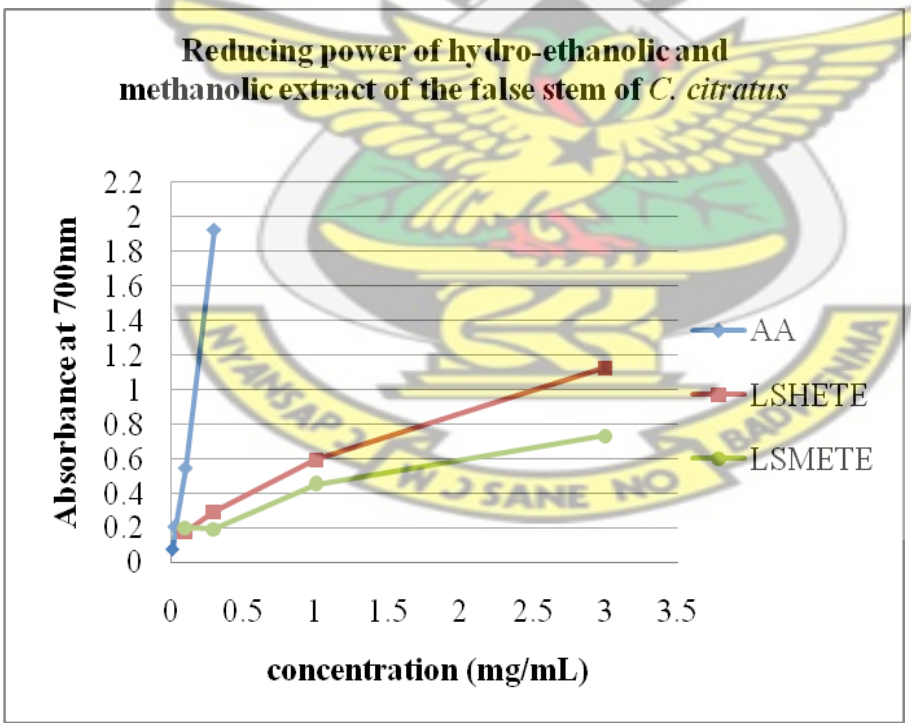
4.3.4 Reducing Power

The plant extracts (0.1 – 3.0 mg/mL) and the reference antioxidant ascorbic acid (0.01- 0.3 mg/ml) dose dependently reduced Fe^{3+} to Fe^{2+} resulting in concentration dependent increase in absorbance (Fig.3.2). Several studies have revealed that reducing power of bioactive compounds is associated with antioxidant activity (Shiddhuraju et al., 2002). It is a measure of the reductive ability of antioxidants and it is evaluated by the transformation of Fe^{3+} to Fe^{2+} in the presence of sample extracts (Huda-Faujan et al., 2009). The results in this study showed that the ferric reducing ability of methanolic and ethanolic extracts of the selected

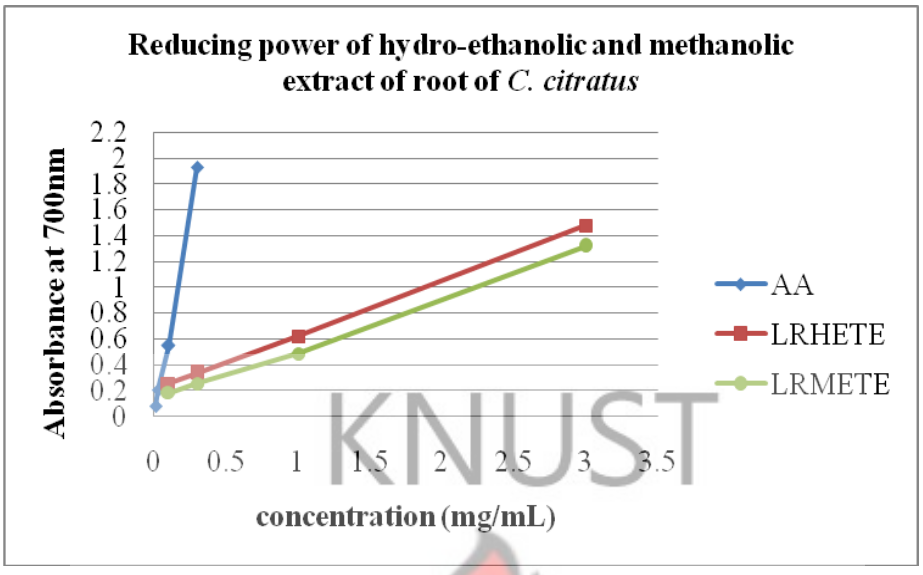
medicinal plants increased with increase in their concentrations (Figure 4.3). Similar observation was reported by Huda-Faujan et al. (2009) who found that the reducing power of methanolic extracts of *Cosmos caudatus* and three others increased with concentration. Interestingly, the ethanolic extract of all the plant extracts showed higher reducing power than the methanolic extracts even though both extracts exhibited reducing capacity they were not appreciable with respect to the standard (ascorbic acid) (figure 4.3). It is conceivable to assume that the phenolic compounds in the plant may be acting as electron donors thereby reducing free radical generation

Figure 2.16 Graphs (a-e) showing reducing powers of selected plant extracts (0.1 - 3.0mg/mL) compared to ascorbic acid (standard)(0.01 - 0.3mg/mL)

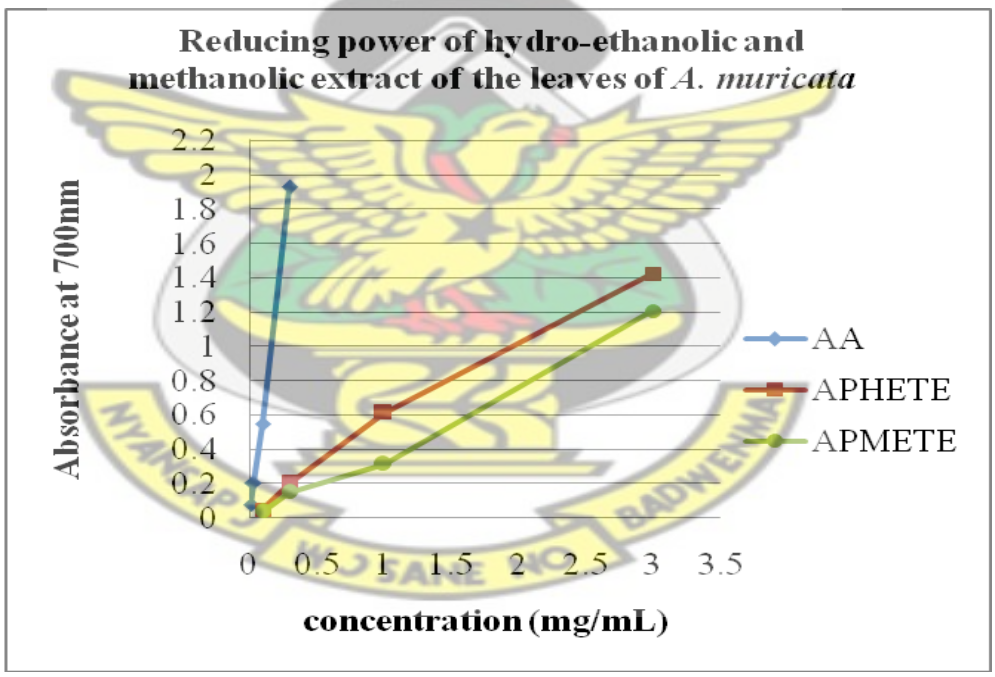
(a)



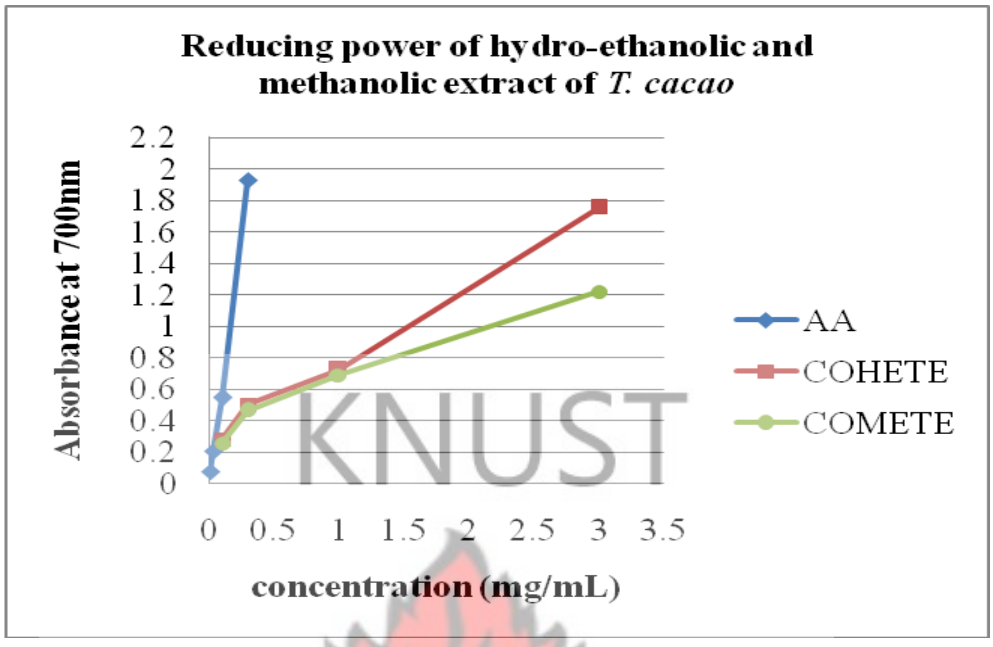
(b)


Reducing power of hydro-ethanolic and methanolic extract of root of C. citratus
Absorbance at 700nm
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0 0.5 1 1.5 2 2.5 3 3.5
concentration (mg/mL)
AA
LRHETE
LRMETE

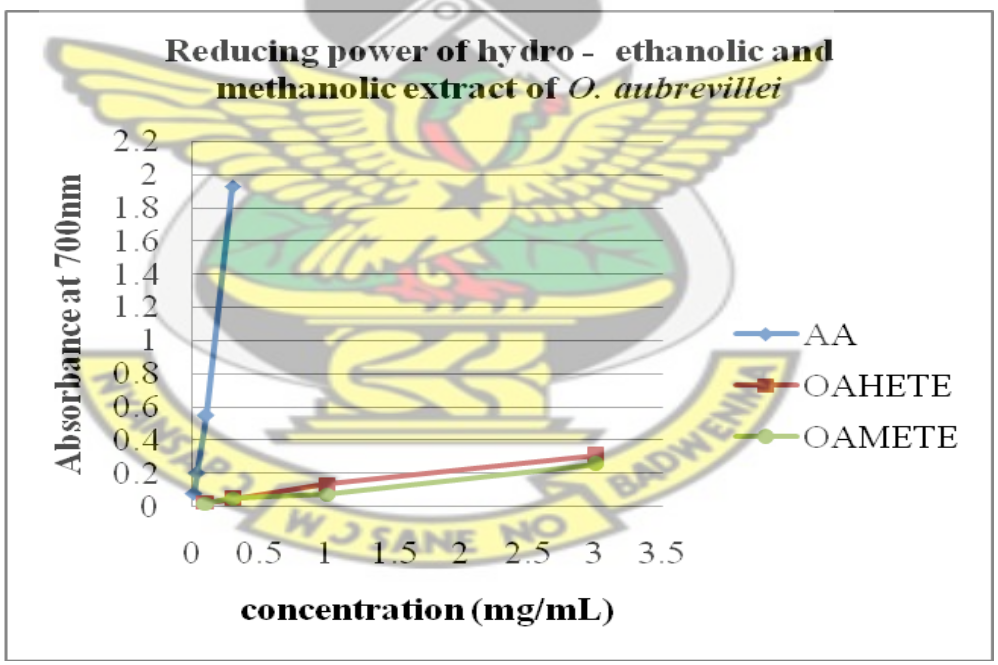
(c)


Reducing power of hydro-ethanolic and methanolic extract of the leaves of A. muricata
Absorbance at 700nm
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0 0.5 1 1.5 2 2.5 3 3.5
concentration (mg/mL)
AA
APHETE
APMETE

(d)



(e)



NB: HET E = ethanolic extract ; METE = methanolic extract

4.3.5 DPPH free radical scavenging activity

DPPH is a free radical compound and has been widely used to test the free radical scavenging ability of various plant extracts. The scavenging activity of plant extracts on DPPH radicals increased with increasing concentrations (Nisha *et al*,2009). IC_{50} value (the amount of Antioxidant material required to scavenge 50% of free radical in the assay system) of ascorbic acid (standard) was observed as 8.3246µg/ml. The smaller the IC_{50} the greater the scavenging activity of the sample. The scavenging ability of ascorbic acid is very high compared with the values obtained for the extracts from all the selected plant. However, the leaves of *Theobroma cacao* showed higher scavenging ability with an IC_{50} of 174.0941µg/ml and 179.9343µg/ml for hydro-ethanolic extract and methanolic extract respectively. On the other hand, the seeds of *Okoubaka aubrevillei* extracts showed the lowest scavenging ability with an IC_{50} of 731.0987µg/ml and 865.6633µg/ml for methanolic extract and hydro-ethanolic extract respectively, as shown in table 4.4 and figure 2.14.

Table 4.4: IC_{50} values of DPPH scavenging activities of different plant extracts.

Test sample	IC_{50} µg/ml	
	Hydro-ethanolic Extract	Methanolic Extract
Ascorbic acid	8.3246	8.3246
Leaves of *Theobroma cacao*	174.0941	179.9343
Leaves of *Annona muricata*	311.2549	285.6519
Root of *Cymbopogon citratus*	303.5980	458.9032
False stem of *Cymbopogon citratus*	512.1105	589.7210
Seeds of *okoubaka aubrevillei*	731.0987	865.6633

The order of RSA of the METE from the selected plants was: Leaves of *T. cacao* > Leaves of *A. muricata* > Root of *C. citratus* > False stem of *C. citratus* > Seeds of *O. aubrevillei* and the HETE from the selected plant showed the different trend. The RSA order was as follows: Leaves of *T. cacao* > Root of *C. citratus* > *Leaves* of *A. muricata* > False stem of *C. citratus* > Seeds of *O. aubrevillei.* The strongest radical scavenging activity was demonstrated by the HETE of the leaves of *T. cacao* (IC_{50}174.0941 µg/ml) and the METE from *O. aubrevillei* showed the least RSA (IC_{50} 865.6633 µg/ml). DPPH is a used as a reagent to evaluate free radical scavenging activity of antioxidants (Oyaizu, 1986), It is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares *et al.*, 1997). The reduction capability of DPPH is determined by the decrease in the absorbance at 517 nm induced by antioxidants. The extracts ability to concentration- dependently reduce DPPH forming yellowish-coloured diphenylpicylhydrazine suggest that the selected plants are a free radical scavenger and acts so by donating an electron or hydrogen radical. The experimental results clearly show that the selected plants are very promising as natural antioxidants.

