IN VITRO ANTIOXIDANT EFFECTS OF DICHLOROMETHANE – METHANOL LEAF BLEND EXTRACTS OF *Clutia abyssinica* AND *Maytenus obscura*.

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DECLARATION

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

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DEDICATION

I dedicate this thesis to my husband (Jackson Guto), my son (Adrian Ondieki), my parents, brothers and sisters for the support and sacrifices you made towards my education.

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ABBREVIATIONS AND ACRONYMS

$[K_3Fe(CN)_6]$	Potassium Ferricyanide
ANOVA	Analysis of Variance
ATP	Adenosine triphosphate
BHA	Butylated Hydroxyl Anisole
BHT	Butylated Hydroxyl Toluene
CAT	Catalase
Cl^-	Chloride ions
Cu^{2+}	Copper (II) ions
DCM	Dichloromethane
DNA	Deoxyribonucleic Acid
DPPH	1,1 –Diphenyl-2-Picrylhydrazyl
Fe^{2+}	Iron (II) ions
Fe^{3+}	Iron (III) ions
Fe_3Cl	Iron (III) chloride
FRAP	Ferric reducing antioxidant power
g	Grams
GAE	Gallic acid equivalents
GPx	Glutathione peroxidase
GRx	Glutathione reductase
GSH	Reduced glutathione
GSSG	Glutathione disulfide
GST	Glutathione-S-transferase
H^+	Hydrogen ions
H_2O	Water
H_2O_2	Hydrogen Peroxide
H_2SO_4	Sulphuric Acid
HCL	Hydrochloric acid
HNO_2	Nitrous acid
HO_2	Hydroperoxyl
HOCL	Hypochlorus acid
IC_{50}	Half maximal inhibitory concentration
KOH	Potassium hydroxide
L-Arg	L-arginine
LDL	Low density lipoprotein
LH	Fatty acid
$LOO^{\cdot}$	Lipid peroxyl
LPIC	Lipid peroxidation inhibition capacity
MCA	Metal Chelating Agent
MDA	Malondialdehyde
MeOH	Methanol
N_2O_3	Dinitrogen trioxide
Na_2CO_3	Sodium carbonate
NADPH	Nicotinamide adenosine dinucleotide phosphate
NaOH	Sodium hydroxide

NDGA	Nordihydro-guaretic Acid
NO or NO^-	Nitric oxide
NO_2	Nitric dioxide
O_2	Superoxide radical
O_3	Ozone
OH	Hydroxyl Radical
$ONOO^-$	Peroxynitrate
ONOOH	Peroxynitrous
ORAC	Oxygen radical absorbance capacity
PG	Propyl Gallate
PL-OH	Phospholipids hydroperoxide
PL-OOH	Phospholipids hydroperoxides
PUFAs	Polyunsaturated fatty acids
$R^\cdot$	Radical
RNS	Reactive nitrogen species
ROH	Hydroxyl fatty acid
ROOH	Hydroperoxide
ROOH	Lipid peroxides
ROOH	Organic Hydroperoxide
ROS	Reactive Oxygen Species
RPM	Rates per minute
SEM	Standard Error of the Mean
SOD	Superoxide dismutase
TBHQ	Tertiary Butyl Hydroquinone
TCA	Trichloroacetic acid