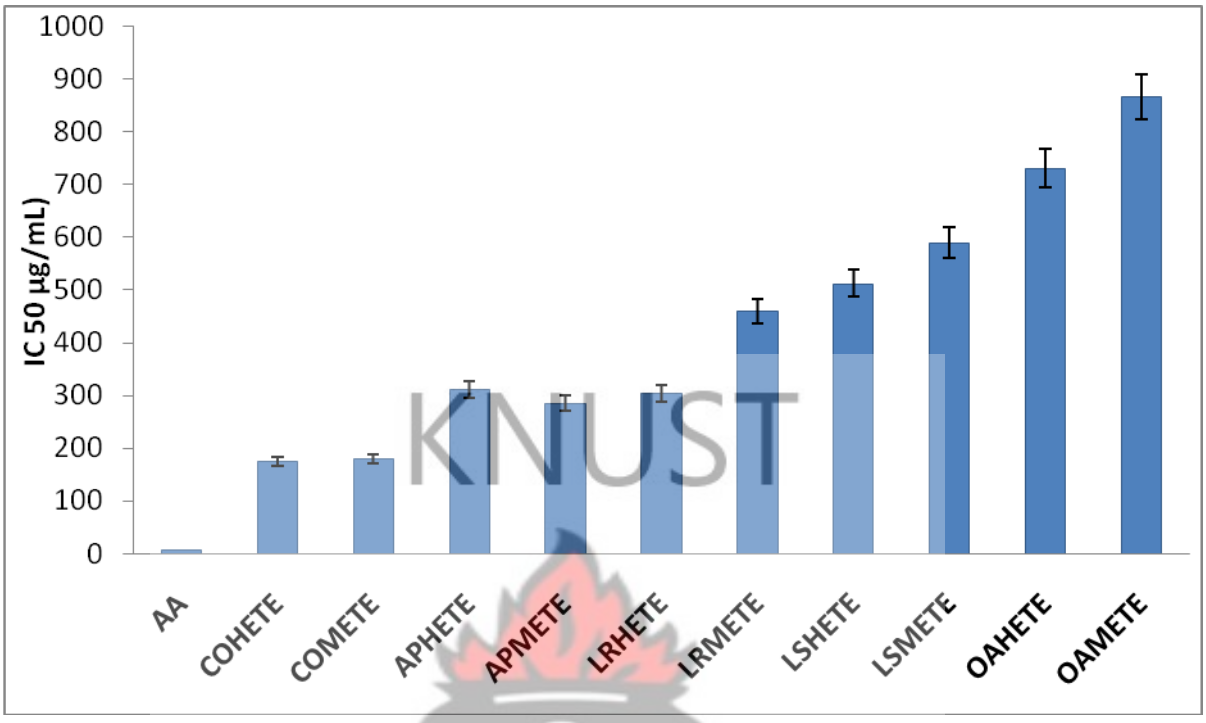


Figure: 2.17 A graphical presentation of IC_{50} µg/mL (50% free radical scavenging) of plant extracts and ascorbic acid determined by DPPH method.[Methanol extracts (METE) and Hydro-Ethanol extracts (HETE) from the selected plants: CO, Leaves of *Theobroma cacao*; LR, Root of *Cymbopogon citratus;* LS, False stem of *Cymbopogon citratus*; AP, Leaves of *Annona muricata;* OA, *Seeds of Okoubaka aubrevillei*.]

4.4 ANTIMICROBIAL TEST

Disease causing bacteria have always been considered a major cause of morbidity and mortality in humans. The appearance of resistant microorganisms paved the way to the occurrence of infections that are only treated by a limited number of antimicrobial agents. Bacterial resistance to antimicrobial agents is a medical problem with public health, socioeconomic, and even political implications (Sharma *et al.*, 2005). In view of the increase in resistance crisis there is an urgent need for new antibacterial compounds in order to fight the emergence of these new resistant pathogens .It is therefore, imperative to ascertain particular plant extracts that are active against the test organisms. The microbial

activity of the various extracts against *Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus* was determined (described under section 3.7) and the results are outlined and discussed in this section.

4.4.1 Susceptibility Test

Susceptibility tests performed on the crude extracts showed that all the extracts of Seeds of *Okoubaka aubrevillei*, False stem of *Cymbopogon citratus*, Root of *Cymbopogon citratus*, Leaves of *Anonna muricata* and Leaves of *Theobroma cacao* have some antimicrobial activity against the test organisms as indicated in the table 4.4 below.

Table 4.5a: Summary of susceptibility test on methanolic extracts.

Crude Extract	***S. aureus***	***E. coli***	***B. substilis***
False stem of *C. citratus*	+	+	+
Root of *C. citratus*	+	+	+
Leaves of *A. muricata*	+	+	+
Leaves of *T. cacao*	+	+	+
Seeds of *O. aubrevillei*	+	+	+

NB: (+) and (-) indicate activity and no activity respectively.

Table 4.5b: Summary of susceptibility test on hydro-ethanolic extracts

Crude Extract	***S. aureus***	***E. coli***	***B. substilis***
False stem of *C. citratus*	+	+	+
Root of *C. citratus*	+	+	+
Leaves of *A. muricata*	+	+	+
Leaves of *T. cacao*	+	+	+
Seeds of *O. aubrevillei*	+	+	+

NB: (+) and (-) indicate activity and no activity respectively.

4.3.2 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations (MICs) of the extracts and the standard (tetracycline) were determined from plots of log of their concentrations against their zones of inhibition using MS Excel (see appendix 2). The plant extracts, generally, had minimum antimicrobial activity against the test organisms with reference to the standard (tetracycline), but the hydro-ethanol plant extracts (HETE) of all the plant, for instance, inhibited the test organisms to a greater extent than the methanol plant extracts (METE) with a minimum inhibitory concentration as low as 30.21µg/ml for *A. muricata* , however, the hydro-ethanol extract of the root of C. citratus had no activity against *S. aureus, E. coli at* lower concentration as shown in table 4.6a and 4.6b below. The results of phytochemical test revealed that the plant extracts contained plants metabolites (saponin, tannins etc.) which are known to have antimicrobial activity. (Akinyemi *et al*.,2006). These may be responsible for the antibacterial activity observed and thus mitigating their use traditionally as medicinal plants for the treatment of microbial diseases.

Table 4.6a: Minimum inhibitory concentration of methanolic extract

Test Organism	Minimum Inhibitory Concentration (µg/ml)					
	AP	**CO**	**LR**	**LS**	**OK**	**TC×10^{-4}**
S. aureus	3861.18	N/A	7622.54	3853.28	287.11	323.00
E. coli	N/A	6249.93	12500.98	1253.83	97.64	67.19
B. substlis	54.13	997.96	1857.83	1104.65	86.97	4.33

Table 4.6b: Minimum inhibitory concentration of hydro-ethanolic extract

Test Organism	Minimum Inhibitory Concentration (µg/ml)					
	AP	**CO**	**LR**	**LS**	**OK**	**TC×10^{-4}**
S. aureus	2627.80	2501.90	N/A	2865.	3061.41	323.00
E. coli	781.13	929.15	N/A	2501.92	N/A	67.18
B. substlis	30.21	99.87	355.02	332.84	1040.73	4.33

NB: N/A= Not Active

CHAPTER FIVE

5 SUMMARY OF FINDINGS, CONCLUSIONS AND RECOMMENDATIONS

Having outlined the methods and findings of the work in the previous chapters, this chapter presents the summary of the major findings of the study and gives suggestions and recommendations for future work in the same area.

5.1 Summary of the findings of the study

From the analysis of the data obtained the following findings were established.

- Hydro-ethanolic extraction gave high percentage extract yield from all the selected medicinal plants.
- Hydro- ethanolic solvent system extracted more phyto-constituent than the methanolic solvent system(as shown in table 4.2)
- The antioxidant properties such as total phenol content, total antioxidant capacity, reducing power were concentration dependent.
- A positive correlation was observed between total phenol content and total antioxidant capacity.
- All the plant extracts had activity against all the test organism (*B. substilis*, *S. aureus*, *E. coli*) at higher concentration, but some were not active against the test organisms at low concentrations.(as shown in table 4.5a-b and 4.6a-b and appendix 1.a- k)
- Hydro-ethanolic extracts had higher inhibition against the test organisms than the methanolic extracts.

5.2 Conclusions

From the results obtained, the following conclusions can be made:

Hydro-ethanolic extraction gave high percentage extract yield from all the selected medicinal plants which suggest that hydro-ethanolic is a preferred solvent system for extracting the phytoconstituents in the selected medicinal plants. Extracts from the selected medicinal plants, all demonstrated appreciable total phenol content, total antioxidant capacity, radical scavenging activity and reducing ability with slight variations compared with the standard antioxidant compounds employed in the study. These findings to a large extent agree with similar works carried out in Nigeria by Akindahunsi and Salawu (2005a, b); Odukoya et al. (2007), Salawu et al. (2006) and in Ghana by Morrison and Twumasi (2010). This observation suggests that the selected medicinal plants are a potential source of natural antioxidant which might help in preventing the progress of various oxidative stresses. All extracts of the plants tested showed varying degree of antibacterial activities against the test bacterial species (appendix 1.a-j). The hydro-ethanol extracts showed higher activity than the methanol extracts, this could be due to the fact that, the hydro-ethanol extracted more phyto-constituent as compared to that of the methanol and might be linked to its higher activity, as reported, recently by Aiyelaagbe et al. (2007) that the presence of some secondary metabolites (such as tannins, alkaloids, flavonoids) in plants extracts inhibited some microorganisms. All the plants extract had the highest inhibition against *B. substilis* (MIC ranging from 30.21µg/ml to 1040 µg/ml for hydro-ethanol extracts and 54.13µg/ml to 1104.64µg/ml for methanol extracts) and lowest inhibition was against *S. aureus* (MIC ranging from 2627.80 µg/ml to 3061.41µg/ml for hydro-ethanol extracts and 287.11µg/ml to 3861.18µg/ml for methanol extracts). However, the methanolic extract of O. aubrevillei had higher microbial activity against the test organisms than the hydro-ethanolic extract, as shown in table 4.6a and b.

5.3 Recommendations

- The phytoconstituents found to be present must be isolated to determine their antioxidant, antimicrobial and antifungal activities.
- The toxic effects of plant extracts must be explored or tested in subsequent works
- Even though the plant extracts have been found to inhibit the growth of *Escherichia coli, Bacillus substilis, and Staphylococcus aureus* at certain concentrations, minimum bacterialcidal concentrations (MBC) must be performed on the plant to ascertain whether it is just inhibitory or can also kill these organisms and other organisms.

REFERENCES

Aiyelaagbe, O.O., Adeniyi, B.A., Fatunsin, O.F. and Arimah, B.D. (2007). *In vitro* Antimicrobial activity and photochemical analysis of Jatropha curcas roots. *Intern. J. Pharmacol*, 3(1), 106-110.

Akindahunsi, A.A. and Salawu, S.O. (2005a). Antioxidant indices of some green leafy vegetables. *Trop. Sci*. (45), 35-45.

Akindahunsi, A.A. and Salawu, S.O. (2005b.) Phytochemical screening and nutrient-antinutrient composition of selected tropical green leafy vegetables. *Afr. J. Biotechnol*. (46), 497-501.

Akinyemi, K.O., Oluwa, O.K. and Omomigbehin, E.O. (2006). Antimicrobial activity of crude extracts of three medicinal plants used in South-West Nigerian folk medicine on some food borne bacterial pathogens. *Afr. J. Trad. CAM* 3(4), 13 – 22.

Ames, B. M. (1983). Dietary carcinogens and anticarcinogens: oxygen radical and degenerative diseases. *Science* 221, 1256-1263.

Amer, J., Ghoti, H., Rachmilewitz, E., Koren, A., Levin, C. and Fibach, E. (2006). Red blood cells, platelets and polymorphonuclear neutrophils of patients with sickle cell disease exhibit oxidative stress that can be ameliorated by antioxidants. *British Journal of Haematology* 132 (1), 108-113.

Ashok, B., Ali, R. (1999). The aging paradox: free radical theory of aging. *Exp Gerontol* 34, 293–303.

Baardseth, P. (1989). Effect of selected antioxidants on the stability of dehydrated mashed potatoes. *Food Add. Contam*; 6, 201-207.

Bánhegyi, G., Mandl, J. (2001). The hepatic glycogenoreticular system. *Pathology & Oncology Research;* **7** (2), 107–110.

Bektas, T., Sokmen, M., Akpulat, H. A. and Sokmen, A. (2005). Free radical scavenging activity of Cassine transvaalensis. *Food Chemistry;* 92(1), 89-92.

Blanco, M.M., Costa, C.A., Freire. A.O., Santos, J.G., Costa, M., (2009). Neurobehavioral effect of essential oil of Cymbopogon citratus in mice. *Phytomedicine;* 16 (2-3), 265–70.

Block, G., Menkes, M. (1989). Ascorbic acid in cancer prevention. In: Moon TE, Micozzi MS., editors. Diet and Cancer Prevention. Investigating the Role of Micronutrients; New York: Marcel Dekker Inc., pp. 341-388.

Bories, C., Loiseau, P., Cortes, D., Myint, S.H., Hocquemiller, R., Gayral, P., Cavé, A. and Laurens, A. (1991). Antiparasitic activity of Annona muricata and Annona cherimolia seeds. *Planta Med.;* 57(5), 434–36.

Buechter D.D. (1988). Free radicals and oxygen toxicity. *Pharm Res*.; (5), 253-60.

Bulger E.M. and Helton W.S., 1998. Nutrient antioxidants in Gastrointestinal Disease. *Gastroentol Clin North Amer*.; 27(2), 403-419.

Cai, Y.Z., Sun, M. and Corke, H. (2003). Antioxidant activity of betalains from plants of the Amaranthaceae. *Journal of Agricultural and Food Chemistry*. 51 (8). 2288-2294.

Campos, M.G., Webby, R.F., Markham, K.R., Mitchell, K.A. and Da Cunha, A.P. (2003). Age induced diminution of free radical scavenging capacity in bee pollens and the contribution of constituent flavonoids. *Journal of Agricultural and Food Chemistry*. (51), 742-745.

Carbonneau, M. A., Le´ger, C. L., Descomps, B., Michel, F. and Monnier, L.(1998). Improvement in the antioxidant status of plasma and low-density lipoprotein in subjects receiving a red wine phenolics mixture. *J. Am. Oil Chem. Soc.*(*75),* 235-240.

Cassina, A. and Radi, R. (1996). Differential inhibitory action of nitric oxide and peroxynitrite on mitochondrial electron transport. *Arch Biochem Biophys.* (328), 309-316.

Chambers, H.F. (1997). Methicillin resistance in *Staphylococci*: molecular and clinical implications. *Clin. Microbiol. Rev*. (10), 781–791.

Cotelle, N., Bernier, J.L., Catteau, J.P., Pommery, J., Wallet, C.J., Gaydou, E.M., (1996). Antioxidant properties of hydroxyflavones. *Free radical Biology and Medicine*. 20 (1), 35-43.

De diego-otero ,Y., Romero-zerbo, Y., El bekay, R., Decara, J., Sanchez, L., Rodriguez-de Fonseca, F., Delarco- Herrera, I.,(2009). Alpha-tocopherol protects against oxidative stress

in the fragile x knockout mouse: an experimental therapeutic approach for the fmr1 deficiency. *Neuropsychopharmacology* 34 (4), 1011–26.

Dekkers, J. C., van Doornen, L. J. P. and Kemper, H. C. G., (19960. The Role of Antioxidant Vitamins and Enzymes in the Prevention of Exercise-Induced Muscle Damage. *Sports Med.* (21), 213-238.

Desta, B., (1993). Ethiopian traditional herbal drugs Part II. Antimicrobial activity of 63 medicinal plants. *J. Ethnopharmacol*. (39), 129-139.

Diaz-Reinoso, B., Moure, A., Dominguez, H. and Parajo, J.C. (2006). Supercritical carbon dioxide extraction and purification of compounds with antioxidant activity. *Journal of Agricultural and Food Chemistry*. (54), 2441-2469.

Diplock, A.T. (1997). Will the 'good fairies' please prove to us that vitamin E lessens human degenerative disease. *Free Radic Res* 27(5), 511-532.

Duthie, G.G., Duthie, S.J. and Kyle, J. A. M. (2000). Plant ployphenols in cancer and heart disease: Implication as nutritional antioxidants. *Nutrition Research Reviews*. (13), 79-106.

Dykes, L. and Rooney, L.W.,(2007). Phenolic compounds in cereal grains and their health benefits. *CFW*. 52(3), 105-111.

Ellnaim, W.M., Kruczynski, Z. and Kaspfak, J. (2003). Investigation of free radical scavenging activity of Ginkgo Biloba L leaves. *Filtotentopia*. (74), 1-6

Fang, Y.Z., Fang, S. and Won, G. (2002). Free radicals, antioxidants and nutrition. *Nutrition;* (18), 872 – 879.

Frankel, E.N. and Meyer, A.S. (2000). The problems of using one-dimensional methods toevaluate multifunctional food and biological antioxidants. *Journal of Science and Food Agriculture*. (80), 1925-1941.

Frank, H. A. and Cogdell, R. J. (1996).Carotenoids in photosynthesis. *Photochem. Photobiol.* (63), 257-264.

Gaziano, J. M. and Hennekens, C. H. (1993).The role of beta-carotene in the prevention of cardiovascular disease. *Ann. NY Acad. Sci.* (691), 148-155

Gems, D., Partridge, L., 2008. Stress-response hormesis and aging: "that which does not kill us makes us stronger. *Cell Metab.* **7** (3), 200–203.

Govindarajan, R., Vijayyakumar, M., Rawat, A.K., Mehrotra, S. (2003) Free radical scavenging potential of Picrorhiza Kurrooa Royale ex Benth. *Indian J Exp Biol* 41 (8), 875 – 879.

Gulam, W., Haseeb, A., (2006). Reactive oxygen species: role in the development of cancer and various chronic conditions. *J. Carcinogenesis*. (5), p. 14.
(www.biomedcentral.com/content/pdf/1477-3163-5-14.pdf (assessed 2011sept. 5).

Gropper, S.S., Smith, J.L., and Grodd, J.L., (2004). *Advanced Nutrition and Human Metabolism* (4th ed.). Belmont, CA. USA: Thomson Wadsworth. pp. 260–275.

Hallé, N., 1987. *Okoubaka* Pellegrin & Normand is really a genus of Santalaceae Muséum. *Bulletin du Muséum national d'Histoire naturelle* **(**4), 355-363

(http//.en.wikipedia.org/wiki/Okoubaka-13-09-11)

Halliwell, B., (1994). Free radicals, antioxidants, and human diseases: curiousity, causes or consequence. *Lancet*. 344 (8924), 721-724.

Halliwell, B. (1996). Uric acid: an example of antioxidant evaluation. In: Cadenas E, Packer L. editors. Handbook of antioxidants. New York: Marcel Dekker. pp. 243–56.

Halliwell B., (1997). Antioxidants and human disease: a general introduction. *Nutr Rev.;* (55), 544–549.

Halliwell B., (1999). Oxygen and nitrogen are pro-carcinogens. Damage to DNA by reactive oxygen, chlorine and nitrogen species: measurement, mechanism and the effects of nutrition. *Mutat Res*.; (443), 37-52.

Halliwell, B., Gutteridge, J. M.C., and Cross, C. E., (1991). Free radicals, antioxidants, and human disease: Where are we now? *Lab. Clin. Med.* (119), 598-620.

Halliwell, B., and Gutteridge, J.M.C., 1999. *Oxygen is a toxic gas an introduction to oxygen toxicity and reactive oxygen species. In: Free Radical in Biology and Medicine;* Oxford University Press: United Kingdom. pp.1-35.

Halender, I.M., Alakomi, H.L., Latva-kala, K., Mattila-Sandhom, T., Pol, I., Smid, E.J., Gorris, L.G.M., and von Wright, A., (1998). Charaterisation of the action of selected essential oil components on gram negative bacteria. *J. Agric. Food Chem*. (46), 3590-3595.

Halvorsen, B. L., Holte, K.and Myhrstad, M.C., (2002). Asystematic screening of total antioxidants in dietary plants. *The journal of Nutrition*. (132), 461-471.

Harman D., (2000). Aging: overview. Ann N Y *Acad Sci* (928), 1–21.

Hasrat, J. A., De Bruyne,T., De Backer, G.P. and Vauquelin, G., Vlietinck, A.J.(1997). Isoquinoline derivatives isolated from the fruit of *Annona muricata* as 5-HTergic 5 HT1A receptor agonists in rats: unexploited antidepressive (lead) products. *J.Pharm. Pharmacol;* 49 (11), 1145–1149.

Hiramatsu, K., Hanaki, H., Ino, T.,Yabuta, K., Oguri, T. and Tenover, F.C.**(**1997). Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. *J. Antimicrob. Chemother.* **(**40**),** 135-136.

Hopia, A. I., Heinonen, M., (1999). Comparison of antioxidant activity of flavonoid aglycones and their glycosides in methyl linoleate. *J. Am. Oil Chem. Soc.*, (*76)*, 139-144.

Huang, D., Ou, B. and Prior, R.L.,(2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*; **(**53), 1841-1856.

Huda-Faujan, N., Noriham, A., Norrakiah, A.S. and Babji, A.S., (2009). Antioxidant activity of plants methanolic extracts containing phenolic compounds. *Afr. J. Biotechnol*. 8(3), 484-489.

Institute of Medicine. Food and Nutrition Board. Dietary Reference Intakes: Vitamin C, Vitamin E, Selenium, and Carotenoids. Washington, DC: National Academy Press, 2000.

Iwu, M.M. (1993). *Handbook of African Medical plants.* Florida : CRC press. P. 367.

Jones, D. P., Kagan, V. E., Aust, S. D., Reed, D. J., and Omaye, S. T., (1995). Impact of nutrient on cellular lipid peroxidation and antioxidant defense system. *Fundam. Appl. Toxicol. (*26), 1-7.

Kirtikar, K.R., and Basu, B.D. (1975). *Indian Medicinal Plants.* Vol. 2. 2nd ed. Bishen Singh Mahendra Pal Singh, Dehradun, India, pp. 894-895.

Kluytmans, J., Van Belkum, A. and Verbrugh, H. (1999). Nasal Carriage of *Staphylococcus aureus*: Epidemiology, Underlying Mechanisms, and Associated Risks. *Clinical Microbiology Reviews*. **10**(3): 505–520

Knight, J.A., (1999). *Free radicals, antioxidants, aging, & disease*. Washington D.C. AACC Press. pp 21–43.

Korycka-Dahl, M.B. and Richardson, T., (1978). Activated oxygen species and oxidation of food constituents. *Crit Rev Food Sci Nutr.;* (10), 209–41.

Krinsky, N. I., (1993). Actions of carotenoids in biological systems. *Annu. Rev. Nutr.* (13), 561-587.

Larson, R.A. (1988). The antioxidants of higher plants. *Phytochemistry*. 27(4), 969-978

Lo¨liger, J., (1991). The use of antioxidants in food. In *Free Radicals and Food Additives*; Aruoma, O. I., Halliwell, B., Eds.; Taylor and Francis: London, pp. 129-150.

Manach, C., Scalbert, A., Morand, C., Remesy, C. and Jimenez, C.,(2004). Polyphenols:Food sources and bioavailability. *Am. J. Clin. Nutr.* (79), 727-747.

Martinez del Rio, C. (1997). Can passerines synthesize vitamin C. *The Auk* 114 (3), 513–16. (http://findarticles.com/p/articles/mi_qa3793/is_199707/ai_n8765385.Retrieved 2 May 2011.)

Mayne, S. T., (1996). Beta-carotene, carotenoids, and disease prevention in humans. *FASEB J.* (10), 690-701.

McVean, M., Kramer-Stickland K. and Liebler, D.C., 1999. Oxidants and antioxidants in ultraviolet-induced nonmelanoma skin cancer. In: Papas AM, editor. Antioxidant status, diet, nutrition, and health. Boca Raton, Fla.: CRC Press. pp. 401–30.

Miller, N.J., Rice-Evans, C.A. (1997). The relative contributions of ascorbic acid and phenolic antioxidants to the antioxidant activity of orange and apple fruit juices and blackcurrant drink. *Food Chem*.; (60), 331-337.

Morrison, J.F. (2009). *Comparative Studies On The In Vitro Antioxidant And Antibacterial Activity Of Methanolic And Hydro-Ethanolic Extracts From Eight Edible Leafy Vegetables Of Ghana*. MPhil.- thesis, Department of Chemistry, Kwame Nkrumah University of Science and Technology p. 182-190

Morrison, J.F. and Twumasi, S.K., (2010). Comparative studies on the *in vitro* antioxidant properties of methanolic and hydro-ethanolic leafy extracts from eight edible leafy vegetables of Ghana. *Afri. Journal of Biotechnology*; 9(32), 5177-5184

Mousumi D. (2008). Clonal propagation and antimicrobial activity of an endemic medicinal plant *Stevia rebaudiana*. *Journal of Medicinal Plants Research*; **2**(2), 45-51

Nakano, M. M. and Zuber, P. (1998). Anaerobic growth of a "strict aerobe" (*Bacillus subtilis*). *Annu. Rev. Microbiol*. **52**: 165-190

Namiki, M., (1990). Antioxidants/antimutagens in food. *Food Science and Nutrition*. (29), 273-300.

Newman, D.J., Cragg G. and Snader, K.M. (2000). The influence of natural products upon drug discovery. *Nat. Prod. Rep*. (17), 175-285.

Nia, R., Paper, D.H., Essien, E.E., Oladimeji, O.H., Lyadi, K.C. and Franz G. (2003). Investigations into *in-vitro* radical scavenging and *in-vivo* anti- inflammatory potential of *Tridax procumbens. Nig. J. of Phsiological sciences* 18 (1-2), 39-43.

Nisha, P., Abdul Nazar, P. and Jayamurthy, P. (2009). A comparative study on antioxidant activities of different varieties of Solanum melongena. *Food and Chemical Toxicology;* 47 (2009), 2640–2644

Nohl, H., Kozlov, A.V., Gille, L., Staniek, K. (2003). Cell respiration and formation of reactive oxygen species: facts and artifacts. *Biochem Soc Trans*.; (31), 1308-11.

Odukoya ,O.A., Inya-Agha, S.I., Sofidiya, M.O. and Ilori, O.O. (2007). Antioxidant activity of selected Nigerian leafy vegetables. *Am. J. Food Technol*. 2(3), 169-175.

Omaye, S. T. and Zhang, P., (1997). Phytochemical interactions: β-Carotene, tocopherol, and ascorbic acid. In *Phytochemicals: Today's Knowledge for Tomorrow's Products* (W. R. Bidlack and S. T. Omaye, Eds.).Technomic Publishing Co., Lancaster, PA. [in press] p. 163.

Oufnac, S.D. (2006). *Determination of antioxidant capacity in corn germ, wheat germ and wheat bran using solvent and microwave-assisted solvent extraction*. Msc.- thesis, Department of Food Science, Louisiana State University and Agricultural and Mechanical College, p. 8

Oyaizu, M., (1986). Studies on products of browning reaction: Antioxidative activities of browning reaction prepared from glucosamine. *Jpn J Nutr. (*44), 307-315.

Palozza, P., Krinsky, N. I., (1992). Antioxidant effects of carotenoids *in vitro* and *in vivo:* An overview. *Methods Enzymol*. (213), 403-420.

Papas, A.M. (1999). Determinants of antioxidant status in humans. In: Papas AM, editor. Antioxidant status, diet, nutrition, and health. Boca Raton, Fla. CRC Press. pp. 21–36.

Pellegrini, P., Serafini, M., Colombi, B., Del Rio, D., Salvatore, S., Bianchi, M., and Brighenti F., (2003). Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. *Journal of Nutrition*. (133), 2812-2819.