ABSTRACT

Oxidative stress is a state of imbalance between free radicals and antioxidants in the body. It is the main cause of several disease conditions such as diabetes, different types of cancers, cardiovascular diseases, inflammation and aging. Oxidative stress is managed by use of antioxidants which can be obtained in the diet or given as supplements. Antioxidants are substances with the ability to prevent oxidation of other molecules in the body by free radicals. Antioxidants react with free radicals making them stable and reducing their ability to react with different cell components. The treatment of oxidative stress has been confined to use of synthetic supplements, which are unaffordable to most Kenyans and are known to possess side effects. This has led to increased demand for herbal products with antioxidant properties that have little side effects, are affordable and more readily available. Different plants that are used as medicinal plants have been tested for antioxidant activity such as *Strychnos henningsii* and *Rosemarinus officinalis*. *Clutia abyssinica* and *Maytenus obscura* though traditionally used, have not been scientifically proven and documented. This study evaluated the *in vitro* antioxidant potential of Dichloromethane: Methanolic extracts of *Clutia abyssinica* and *Maytenus obscura*. Different antioxidant assays were done including free radical scavenging activity by using 1,1 –Diphenyl-2-Picrylhydrazyl (DPPH), total ferric reducing power and hydrogen peroxide scavenging activity. The DCM: MeOH extracts of both plants demonstrated a significant level of DPPH scavenging activity with the highest percentage of 80%, 82.57% and 91.77% for *C. abyssinica, M. obscura* and ascorbic acid respectively. Ascorbic acid demonstrated the lowest value of IC_{50} of 0.044 and 0.087, 0.065 for *C. Abyssinica* and *M. Obscura* respectively. They also demonstrated reducing power that increased with increase in concentration. Different levels of hydrogen peroxide scavenging activity were also demonstrated by the extracts depending on the concentration. There were significant differences between the extracts and the standard. The DCM: MeOH extracts of *C. abyssinica* and *M. obscura* demonstrated significant antioxidant activity. The DCM: MeOH extracts of *C. abyssinica* and *M. obscura* can therefore be an alternative source of antioxidants for management of different problems that rise due to oxidative stress. The present study, therefore, scientifically validates and supports the traditional use of *C. abysssinica* and *M. obscura* in the management of oxidative stress.

CHAPTER ONE
INTRODUCTION

1.1 Background information

Living organisms that carry out aerobic metabolism are exposed to dangers associated with free radicals, reactive oxygen species (ROS) and reactive nitrogen species (RNS). Free radicals are unstable and reactive molecules such as super oxide radical, nitric oxide, hydrogen peroxide and hydroxyl radical, which are released as by-products of aerobic metabolism (Ebrahimzadeh *et al*., 2009). Accumulation of free radicals leads to oxidation of different biomolecules in the body such as DNA, proteins and lipids, causing injuries to different body tissues and induces oxidative damage or stress (Ahmad *et al*., 2013; Ofeimun *et al*., 2014). Most aerobic organisms have defense mechanisms to protect the body from harmful effects caused by the free oxygen radicals. Failure of the antioxidant defense system results in oxidative stress.

Oxidative stress is a state of imbalance between oxidants and antioxidants (high levels of oxidants than antioxidants that neutralize their harmful effects). It is the main cause of several disease conditions such as diabetes, different types of cancers, cardiovascular diseases, inflammation and aging (Esmaeili *et al*., 2009; Lacine *et al*., 2013).

Antioxidants help in the management of oxidative stress by preventing oxidation of other molecules in the body by free radicals (Hamid *et al*., 2010; Krishnaveni, 2014). They react with free radicals to make them stable and reduce their ability to react with different cell components (Nandini and Sreemoyee, 2014).

Based on their functions, antioxidants can be classified as enzymatic and non-enzymatic. Enzymatic antioxidants are involved in breakdown and removal of free radicals from the body through different steps that require cofactors such as Copper, Zinc and Iron. They are produced naturally in the body and examples include superoxide dismutase and catalase (Birben *et al*., 2012). Non-enzymatic antioxidants help in interrupting free radical chain reactions. They include vitamin C, E and carotenoids among others and they are found in food supplements.