Percival, M., Pellegrini, P., Serafini, M., Colombi, B., Del Rio, D., Salvatore, S. and Bianchi M. (1998). Antioxidants. *Clin. Nutr. Insights*. (31) 1-4.

Perez-Jimenez, J. and Saura-Calixto, F. (2005). Literature data may underestimate the actual antioxidant capacity of cereals. *Journal of Agricultural and Food Chemistry*. (53), 5036-5040.

Pinzino, C., Capocchi, A., Galleschi, L., Saviozzi, F., Nanni, B. and Zandomeneghi, M., (1999). Aging, free radicals, and antioxidants in wheat seeds. *Journal of Agricultural and Food Chemistry*. (47), 1333-1339.

Poderoso, J.J., Carreras, M.C., Lisdero, C., Riobó, N., Schöpfer, F. and Boveris, A., (1996). Nitric oxide inhibits electron transfer and increases superoxide radical production in rat heart mitochondria and submitochondrial particles. *Arch Biochem Biophys* . (328), 85-92.

Poulson, H.E., Prieme, H. and Loft, S. (1998). Role of oxidative DNA damage in cancer initiation and promotion. *European journal of cancer prevention;* 7(1), 9-16.

Prieto, P., Pineda, M. and Anguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdaenum complex: specific application to the determination of vitamin E. *Anal. Biochem*. 269(2), 337-341.

Prior, R.L., Wu, X. and Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*. (53), 4290-4302.

Reynolds, J.E.F.,(19960. *Martindale-the Extra Pharmacopeia*, 31st ed. Royal Pharmaceutical Society of Great Britain. London.

Reische, D.W., Lillard, D.A. and Eintenmiller, R.R.,(1998). *Antioxidants in food lipids*. In: Ahoh, C.C., Min, D.B. (Eds.). Chemistry, Nutrition and Biotechnology. Marcel Dekker, New York, pp 423-448

Rice-Evans, C.A., Miller, N.J., Bolwell, P.J., Bramley,P.M. and Pridham, J.B. (1995). The relative antioxidant activities of plant derived polyphenolic flavonoids. *Free Radical Res.* (22), 375-383.

Rodrigues, E., Carlini, E.A. (2006). Plants with possible psychoactive effects used by the Krahô Indians, Brazil. *Revista Brasileira de Psiquiatria* 28(4), 277-282.

Sanchez-Moreno C., (2002). C. Review: Methods used to evaluate the free radical scavenging activity in foods and biological systems. *Food Science Technology International.* (8), 121-137.

Sartor, V., Henderson, P. T. and Schuster, G. B. (1999). Radical cation transport and reaction in RNA/DNA hybrid duplexes: effect of global structure on reactivity. *J. Am. Chem. Soc.*, (*121),* 11027-11033.

Selitrennikoff, C. (1992). *Screening for antifungal drugs*. In: Finkelstein, D. and Ball, C. (Eds.). *Biotechnology of Filamentous Fungi. Technology and Products*. Boston: Butterworth Heinemann. P. 189–217.

Serafini, M., Maiani, G. and Ferro-Luzzi, (1998). A. Alcohol-free red wine enhances plasma antioxidant capacity in *humans. J. Nutr. , 128* (6), 1003-1007.

Sharma, R., Sharma, C.L. and Kapoor, B.,(20050. Antibacterial resistance: current problems and possible solutions. *Indian Journal of Medical Sciences*. 59 (3), 120–129.

Shiddhuraju, P., Mohan, P.S. and Becker, K. (2002). Studies on antioxidant activity of Indian Laburnum (*Cassia fistula* L.): a preliminary assessment of crude extracts from stem back leaves, flower and fruit pulp. *Food Chem*. (79), 61-7.

Singleton, V.L. and Skinkard, K. (1977). Total phenol analyses; automation and comparison with manual methods. *Am J Enol Vit* (28**),** 49-55.

Soares, J.R., Dinis, T.C., Cunha, A.P. and Almeida, L.M. (1997). Antioxidant activities of some extracts of Thymus zygis. *Free Radic Res.* 26(5)**,** 469-478.

Stanner, S.A., Hughes, J., Kelly, C.N. and Buttriss, J., (2003) A review of the epidemiological evidence for the 'antioxidant hypothesis. *Public Health Nutrition*. 7(3), 407-422.

Stief, T. W. (2000). The blood fibrinolysis/deep-sea analogy: a hypothesis on the cell signals singlet oxygen/photons as natural antithrombotic. *Thromb Res. (* 99), 1–20

Stief T. W., (2003). The physiology and pharmacology of singlet oxygen. *Med Hypoth;* (60), 567–72.

Stratil , P., Klejdus, B. and Kuban, V. (2006). Determination of total content of phenolic compounds and their antioxidant activity in vegetables–Evaluation of spectrophotometric methods. *Journal of Agricultural and Food Chemistry*. (54), 607-616.

Stryer, Lubert (1995). *Biochemistry, 4th Edition*. W.H. Freeman and Company. pp. 732.

Traber, M.G., (2006). Vitamin E. In: Shils ME, Shike M, Ross AC, Caballero B, Cousins R, eds. Modern Nutrition in Health and Disease. 10th ed. Baltimore, MD: Lippincott Williams & Wilkins, pp.396-411

Verhagen, H., Buijsse, B., Jansen, E. and Bueno-de-Mesquita, B., (2006). The state of antioxidant affairs. *Nutr Today*. (41), 244-50.

Vogt, R. L., Dippold, L. (2005). Escherichia coli O157:H7 Outbreak Associated with Consumption of Ground Beef. *Public Health Reports.* **120**: 174-178

Waladkhani A.R. and Clemens M.R., (1998). Effect of dietary phytochemicals on cancer development. *Int. J. Mol. Med*.; (1), 747–753.

Wallace, S.S., (1997). Oxidative damage to DNA and its repair, in Oxidative Stress and Molecular Biology of Antioxidant Defenses. Cold Spring Harbor Laboratory Press: Plainview, NY. pp.49-90.

Wright, J. S., Johnson, E. R. and DiLabio, G. A.,(2001). Predicting the activity of phenolic antioxidants: Theoretical method, analysis of substituent effects, and application to major families of antioxidants. *J. Am. Chem. Soc.;* (*123)*, 1173-1183.

Wu, X., Beecher, G.R., Holden, J.M., Haytowitz, D.B., Gebhardt, S.E. and Prior, R.L., (2004). Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *Journal of Agricultural and Food Chemistry*. (52), 4026-4037.

Ziegler, R. G., (1989). A review of epidemiologic evidence that carotenoids reduce the risk of cancer. *J. Nutr. (*119), 116-122.

Zhou, K., Liangli,Y. (2004). Effects of extraction solvent on wheat bran antioxidantactivity estimation. *Lebensm.-Wiss. –Technol*. (37), 717-721.

Zhou, K.K., Su, L. and Yu, L. (2004). Phytochemicals and antioxidant properties in wheat bran. *Journal of Agricultural and Food Chemistry*. (52), 6108-6114.

hptt:// www.websters-online-dictionary.org/definition/english/Su/Superoxide.html (Accessed-31/08/11)

hptt:// www. vita-dose.com/structure-of-vitamin-e (Accessed-01/06/11)

hptt:// en.wikipedia.org/wiki/Vitamin C (Accessed-02/06/11)

hptt:// en.wikipedia.org/wiki/*Annona muricata* (Accessed – 31/o8/11)

hptt:// www.nutrition-and you.com/lemongrass.html(Accessed- 31/08/11)

hptt:// www.kew.org/plants-fungi/Cymbopogon-citratus.htm (Accessed-31/08/11)

hptt://www.mobot.org/gardeninghelp/plantfinder/plant.asp?code=A504(Accessed-31/08/11)

hptt://www. database.prota.org/PROTAhtml/Okoubaka%20aubrevillei_En.htm (Accessed -13-09-11)

hptt:// en.wikipedia.org/wiki/Okoubaka (Accessed-13-09-11)

hptt:// www.tropilab.com/soursop.html (Accessed -16/08/11)

hptt:// www.rain-tree.com/reports/graviola-techreport.pdf (Accessed-31/08/11)

hptt://www.cancerplants.com/medicinal_plants/annona_muricata.html(Accessed-15/05/11)

hptt:// www.vitaminstuff.com/vitamin-e html (Accessed-15/08/11)

hptt://www.johnnyseeds.com/Assets/Information/LemongrassPlants.pdf(Accessed-14-09-11)

http://en.wikipedia.org/wiki/file. Cacao (Accessed 10/04/12)

http://en.wikipedia.org/wiki/Theobroma_cacao (Accessed 10/04/12)

http://theida.com/essential-oils/lemongrass-Cymbopogon-citratus (Accessed 10/04/12)

APPENDICES

1.0 Determination of Minimum Inhibitory Concentration

Below are tables for the average of the zones of inhibition obtained for the various plant extracts and tetracycline (standard)

1. A Results for zone of inhibition of methanol extract

Concentration (mg/mL)	**Average zone of inhibition (mm)**		
Plant Extracts False stem of *C. citratus*	*S. aureus*	*E. coli*	*B. substlis*
100	11	13	9
50	7	11	8
25	5	8.5	6
12.5	4.5	7	5

(B)

Concentration (mg/mL)	**Average zone of inhibition** (mm)		
Plant Extracts Root of *C. citratus*	***S. aureus***	***E. coli***	***B. substlis***
100	13	6	12
50	9	4	9
25	7	NA	7
12.5	NA	NA	6

(C) Results for zone of inhibition of methanolic extract

Concentration (mg/mL)	**Average zone of inhibition (mm)**		
Plant Extracts Leaves of *A. muricata*	***S. aureus***	***E. coli***	***B. substlis***
100	8.5	NA	29
50	6.5	NA	27
25	5	NA	24
12.5	4.5	NA	21

(D)

Concentration (mg/mL)	**Average zone of inhibition (mm)**		
Plant Extracts Seeds of *O. aubrevillei*	***S. aureus***	***E. coli***	***B. substlis***
100	12.5	20	15
50	11.5	18	14
25	9.5	NA	12.5
12.5	NA	NA	10.5

(E) Results for zone of inhibition of methanolic extract

Concentration(mg/mL)	**Average zone of inhibition (mm)**		
Plant Extracts Leaves of *T. cacao*	***S. aureus***	***E. coli***	***B. substlis***
100	NA	8	11
50	NA	6	10
25	NA	4.5	8
12.5	NA	NA	6

(F) Results for zone of inhibition of hydro-ethanolic extract

Concentration(mg/mL)	**Average zone of inhibition (mm)**		
Plant Extracts False stem of *C. citratus*	***S. aureus***	***E. coli***	***B. substlis***
100	10.5	14	11
50	8	12	9
25	6	8	8
12.5	4.5	6.5	7

(G) Results for zone of inhibition of hydro-ethanolic extract

Concentration(mg/mL)	**Average zone of inhibition (mm)**		
Plant Extracts Root of *C. citratus*	***S. aureus***	***E. coli***	***B. substlis***
100	NA	NA	12
50	NA	NA	10
25	NA	NA	9
12.5	NA	NA	7.5

(H)

Concentration(mg/mL)	**Average zone of inhibition (mm)**		
Plant Extracts Leaves of *A. muricata*	***S. aureus***	***E. coli***	***B. substlis***
100	14	15	31
50	12	14	26
25	10	13	20
12.5	8	11	13

(I) Results for zone of inhibition of hydro-ethanolic extract

Concentration(mg/mL)	**Average zone of inhibition (mm)**		
Plant Extracts Leaves of *T. cacao*	***S. aureus***	***E. coli***	***B. substlis***
100	14	14	15.5
50	11.5	11	14
25	9	9	12
12.5	6	8	11

(J)

Concentration(mg/mL)	**Average zone of inhibition (mm)**		
Plant Extracts Seeds of *O. aubrevillei*	***S. aureus***	***E. coli***	***B. substlis***
100	8.5	NA	19
50	7	NA	16
25	5	NA	14
12.5	4.5	NA	10

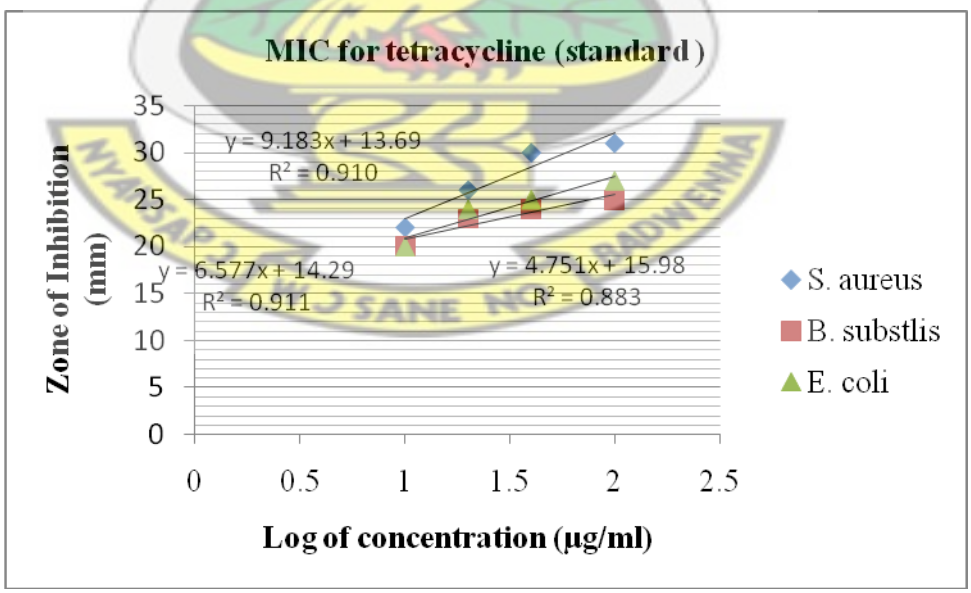
(k) Results for zone of inhibition of Tetracycline (standard)

Concentration(µg/mL)	**Average zone of inhibition (mm)**		
Tetracycline	***S. aureus***	***E. coli***	***B. substlis***
100	31	27	25
50	30	25	24
25	26	24	23
12.5	22	20	20

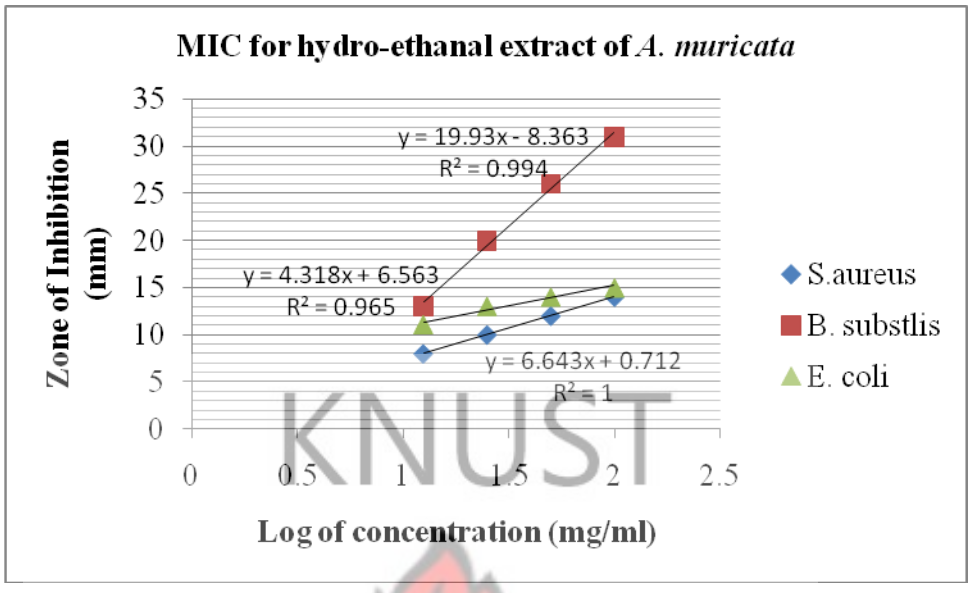
2.0 Graphs Show minimum inhibition

The graphs below were obtained by plotting the average zones of inhibition against the Log of concentration of the various extracts and sample using MS Excel. The minimum inhibitory concentrations were obtained by finding the values of the Log of concentration where the ordinate is zero.

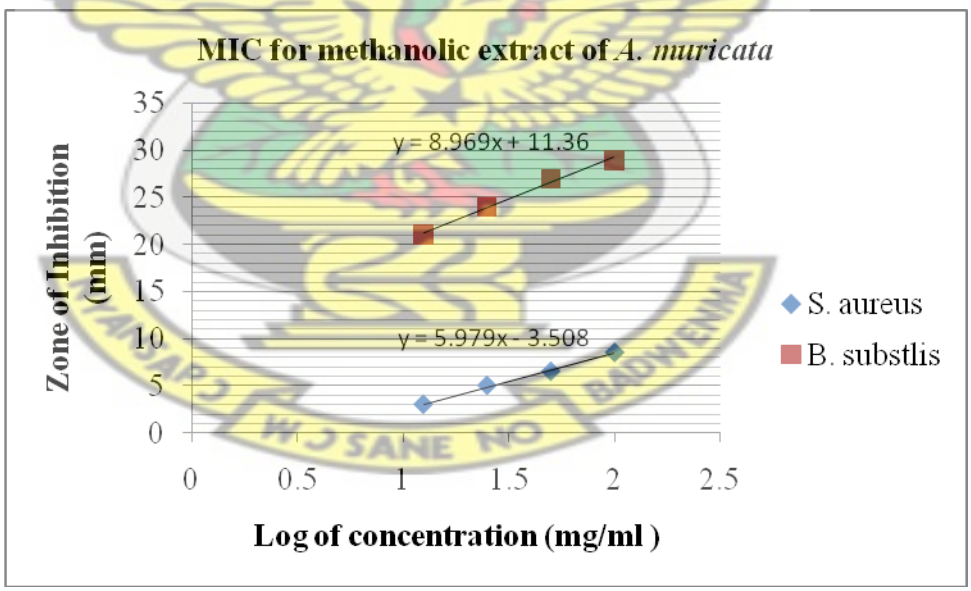
(A)



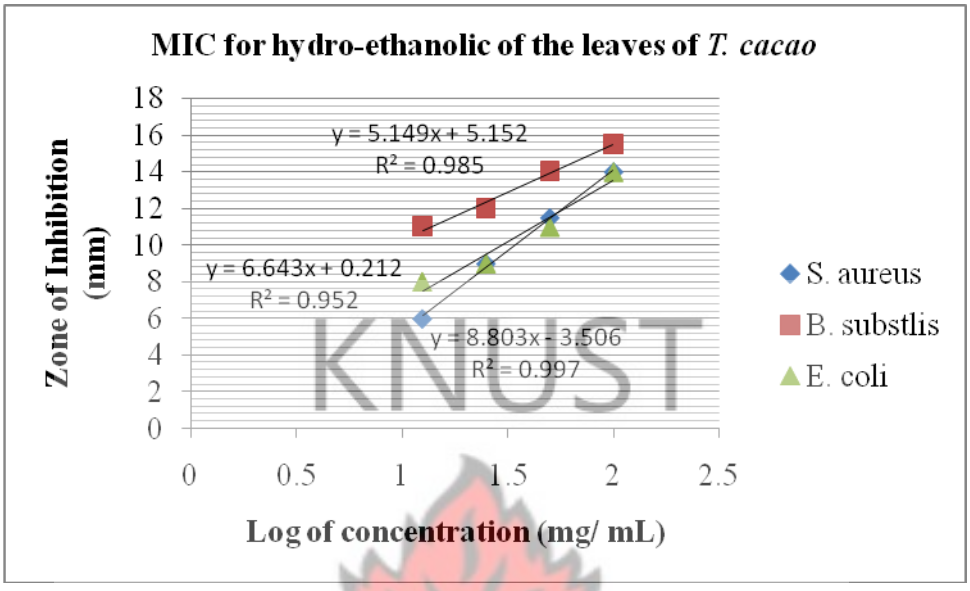
(B)


MIC for hydro-ethanal extract of A. muricata
Zone of Inhibition (mm)
35
30
25
20
15
10
5
0
y = 19.93x - 8.363
R² = 0.994
y = 4.318x + 6.563
R² = 0.965
y = 6.643x + 0.712
R² = 1
0
0.5
1
1.5
2
2.5
Log of concentration (mg/ml)
S.aureus
B. substlis
E. coli

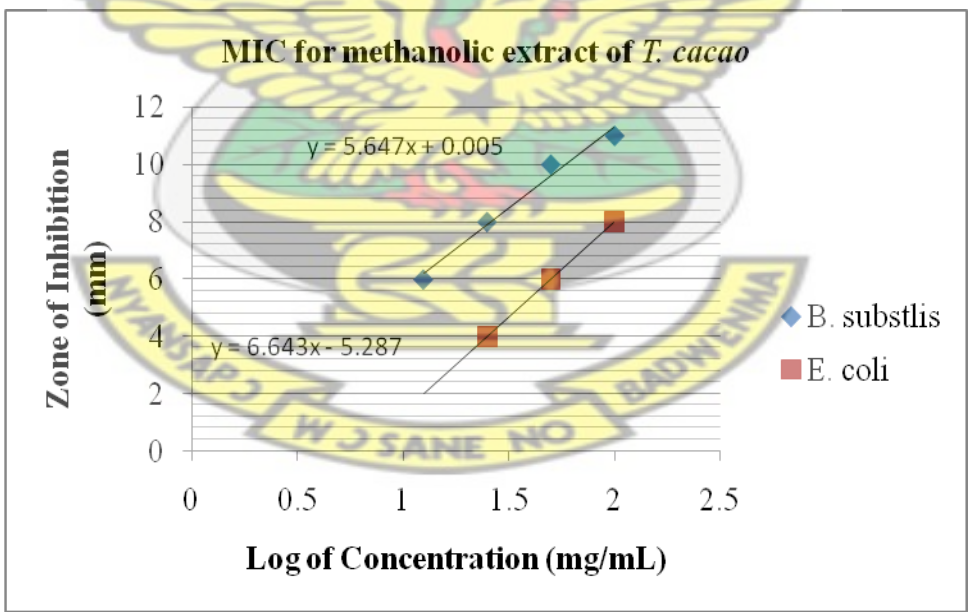
(C)


MIC for methanolic extract of A. muricata
Zone of Inhibition (mm)
35
30
25
20
15
10
5
0
y = 8.969x + 11.36
y = 5.979x - 3.508
0
0.5
1
1.5
2
2.5
Log of concentration (mg/ml)
S. aureus
B. substlis

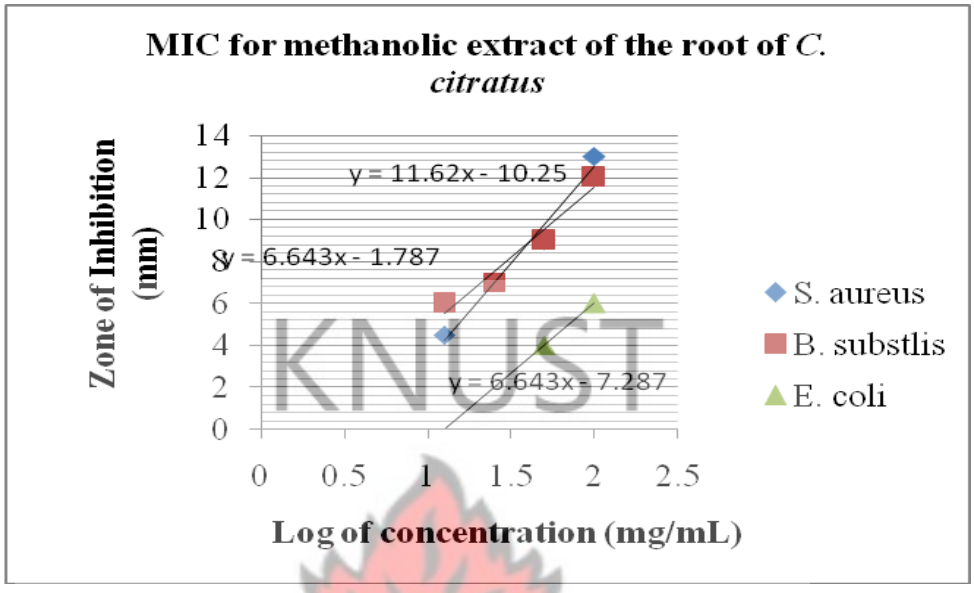
(D)


MIC for hydro-ethanolic of the leaves of T. cacao
Zone of Inhibition (mm)
18
16
14
12
10
8
6
4
2
0
y = 5.149x + 5.152
R² = 0.985
y = 6.643x + 0.212
R² = 0.952
y = 8.803x - 3.506
R² = 0.997
0
0.5
1
1.5
2
2.5
Log of concentration (mg/ mL)
S. aureus
B. substlis
E. coli

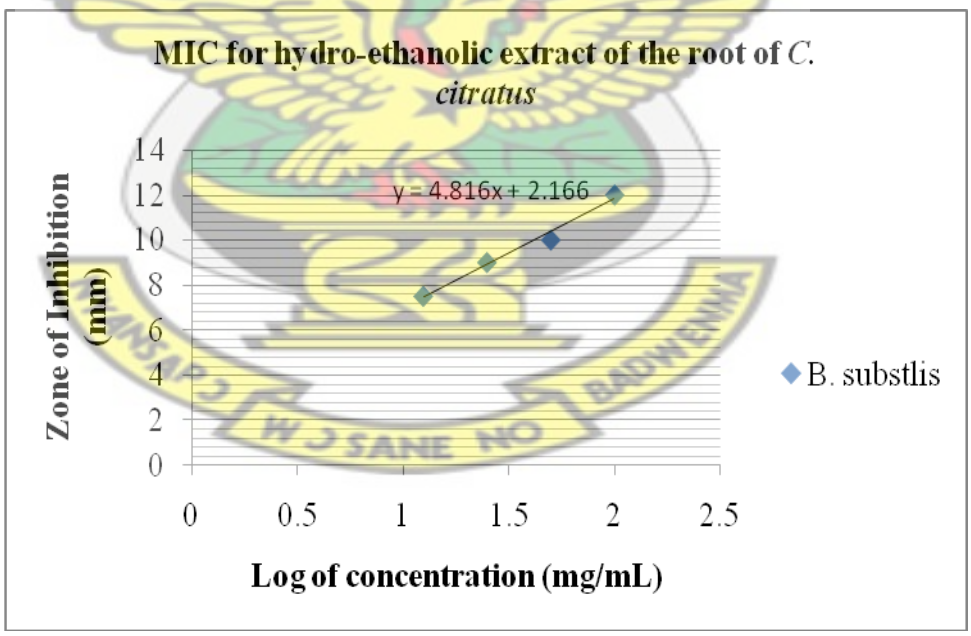
(E)


MIC for methanolic extract of T. cacao
Zone of Inhibition (mm)
12
10
8
6
4
2
0
y = 5.647x + 0.005
y = 6.643x - 5.287
0
0.5
1
1.5
2
2.5
Log of Concentration (mg/mL)
B. substlis
E. coli

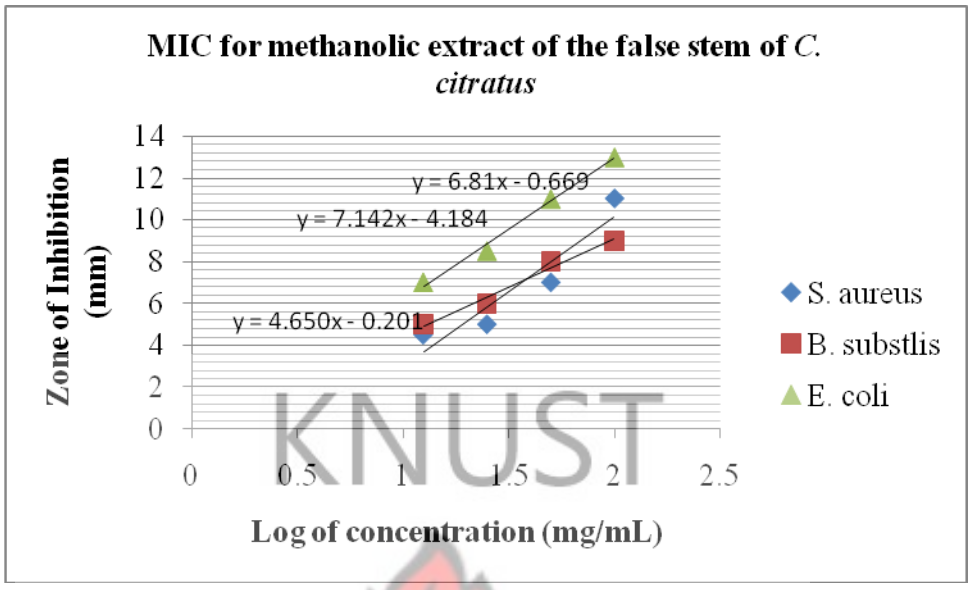
(F)