Antioxidants are classified as natural and synthetic based on their source. Natural antioxidants are endogenously produced in the body and are obtained from natural sources such as vegetables, fruits and herbs (Hamid *et al.*, 2010). Synthetic antioxidant supplements are used in the conventional management of oxidative stress. Such supplements include BHA-butylated hydroxyl anisole, BHT-butylated hydroxyl toluene and propyl gallate (Oyedemi *et al.*, 2010). However, they are unaffordable, not easily accessible and are suspected to be responsible for liver damage and carcinogenesis in laboratory animals (Moulisha *et al.*, 2010). There is, therefore, the need to develop more affordable, accessible and safe antioxidants.

There are different herbs that have been used as a source of antioxidants they include *Rosemarinus officinalis* (Lamiacea) leaf extract and *Zingiber officinale* (Zingiberaceae). They have been found to be good radio-protectors and have been found to be non-toxic compared to most synthetic protectors (Asif, 2015). Antioxidants from natural sources and phytochemicals have the advantage of low toxicity (Asif, 2015). Extracts of

S*trychnos henningsii* have been shown to possess antioxidant potential dependent on the concentration, hence they can be used as antioxidants (Oyedemi *et al.*, 2010). According to studies carried out by Monica *et al*. (2010), sap from the tree trunk of the species *Croton lechleri* sampled in Peru showed high antioxidant activity. In South America, this sap has long been used as an antifungal, antiseptic, antiviral and anti-haemorrhagic and in wound healing.

Clutia abyssinica and *Maytenus obscura* are used traditionally for management of oxidative damage/stress and different oxidative stress-related disease conditions. However, this has not been scientifically evaluated and documented. It is against this background that this study was designed to determine *in vitro* antioxidant potential of Dichloromethane (DCM): Methanol (MeOH) leaf blend extracts of *Clutia abyssinica* and *Maytenus obscura.*

1.2 Problem statement and justification

Accumulation of free radicals leads to oxidative stress, which is the basis of the development of different disease conditions in the body. Some of these include cardiovascular diseases, cancers, Parkinson's disease, Alzheimer, arthritis, atherosclerosis, diabetes and ageing (Purabi *et al*., 2011; Boguslaw, 2012). Therefore, oxidative stress poses a great threat to human health and population. Reduction of such diseases incidences can mainly be done by management of oxidative stress, hence there is need for antioxidants supplements to manage such conditions (Ahmad *et al*., 2013).

Commercially available antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have proved to be harmful and carcinogenic (Rabia *et al.,* 2015). Due to their toxic effects, restrictions have been imposed on their use (Lacine *et al*., 2013). They are also not easily accessible and affordable to the poor (Asif, 2015). Consequently, researchers have turned on the search for antioxidants from natural sources, because they are considered to be safe, have higher therapeutic value and long life (Sreeramulu *et al*., 2013).

Medicinal plants have had a great contribution in providing the health benefits to man including traditional use of these two plants under study as antioxidants. However, this has not been documented and confirmed. It is, therefore, imperative to bioscreen the DCM: MeOH blend extracts of *C. abyssinica* and *M. obscura* for *in vitro* antioxidant activity to confirm, document and validate their traditional use in preventing or managing oxidative stress-related disorders.

1.3 Research questions

(i) Do the DCM: MeOH leaf blend extracts of *Clutia abyssinica* and *Maytenus obscura* have *in vitro* DPPH free radical scavenging activities?

(ii) Do the DCM: MeOH leaf blend extracts of *Maytenus obscura* and *Clutia abysinica* have *in vitro* total ferric reducing power activities?

(iii) Do the DCM: MeOH leaf blend extracts of *Maytenus obscura* and *Clutia abysinica* have *in vitro* hydrogen peroxide scavenging activities?

(iv) What is the total phenolic content of the DCM: MeOH leaf blend extracts of *Clutia abyssinica* and *Maytenus obscura*?

(v) What is the qualitative phytochemical composition of the DCM: MeOH leaf blend extracts of *Clutia abyssinica* and *Maytenus obscura*?

1.4 Objectives

1.4.1 General objective

To determine the *in vitro* antioxidant activities of DCM: MeOH leaf blend extracts of *Clutia abysinicca* and *Maytenus obscura.*

1.4.2 Specific objectives

(i) To determine the *in vitro* DPPH radical scavenging activity of DCM: MeOH leaf blend extracts of *Clutia abyssinica* and *Maytenus obscura*.

(ii) To determine the *in vitro* total ferric reducing power of the DCM: MeOH leaf blend extracts of *Clutia abyssinica* and *Maytenus obscura*.

(iii) To determine the *in vitro* hydrogen peroxide scavenging activities of DCM: MeOH leaf blend extracts of *Clutia abyssinica* and *Maytenus obscura*.

(iv) To determine the total phenolic content of the DCM: MeOH leaf blend extracts of *Clutia abyssinica* and *Maytenus obscura*.

(v) To determine the qualitative phytochemical composition of DCM: MeOH leaf blend extracts of *Clutia abyssinica* and *Maytenus obscura.*

CHAPTER TWO
LITERATURE REVIEW

2.1 Oxidative stress

Oxidative stress is a state of physiological stress in the body that arises due to an imbalance between the production of free radicals (reactive oxygen species, ROS) and the ability of the body to counteract or detoxify their harmful effects through neutralization by antioxidants (Helmut, 2015). Living organisms that depend on aerobic metabolism as a source of energy produce reactive oxygen species. Lifestyle and other environmental factors such as poor diet, smoking, stress, and lack of sleep, pollution, infections and radiations also contribute to production of high levels of free radicals (Anu *et al*., 2014). Free radicals are highly reactive molecules due to presence of unpaired electrons, they include hydroxyl radical (OH), hydrogen peroxide (H_2O_2), hydroperoxide (ROOH), super oxide radical and nitric oxide radical (Nabavi *et al.*, 2009; Ahmad *et al*., 2013).

Aerobic organisms possess antioxidant defense system which include, dietary antioxidant system which relies on the diet and antioxidant enzyme system provided by different enzymes such as catalase, superoxide, dismutase and peroxides. The defense system reduces the harmful effects caused by free radicals (Ahmad *et al*., 2013). Failure of the antioxidant defense system due to abnormalities leads to accumulation of free radicals at the cell membranes causing lipid peroxidation and also exposes them to oxidative damage (Ali *et al*., 2015). Accumulation of free radicals can also affect DNA by interfering with its duplication and alterations in its structure, giving rise to different mutations. This encourages the onset of different diseases conditions that arise due to

oxidative damage such as diabetes, cancer, arthritis and inflammatory diseases (Oyedemi and Afolayan, 2011; Devi and Muthu, 2014). However, free radicals in their right amount help the body in performing different physiological functions such as health ageing (Sukru *et al*., 2012). Antioxidants have been shown to prevent oxidative damage caused by free radicals, thus preventing the occurrence of different disease conditions such as diabetes, cancer and aging (Patel *et al*., 2012).

2.2 Free radicals

Free radicals are molecules that contains one or more unpaired electron (s) and they are capable of existing on their own (Pham-Huy *et al*., 2008; Sen *et al*., 2010). This makes them unstable and reactive, hence to stabilize they have to donate or accept electron (s) from other molecules (Lobo *et al*., 2010). They include different reactive species such as reactive oxygen and nitrogen species (radicals and non-radicals). Examples of these radicals include hydroxyl radical, superoxide radical, hydrogen peroxide, nitric oxide radical among others (Sivanandham, 2011).

2.2.1 Superoxide radical ($O_2^{\bullet -}$)

The superoxide radical is produced in different processes in the body such as; electron transport chain in oxidative phosphorylation that generates energy in form of ATP in the body, enzymatic reduction of oxygen by different enzymes such as xanthine oxidases and cycloxigenase among others, redox recycling and by phagocytic cells such as white blood cells (Knight, 1998; Eboh, 2014). It can also be formed through non-

enzymatic reactions involving electron transfer to molecular oxygen as shown in the equation below (Birben *et al*., 2012).

$$O_2 + e^- \rightarrow O_2^{\bullet -}$$

It can exist in two forms either as hydroperoxyl radical (HO_2) or $O_2^{\bullet -}$. Two molecules of superoxide radical can react leading to formation of hydrogen peroxide through a dismutation reaction catalyzed by superoxide dismutase enzyme (Rahman *et al*., 2012; Phaniendra *et al*., 2015).