MIC for methanolic extract of the root of C. citratus
Zone of Inhibition (mm)
14
12
10
8
6
4
2
0
y = 11.62x - 10.25
y = 6.643x - 1.787
y = 6.643x + 7.287
0
0.5
1
1.5
2
2.5
Log of concentration (mg/mL)
S. aureus
B. substlis
E. coli

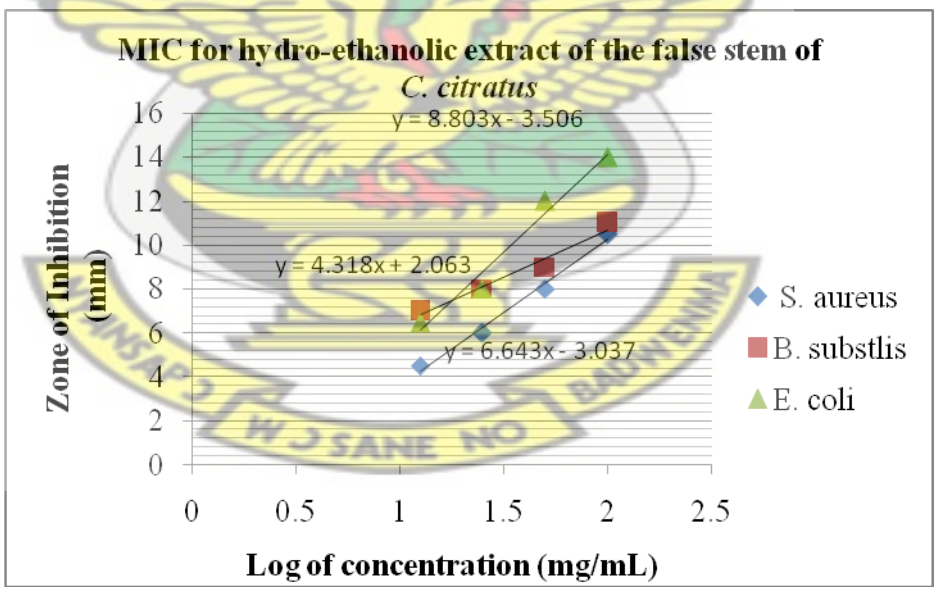
(G)


MIC for hydro-ethanolic extract of the root of C. citratus
Zone of Inhibition (mm)
14
12
10
8
6
4
2
0
y = 4.816x + 2.166
0
0.5
1
1.5
2
2.5
Log of concentration (mg/mL)
B. substlis

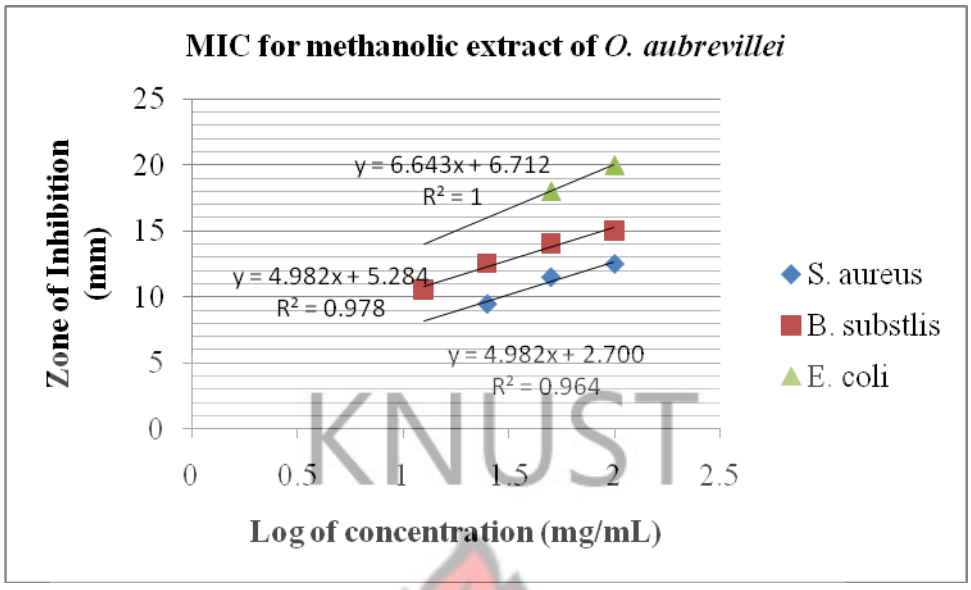
(H)


MIC for methanolic extract of the false stem of C. citratus
Zone of Inhibition (mm)
14
12
10
8
6
4
2
0
y = 6.81x - 0.669
y = 7.142x - 4.184
y = 4.650x - 0.201
0
0.5
1
1.5
2
2.5
Log of concentration (mg/mL)
S. aureus
B. substlis
E. coli

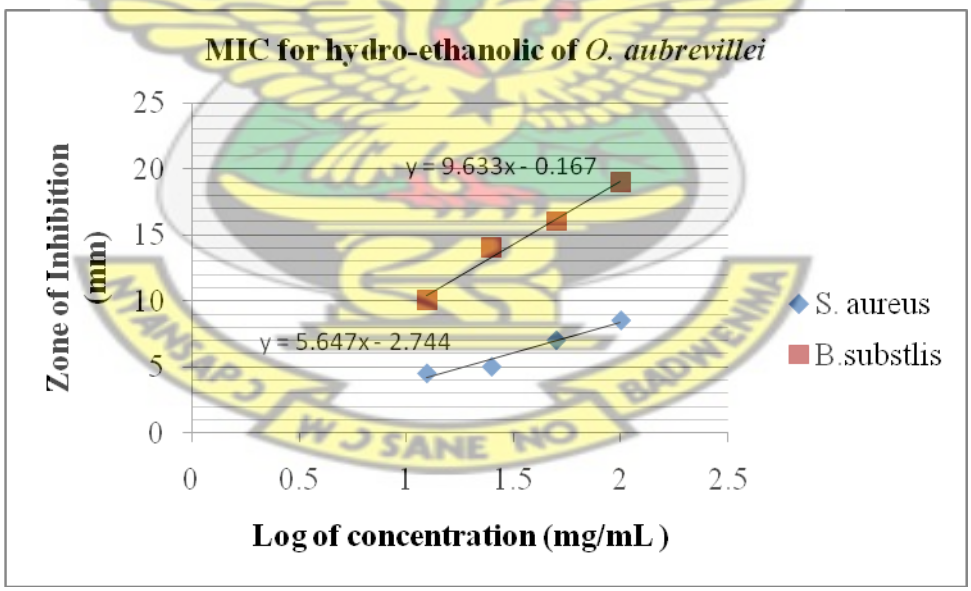
(I)


MIC for hydro-ethanolic extract of the false stem of C. citratus
Zone of Inhibition (mm)
16
14
12
10
8
6
4
2
0
y = 8.803x - 3.506
y = 4.318x + 2.063
y = 6.643x - 3.037
0
0.5
1
1.5
2
2.5
Log of concentration (mg/mL)
S. aureus
B. substlis
E. coli

(J)

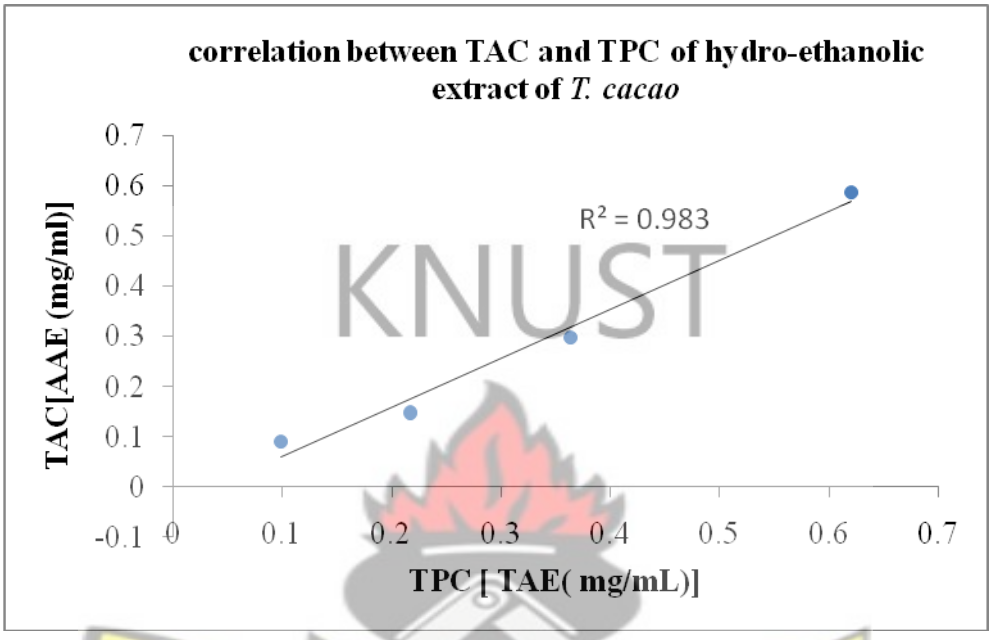

MIC for methanolic extract of O. aubrevillei
Zone of Inhibition (mm)
25
20
15
10
5
0
y = 6.643x + 6.712
R² = 1
y = 4.982x + 5.284
R² = 0.978
y = 4.982x + 2.700
R² = 0.964
0
0.5
1
1.5
2
2.5
Log of concentration (mg/mL)
S. aureus
B. substlis
E. coli

(K)

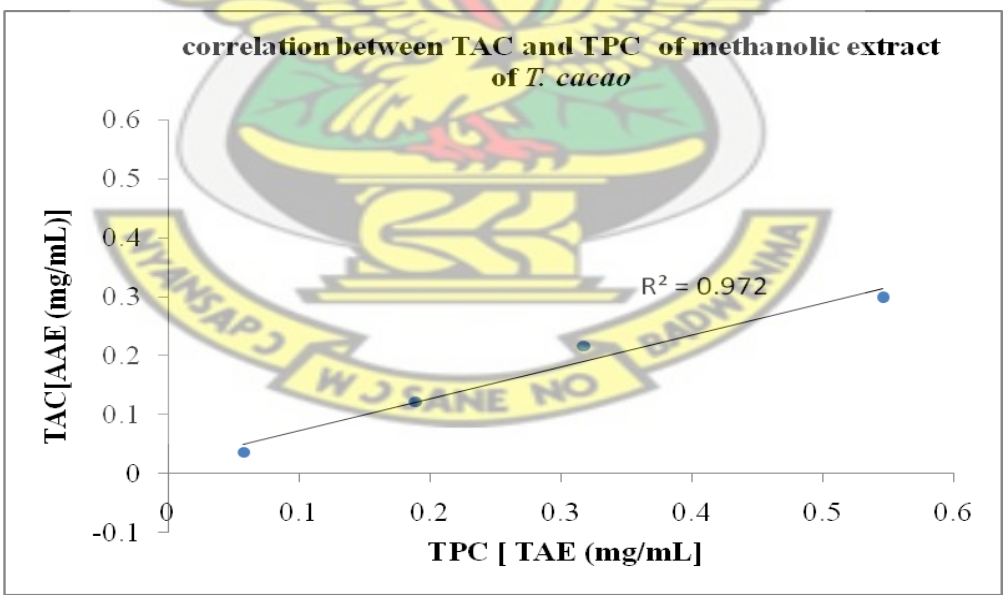

MIC for hydro-ethanolic of O. aubrevillei
Zone of Inhibition (mm)
25
20
15
10
5
0
y = 9.633x - 0.167
y = 5.647x - 2.744
0
0.5
1
1.5
2
2.5
Log of concentration (mg/mL)
S. aureus
B.substlis

3.0 Graphs showing the Correlation between TPC and TAC of the various plants extracts

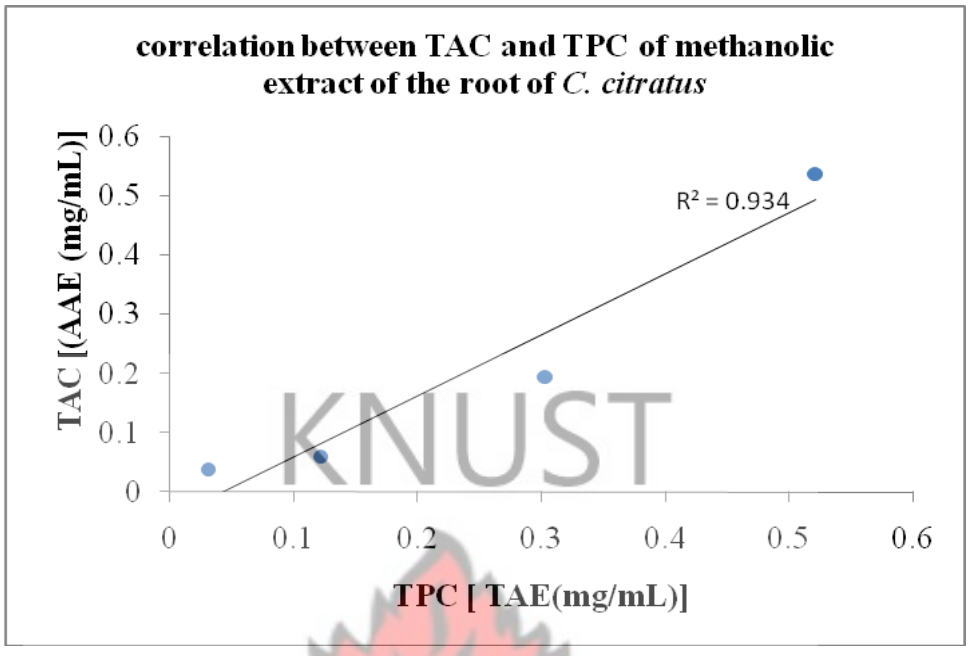
(A)