2.2.2 Hydroxyl radical ($OH^{\bullet}$)

This radical is produced in some reactions of transition metals with hydrogen peroxide during the fenton reaction and decomposition of peroxynitrite (Valko *et al*., 2004).

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + {}^{\bullet}OH + OH^-$$

It can also be formed when hydrogen peroxide reacts with $O_2^{\bullet}$ in the presence of iron initiating the Haber-weiss reaction (Gutowski and Kowalczyk, 2013).

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

The radical has a three electron reduction state, is highly reactive, short-lived and attacks most cellular components such as lipids, proteins and DNA (Sivanandham, 2011; Gutowski and Kowalczyk, 2013)).

2.2.3 Hydrogen peroxide (H_2O_2)

Hydrogen peroxide is mainly produced during dismutation reactions catalyzed by the enzyme superoxide dismutase as in the equation below (Eboh, 2014).

$$2O^{2\cdot} - + 2H+ \rightarrow H_2O_2 + O_2$$

When produced it is acted upon by catalase or glutathione peroxidase producing water and oxygen. It has two electron reduction state, is lipid soluble hence able to diffuse across membranes. It does not directly affect DNA, but when it can produce hydroxyl radical in the presence of metal ions such as Fe^{2+} or Cu^{2+} as in the reaction above involving hydroxyl radical. Hydroxyl radical can cause modification in DNA (single or double stranded DNA breaks) and cross-linking of protein. Hydrogen peroxide acts as a substrate in oxidation reactions such as those involving the synthesis of complex organic molecule (Kumar, 2011; Sivanandham, 2011).

2.2.4 Hypochlorous acid

It is an antibacterial agent, which is produced by immune cells such as the neutrophils when activated to aid in the process of phagocytosis (Turrens, 2003). It is formed when hydrogen peroxide reacts with chloride ions in a reaction catalyzed by myeloperoxidase enzyme (Eboh, 2014).

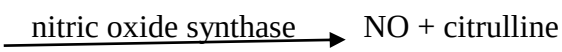
It can form hydroxyl radical in the presence of transition metals and by reacting with superoxide dismutase (Phaniendra *et al*., 2015).

$$Fe^{2+} + HOCl \rightarrow Fe^{3+} + Cl^{-} + OH^{\bullet}$$

$$HOCl + O_2^{\bullet -} \rightarrow OH^{\bullet} + Cl^{-} + O_2$$

2.2.5 Nitric oxide

This is a reactive nitrogen species that is produced through enzymatic reaction of nitric oxide synthase from amino acid arginine as in the equation below (Nimse and Pal, 2015).



It is an important molecule that acts as a signaling molecule in different biological processes such as neural transmission, regulation of blood pressure and defense mechanisms. It is both water and lipid soluble, hence it can easily diffuse through membranes. During the respiratory burst it reacts with superoxide to produce peroxynitrite a more potent oxidant (Valko *et al*., 2007).

2.2.6 Peroxynitrite ($ONOO^-$)

This is a very strong oxidant capable of attacking different biological membranes and it is formed from reaction of nitric oxide ($NO^{\cdot}$) and superoxide radical ($O_2^{\bullet -}$) as in the reaction below (Nimse and Pal, 2015).

It can react with carbon monoxide forming peroxynitrous (ONOOH), which undergoes hemolysis to forming products such as NO_2 and $OH^{\bullet}$ which are capable of attacking cellular components (Phaniendra *et al*., 2015).

2.3 Sources of free radicals

2.3.1 Endogenous sources

The mitochondria is an intracellular organelle involved in respiration and energy production, through the process of electron transport chain (Valko *et al*., 2004). The electron transport chain is composed of four complexes that is complex I, II, III and IV. These complexes are involved in electron transfer to the final acceptor oxygen (Phaniendra *et al*., 2015).

Oxidation-reduction reactions occur through the four complexes, in which case some molecules donate electrons while others accept releasing energy in the process. During the electron transfer, some electrons accidentally leak out and react with oxygen forming superoxide radical (Valko *et al*., 2004). The superoxide radical is neutralized to hydrogen peroxide by superoxide dismutase. Hydrogen peroxide can dissociate and form hydroxyl radical. Besides, the superoxide radical can react with nitric oxide forming peroxynitrite. Both hydroxyl and peroxynitrite radicals are strong oxidants that can cause damage to different cell components (Lobo *et al*., 2010; Phaniendra *et al*., 2015).

The enzyme cytochrome oxidase found in the mitochondria, is involved in reduction reactions of oxygen in the electron transport chain. Accidentally, during these reactions superoxide radical is produced. Also incomplete reduction of oxygen in this process leads to production of free radicals such as hydroxyl and hydrogen peroxide (Valko *et al*., 2004; Phaniendra *et al*., 2015).

During the process of phagocytosis, the white blood cells such as macrophages, monocytes and neutrophils produce a high amount of superoxide to help in providing antibacterial activity, but in the process superoxide is converted to hydrogen peroxide by enzymatic activity of superoxide dismutase. The hydrogen peroxide may partially dissociate and form hydroxyl radical, a more potent oxidant (Knight, 2000; Wu and Cederbaum, 2003).

Different enzymes catalyze reactions that lead to production of free radicals, which include instance xanthine oxidase that is involved in oxidation of purines. It is involved in the degradation of hypoxanthine to xanthine, and xanthine to uric acid (Knight, 1998). In the process it leads to the production of superoxide radical which is neutralized by superoxide dismutase producing hydrogen peroxide (Valko *et al*., 2004). An example of a reaction catalyzed by xanthine oxidase is illustrated below (Bagchi and Puri, 1998).

$$\text{Xanthine} + O_2 + H_2O \xrightarrow{\text{xanthine oxidase}} \text{urate} + O_2^{\cdot -} + 2H^+$$

NADPH oxidase complex is a group of enzymes present in the macrophages and neutrophils. When these immune cells are activated, NADPH oxidase is activated, producing superoxide and hydrogen peroxide radicals that aid in phagocytosis of the foreign or harmful particle (Valko *et al*., 2007). Myeloperoxidase enzyme catalyzes the reaction of chloride ions and hydrogen peroxide leading to formation of hypochlorous

radical, an antibacterial agent produced by immune cells to kill invading pathogens (Turrens, 2003).

During the process of xenobiotic metabolism, which is the breakdown of different molecules in the liver such as drugs there is production of free radicals. This process normally involve different cytochrome P450 molecules using molecular oxygen to catalyze their reactions hence, in the process reactive oxygen species are produced. The amount of reactive oxygen species produced may vary depending on the compound being degraded and the type of cytochrome P450 molecule involved (Wu and Cederbaum, 2003). The following is an example of a reaction catalyzed by cytochrome P450 in the breakdown of a drug (Quinone) (Bagchi and Puri, 1998).

$$NADPH + 2quinone \xrightarrow{\text{cytochrome P450 reductase}} NADP^{+} + 2semiquinone^{-} + H^{+}$$

Inflammation is the process by which the body's immune system responds to a stimuli or foreign particle (Bala and Haldar, 2013). During this process different immune cells such as neutrophils and macrophages are activated to undergo oxidative burst, which is a series of reactions that a phagocytic cell goes through when exposed to a foreign particle leading to production of free radicals such as superoxide and nitric oxide that aid in destruction of the foreign particle or organism. During oxidative burst free radicals are produced in large amounts, hence there is a possibility that there will be excess free radicals that will create an imbalance unless neutralized by antioxidants (Valko *et al*., 2007; Bala and Haldar, 2013).