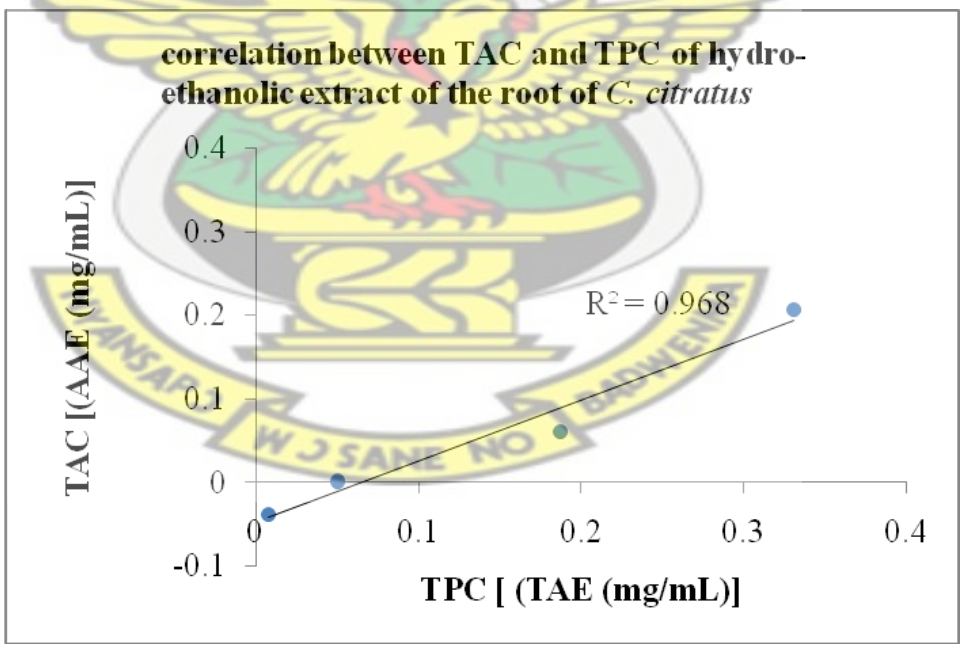
(B)



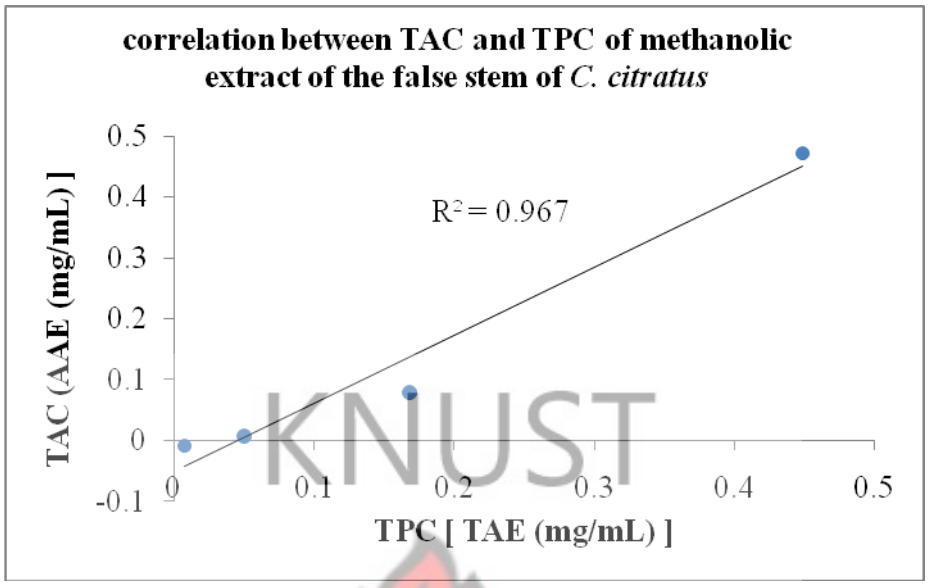
(C)


correlation between TAC and TPC of methanolic extract of the root of C. citratus
TAC [(AAE (mg/mL)]
0.6
0.5
0.4
0.3
0.2
0.1
0
R² = 0.934
0
0.1
0.2
0.3
0.4
0.5
0.6
TPC [TAE(mg/mL)]

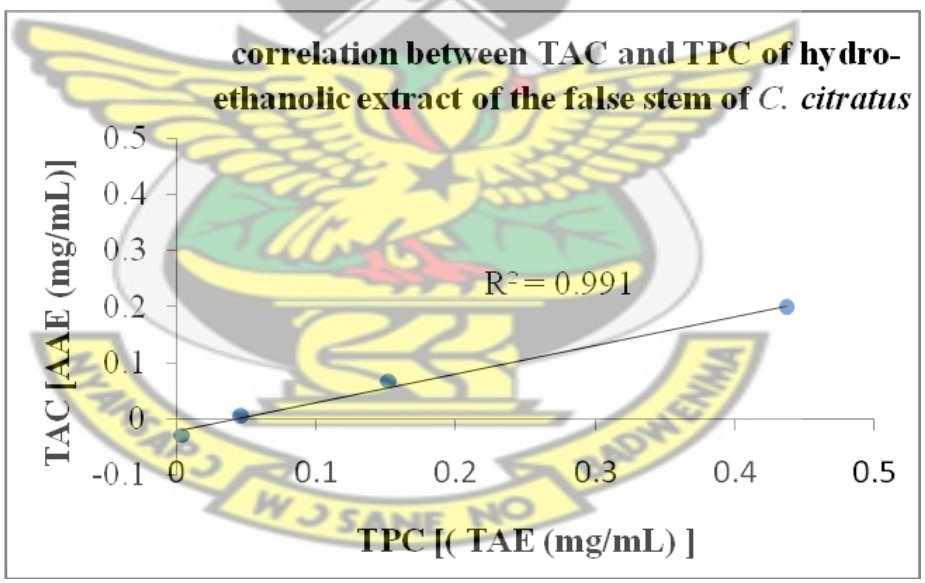
(D)


correlation between TAC and TPC of hydro-ethanolic extract of the root of C. citratus
TAC [(AAE (mg/mL)]
0.4
0.3
0.2
0.1
0
-0.1
R² = 0.968
0
0.1
0.2
0.3
0.4
TPC [(TAE (mg/mL)]

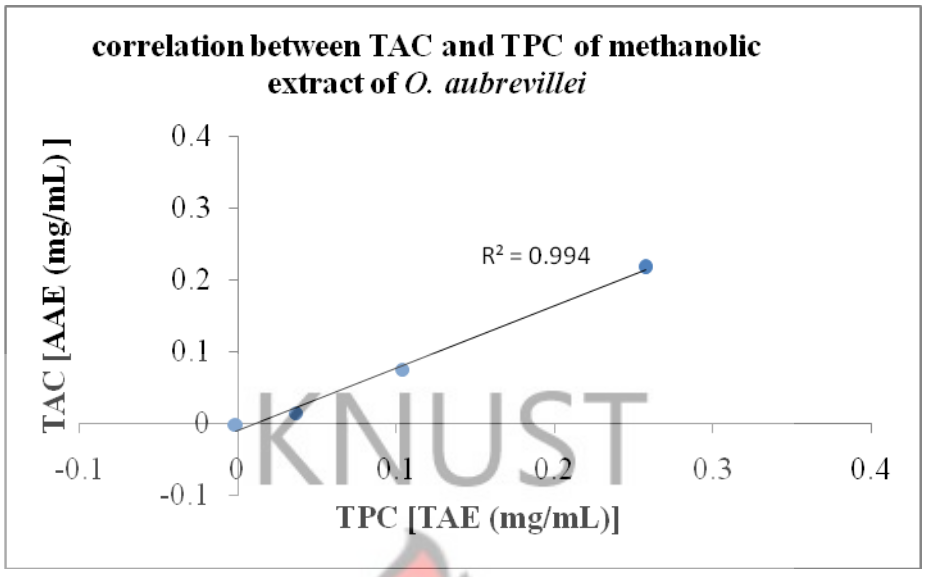
(E)


correlation between TAC and TPC of methanolic extract of the false stem of C. citratus
TAC (AAE (mg/mL)]
TPC [TAE (mg/mL)]
R² = 0.967
0.5
0.4
0.3
0.2
0.1
0
-0.1
0
0.1
0.2
0.3
0.4
0.5

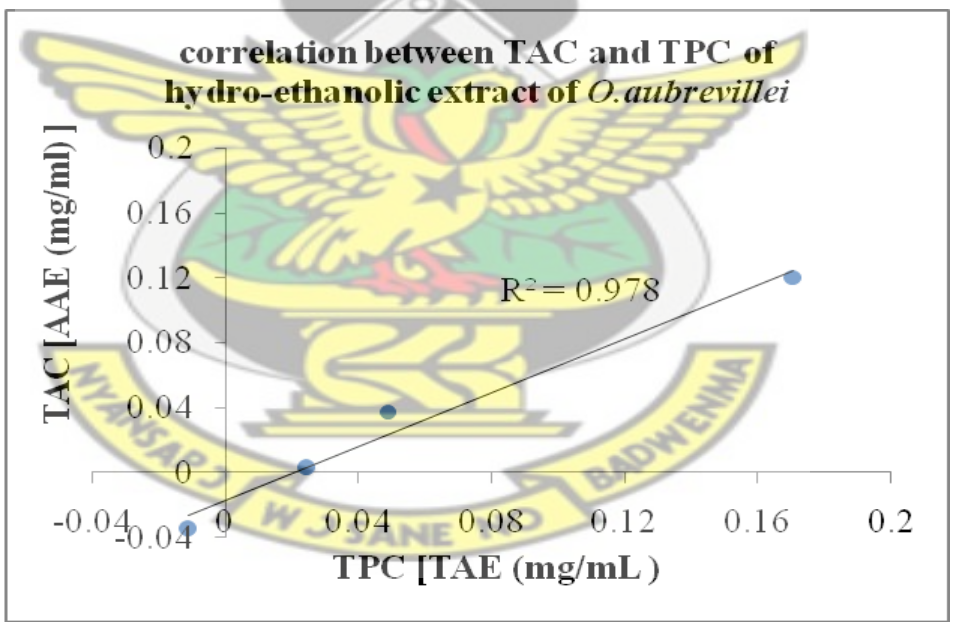
(F)


correlation between TAC and TPC of hydro-ethanolic extract of the false stem of C. citratus
TAC [AAE (mg/mL)]
TPC [(TAE (mg/mL)]
R² = 0.991
0.5
0.4
0.3
0.2
0.1
0
-0.1
0
0.1
0.2
0.3
0.4
0.5

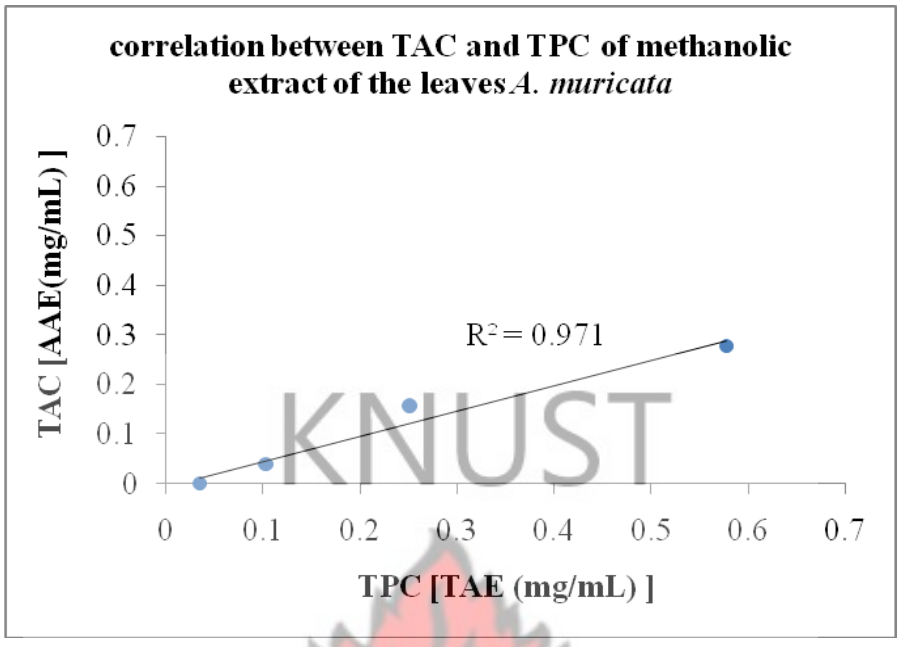
(G)


correlation between TAC and TPC of methanolic extract of O. aubrevillei
TAC [AAE (mg/mL)]
TPC [TAE (mg/mL)]
R² = 0.994
0.4
0.3
0.2
0.1
0
-0.1
-0.1
0
0.1
0.2
0.3
0.4

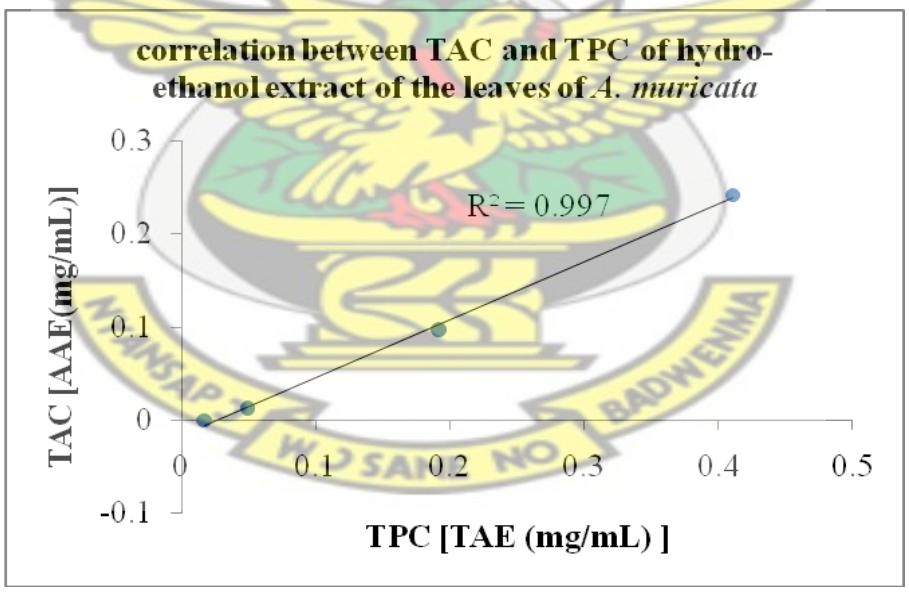
(H)


correlation between TAC and TPC of hydro-ethanolic extract of O.aubrevillei
TAC [AAE (mg/ml)]
TPC [TAE (mg/mL)
R² = 0.978
0.2
0.16
0.12
0.08
0.04
0
-0.04
-0.04
0
0.04
0.08
0.12
0.16
0.2