2.3.2 Exogenous sources

Diets rich in high levels of calories such as sugars and refined carbohydrates increases the level of sugars in the body (Mohammed *et al*., 2015). This means that the mitochondria has to work more so as to break down the sugars. The levels of free radicals such superoxide dismutase will increase due increased mitochondrial respiration. However, diets rich in antioxidants such as vegetables and fruits help the body in neutralizing different oxidants in the body. This means that the levels of free radicals will be high where diets lack or contain small amounts of antioxidants (Mohammed *et al*., 2015). High levels of fat in diets leads to increased levels of hydrogen peroxide. This occurs when fatty acids are degraded by peroxisomes into hydrogen peroxide producing heat in the process (Elochukwu, 2015).

Ionizing radiations such as those from x-rays, cellphones and hair dryers contain particulate matter such as electrons and photons. These particles release their energy to different cell components through process such as oxidation and reduction, which involves transfer of electrons. In the process free radicals are formed (Rahal *et al*., 2014). Radiations may also cause ionization of water found in cells leading to formation of hydroxyl radical and hydrogen atoms (Valko *et al*., 2004). Oxygen can combine with the hydrogen radical producing hydroperoxyl, a very strong and potent oxidant. Radiation effects are usually higher in oxygenated cells than those deficient of oxygen, due to presence of oxygen that initiates a number of reactions that lead to free radical formation (Rahal *et al*., 2014).

Different pathogenic infections increase the level of free radicals in the body such bacterial infections. Presence of invading bacteria in the body leads to activation of the white blood cells (neutrophils) to produce oxygen radicals which aid in killing the invading bacteria. In such situations there is increased levels of oxygen radicals which are harmful to the body. Besides when produced in excess, they react with each other producing more active oxidants (Sharma and Clark, 1998).

Exposure to different pollutants in the environment such as air and water pollution leads to activation of cells of the immune system so as to fight the foreign particles. The cells themselves produce free radicals to eliminate the foreign particles and in the process there is an increased production of free radicals in the body. Foods that we use contain chemicals some of which were used as pesticides and fertilizers, which may contain free radicals or produce free radicals when ingested. These radicals either react with others producing more destructive radicals or can directly react with different cellular components leading to damage (Sharma and Clark, 1998; Mohammed *et al*., 2015).

Tobacco smoke contains a lot of oxidants that cause oxidative damage to the lungs during smoking. These oxidants deplete antioxidants in lungs thus leading to loss of balance between oxidants and antioxidants. Besides, smokers have high levels of neutrophils in their respiratory tract that fights foreign particles. The high levels of neutrophils can themselves lead to increased levels of free radicals through activation of processes such as inflammation and respiratory burst (Lobo *et al*., 2010).

When one is under stress there is increased production of stress hormone cortisol and catecholamine which themselves degenerate into free radicals, which can directly or indirectly lead to oxidative damage. Stress increases the body's need for energy, meaning that the processes of energy production will be activated, which themselves leads to production of free radicals as by-products. Insufficient or lack of sleep increases oxidation leading to increased levels of oxidants (Rahal *et al*., 2014).

2.4 Beneficial roles of free radicals

Signal transduction is process by which cells communicate which can be either intracellular or extracellular. Different free radicals help in this process, for example, nitric oxide (NO) is an intercellular messenger (neurotransmitter) for modulating blood flow, thrombosis, and neural activity also in mediating immune responses (El-Bahr, 2013).