(I)


correlation between TAC and TPC of methanolic extract of the leaves A. muricata
TAC [AAE(mg/mL)]
TPC [TAE (mg/mL)]
R² = 0.971
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7

(J)


correlation between TAC and TPC of hydro-ethanol extract of the leaves of A. muricata
TAC [AAE(mg/mL)]
TPC [TAE (mg/mL)]
R² = 0.997
0.3
0.2
0.1
0
-0.1
0
0.1
0.2
0.3
0.4
0.5

4.0 REDUCING POWER

The absorbance (700nm) of the various plant extracts and ascorbic acid (standard) at different concentrations are shown in the table below

Conc.(mg/mL)	Ascorbic acid		
	A	B	C
0.01	0.0803	0.0833	0.0743
0.03	0.2002	0.2173	0.2013
0.10	0.5794	0.5312	0.5349
0.30	1.9220	1.9005	1.9640

Conc.(mg/mL)	Methanol extract of the leaves of *Theobroma cacao*		
	A	B	C
0.1	0.2344	0.2652	0.2723
0.3	0.4432	0.5012	0.4510
1.0	0.6843	0.7043	0.6821
3.0	1.5642	0.9974	1.0962

Conc.(mg/mL)	Hydro - ethanolic extract of the leaves of *Theobroma cacao*		
	A	B	C
0.1	0.2494	0.2573	0.3416
0.3	0.4148	0.5383	0.5503
1.0	0.7143	0.7699	0.6988
3.0	1.5870	1.8233	1.8572

Conc.(mg/mL)	Methanolic extract of the seeds of *Okuobaka aubrevillei*		
	A	B	C
0.1	0.0479	-0.0366	0.0291
0.3	0.0541	0.0366	0.0436
1.0	0.0338	0.0767	0.1392
3.0	0.2050	0.2539	0.2843

Conc.(mg/mL)	Hydro - ethanolic extract of the seeds of *Okuobaka aubrevillei*		
	A	B	C
0.1	0.0320	0.0153	0.0291
0.3	0.0391	0.0551	0.0436
1.0	0.1484	0.1180	0.1392
3.0	0.4504	0.1975	0.2843

Conc.(mg/mL)	Hydro - ethanolic extract of the leaves of *Annona muricata*		
	A	B	C
0.1	0.0494	0.0573	0.0416
0.3	0.2148	0.2383	0.1503
1.0	0.6143	0.5699	0.6488
3.0	1.587	1.4233	1.2572

Conc.(mg/mL)	Methanolic extract of the leaves of *Annona muricata*		
	A	B	C
0.1	0.0465	0.0323	0.0350
0.3	0.1971	0.0889	0.1582
1.0	0.2275	0.3477	0.3610
3.0	1.1628	1.2631	1.1907

Conc.(mg/mL)	Hydro - ethanolic extract of false stem of *Cymbopogon citratus*		
	A	B	C
0.1	0.1797	0.1505	0.2115
0.3	0.3169	0.2817	0.2820
1.0	0.4948	0.5436	0.7422
3.0	1.3037	0.7340	1.3440

Conc.(mg/mL)	Methanolic extract of false stem of *Cymbopogon citratus*		
	A	B	C
0.1	0.2024	0.1915	0.2094
0.300	0.2352	0.2917	0.0591
1.000	0.5360	0.3816	0.4417
3.000	0.7239	0.7133	0.7662

Conc.(mg/mL)	Methanolic extract of root of *Cymbopogon citratus*		
	A	B	C
0.1	0.1112	0.2363	0.2070
0.3	0.2709	0.2738	0.2168
1.0	0.3430	0.5922	0.5162
3.0	1.4884	1.6368	0.8241

Conc.(mg/mL)	Hydro - ethanolic extract of root of *Cymbopogon citratus*		
	A	B	C
0.1	0.2426	0.2802	0.2365
0.3	0.2438	0.3752	0.389
1.0	0.8481	0.7970	0.8140
3.0	1.7830	1.2367	1.0092

5.0 TOTAL ANTIOXIDANT CAPACITY

The absorbance (695nm) of the various plant extracts and ascorbic acid (standard) at different concentrations are shown in the table below

Conc.(mg/mL)	Ascorbic acid		
	A	B	C
0.01	0.0367	0.0468	0.1488
0.03	0.0982	0.1027	0.1253
0.10	0.368	0.2854	0.2744
0.30	0.6639	0.7592	1.0964

Conc.(mg/mL)	Methanolic extract of the seeds of *Okoubaka aubrevillei*		
	A	B	C
0.1	0.0190	0.0618	0.0215
0.3	0.0642	0.0922	0.0801
1.0	0.2833	0.1780	0.2598
3.0	0.6488	0.7338	0.4684

Conc.(mg/mL)	Hydro - ethanolic extract of seeds *Okoubaka aubrevillei*		
	A	B	C
0.1	0.0456	0.0526	0.0557
0.3	0.0674	0.0698	0.1022
1.0	0.3165	0.4709	0.3298
3.0	0.6946	0.6649	0.7971

Conc.(mg/mL)	Hydro - ethanolic extract of the root of *Cymbopogon citratus*		
	A	B	C
0.1	0.0134	0.0259	0.0212
0.3	0.0465	0.0448	0.0912
1.0	0.2450	0.2390	0.2677
3.0	1.2778	1.3644	1.2412

Conc.(mg/mL)	Methanolic extract of root of *Cymbopogon citratus*		
	A	B	C
0.1	-0.0573	-0.0616	-0.0653
0.3	0.0178	0.0323	0.0704
1.0	0.2043	0.2017	0.2028
3.0	0.5265	0.5990	0.6471

Conc.(mg/mL)	Hydro - ethanolic extract of false stem of *Cymbopogon citratus*		
	A	B	C
0.1	0.0487	-0.0361	0.0293
0.3	0.1871	0.0905	0.1603
1.0	0.5399	0.4618	0.3567
3.0	0.7716	0.7750	0.7914

Conc.(mg/mL)	Methanolic extract of the false stem of *Cymbopogon citratus*		
	A	B	C
0.1	-0.0446	-0.0242	-0.0468
0.3	0.0426	0.0702	0.0355
1.0	0.2058	0.2594	0.3041
3.0	0.5083	0.5488	0.6427

Conc.(mg/mL)	Hydro - ethanolic extract of the leaves *Annona muricata*		
	A	B	C
0.1	0.1237	0.0979	0.0917
0.3	0.2065	0.2026	0.1914
1.0	0.3752	0.6781	0.6219
3.0	1.7039	1.7314	0.9744

Conc.(mg/mL)	Methanolic extract of the leaves of *Annona muricata*		
	A	B	C
0.1	0.0522	0.0457	0.0356
0.3	0.0678	0.0922	0.0574
1.0	0.2709	0.3088	0.3065
3.0	0.6649	0.7914	0.5946

Conc.(mg/mL)	Hydro - ethanolic extract of the leaves *Theobroma cacao*		
	A	B	C
0.1	0.0487	0.0368	0.0393
0.3	0.1871	0.0995	0.1663
1.0	0.5399	0.5618	0.5567
3.0	0.7916	0.8750	0.7915

Conc.(mg/mL)	Methanolic extract of the leaves *Theobroma cacao*		
	A	B	C
0.1	0.0456	0.0526	0.0557
0.3	0.0674	0.0698	0.1022
1.0	0.3165	0.4709	0.3298
3.0	0.6946	0.6649	0.7971

6.0 TOTAL PHENOL CONTENT

The absorbance (760nm) of the various plant extracts and ascorbic acid (standard) at different concentrations are shown in the table below

Conc.(mg/mL)	Tannic acid		
	A	B	C
0.01	0.0561	0.0702	0.0623
0.03	0.1412	0.1601	0.1657
0.10	0.3126	0.3401	0.3243
0.30	0.8425	0.8049	0.7645

Conc.(mg/mL)	Hydro - ethanolic extract of the seeds *Okuobaka aubrevillei*		
	A	B	C
0.1	0.0137	0.1456	0.021
0.3	0.1499	0.1608	0.1488
1.0	0.2722	0.3729	0.3141
3.0	0.567	0.8478	0.6921

Conc.(mg/mL)	Methanolic extract of the seeds *Okuobaka aubrevillei*		
	A	B	C
0.1	0.0173	0.0681	0.0204
0.3	0.1268	0.1206	0.1241
0.1	0.1874	0.1959	0.1692
0.3	0.4618	0.4528	0.5467

Conc.(mg/mL)	Hydro - ethanolic extract of the leaves *Annona muricata*		
	A	B	C
0.1	0.176	0.106	0.1601
0.3	0.328	0.2974	0.3352
1.0	0.7052	0.6877	0.6732
3.0	1.7579	1.7372	0.9967

Conc.(mg/mL)	Methanol extract the leaves *Annona muricata*		
	A	B	C
0.1	0.0829	0.0651	0.0687
0.3	0.1909	0.1761	0.1658
1.0	0.4418	0.4884	0.3926
3.0	1.1381	1.1582	1.1496

Conc.(mg/mL)	Methanolic extract of the false stem of *Cymbopogon citratus*		
	A	B	C
0.1	0.0652	0.134	0.1213
0.3	0.1873	0.191	0.1762
1.0	0.5009	0.509	0.6124
3.0	0.8317	1.319	1.1012

Conc.(mg/mL)	Hydro- ethanolic extract of false stem of *Cymbopogon citratus*		
	A	B	C
0.1	0.0715	0.063	0.1094
0.3	0.1843	0.1783	0.1987
1.0	0.4597	0.4646	0.5189
3.0	1.1621	1.1809	1.1629

Conc.(mg/mL)	Hydro - ethanolic extract of the root of *Cymbopogon citratus*		
	A	B	C
0.1	0.1636	0.1316	0.1213
0.3	0.3352	0.324	0.4321
1.0	0.844	0.8489	0.7563
3.0	1.3457	1.3113	1.4174

Conc.(mg/mL)	Methanolic extract of the root of *Cymbopogon citratus*		
	A	B	C
0.1	0.0824	0.0853	0.0796
0.3	0.1898	0.1934	0.1795
1.0	0.4906	0.5082	0.5854
3.0	0.8793	0.9001	0.8765

Conc.(mg/mL)	Methanolic extract of the leaves *Theobroma cacao*		
	A	B	C
0.1	0.2036	0.2054	0.2091
0.3	0.5254	0.5355	0.5324
1.0	0.8936	0.8947	0.7649
3.0	1.3936	1.495	1.3721

Conc.(mg/mL)	Hydro - ethanolic extract of leaves *Theobroma cacao*		
	A	B	C
0.1	0.2531	0.3192	0.3525
0.3	0.5932	0.6231	0.5875
1.0	0.8997	0.9967	1.0021
3.0	1.6741	1.4998	1.6342

7.0 DPPH FREE RADICAL SCAVENGING ACTIVITY

The absorbance (517nm) of the various plant extracts and ascorbic acid (standard) at different concentrations are shown in the table below

Conc.(mg/mL)	Ascorbic acid		
	A	B	C
0.01	0.1362	0.1385	0.1406
0.03	0.1145	0.1104	0.1084
0.10	0.0676	0.0731	0.0646
0.30	0.0484	0.0484	0.0494

Conc.(mg/mL)	Methanolic extract of the leaves *Annona muricata*		
	A	B	C
0.1	0.3103	0.2012	0.2114
0.3	0.1763	0.1467	0.1627
1.0	0.1107	0.1253	0.1132
3.0	0.0787	0.0892	0.0577

Conc.(mg/mL)	Hydro - ethanolic extract of the leaves *Annona muricata*		
	A	B	C
0.1	0.2986	0.2728	0.2755
0.3	0.1021	0.1057	0.1127
1.0	0.0575	0.0511	0.0511
3.0	0.0419	0.0277	0.0344

Conc.(mg/mL)	Methanolic extract of the false stem of *Cymbopogon citratus*		
	A	B	C
0.1	0.2929	0.3148	0.3021
0.3	0.1567	0.2387	0.2295
1.0	0.0909	0.0798	0.0937
3.0	0.0567	0.0687	0.0629

Conc.(mg/mL)	Hydro- ethanolic extract of false stem of *Cymbopogon citratus*		
	A	B	C
0.1	0.2937	0.3802	0.2275
0.3	0.1883	0.1961	0.2327
1.0	0.0498	0.047	0.0449
3.0	0.0468	0.0393	0.0427

Conc.(mg/mL)	Methanolic extract of the leaves of *Theobroma cacao*		
	A	B	C
0.1	0.2186	0.2928	0.1985
0.3	0.1221	0.1057	0.1327
1.0	0.0675	0.0571	0.0519
3.0	0.0429	0.0377	0.0384

Conc.(mg/mL)	Hydro - ethanolic extract of the leaves of *Theobroma cacao*		
	A	B	C
0.1	0.2109	0.3103	0.1201
0.3	0.1734	0.1633	0.1593
1.0	0.0787	0.1056	0.0793
3.0	0.0647	0.0687	0.069

Conc.(mg/mL)	Methanolic extract of the root of *Cymbopogon citratus*		
	A	B	C
0.1	0.2884	0.2914	0.3047
0.3	0.1691	0.1727	0.2603
1.0	0.0338	0.0355	0.0339
3.0	0.0333	0.0248	0.0259

Conc.(mg/mL)	Hydro - ethanolic extract of the root of *Cymbopogon citratus*		
	A	B	C
0.1	0.2495	0.3425	0.2171
0.3	0.1407	0.1716	0.1618
1.0	0.0177	0.0263	0.0211
3.0	0.0167	0.0209	0.0155

Conc.(mg/mL)	Methanolic extract of the seeds of *Okuobaka aubrevillei*		
	A	B	C
0.1	0.3308	0.3446	0.3204
0.3	0.3101	0.3011	0.3131
1.0	0.1631	0.1002	0.1854
3.0	0.03473	0.0342	0.0132

Conc.(mg/mL)	Hydro - ethanolic extract of the seeds of *Okuobaka aubrevillei*		
	A	B	C
0.1	0.3533	0.2461	0.2445
0.3	0.2065	0.2105	0.2523
1.0	0.1158	0.2496	0.1134
3.0	0.0916	0.0917	0.1111