Free radicals are also involved in modulating and regulation of different activities in signal transduction processes during transduction of intercellular information, such as NO controls vasodilation and neurotransmission through activation of soluble guanylated cyclase (Durackova, 2010). At low concentrations, superoxide or hydrogen peroxide positively affect cell proliferation and survival through regulation of signal transduction, but at high concentrations, they stimulate signal transduction pathways for cell apoptosis or necrosis (Durackova, 2010; Sivanandham, 2011).

In immune defense, the phagocytic cells such as macrophages, monocytes and neutrophils produce free radicals that help in killing the invading pathogens. Individuals suffering from granulomatous disease, have defective membrane-bound NADPH oxidase system, hence they are not able to produce superoxide radical resulting in prolonged infection (Pham- Huy *et al*., 2008). Other functions of superoxide radical include cell growth regulation and in attacking various invading pathogens that induce inflammation. Macrophages produce hydrogen peroxide and use it in destroying invading bacteria and other foreign pathogens (El-Bahr, 2013).

2.5 Harmful effects of free radicals

At high levels free radicals cause oxidative damage to different molecules, cells and tissues. The main cell components that are affected are the lipid, proteins and DNA (Durackova, 2010) The hydroxyl radical is known to react with all components of the DNA molecule, proteins and lipids which leads to modification of these molecules which will thus affect their normal physiological function (Pham-Huy *et al*., 2008; Nimse and Pal, 2015).

Free radicals cause oxidative degradation of lipids (lipid peroxidation). Which is the process in which free radicals take electrons from lipids in cell membranes (mainly the polyunsaturated fatty acids PUFAs) resulting in cell damage (Birben *et al*., 2012).

This disrupts the membrane lipid bilayer arrangement, altering activity of membrane-bound receptors and enzymes and increase tissue permeability, hence loss of function

such as ability to transport oxygen, nutrients and water to the cells (Repetto *et al*., 2012). During this process of lipid peroxidation a number of different toxic by-products are formed which affects different sites far from the area of generation (Devasagayam *et al*., 2004). The different products of lipid peroxidation such as malondialdehyde (MDA), isoprostanes, are used as biomarkers of oxidative stress in tissues. Malondialdehyde a carbonyl, is both mutagenic and carcinogenic (Zegarac, 2015).

Protein molecules are highly susceptible to oxidative damage. Damage to proteins can be induced either directly by ROS or indirectly by reaction of secondary by-products produced during oxidative stress (Gutowski and Kowalczyk, 2013). Free radicals such as hydroxyl radical oxidize proteins leading to protein fragmentation of peptide chains, cross-linking of proteins, alteration of the electric charge of proteins and oxidation of specific amino acids leading to alterations of signal transduction mechanisms, transport systems, or enzyme activities and increased susceptibility to proteolysis by degradation by specific proteases (Sivanandham, 2011).

DNA is the genetic material that regulates growth and development. Different free radicals can attack and modify DNA through degradation of bases, purine, pyrimidine bases, single or double DNA breaks, gene sequence amplification and mutations (Valko *et al*., 2007). The radicals that cause severe damage include, nitric oxide which inactivates the DNA repair enzyme formamidopyrimidine-DNA glycolyase. Hypochlorous radical as well may chlorinate the DNA purines. Hydroxyl radical can

also attack DNA by oxidizing guanine and thymine to 8-hydroxy- 2-deoxyguanosine and thymine gylcol respectively (Sivanandham, 2011).

Transcription factors' binding sites are susceptible to free radicals attack which may lead to modification of transcription factors hence changing the expression of related genes (Birben *et al*., 2012). The mitochondria DNA is susceptible to free radical attack because of lack of protective protein enzymes and the high levels of free radicals generated in the mitochondria during electron transport. Free radical damage to mitochondria DNA is taught to be a contributing factor to the process of aging (El-Bahr, 2013). These mutations if not repaired during normal cell division, mutant/abnormal cells will arise, which are an indication of cancer development (Valko *et al*., 2007).

Free radical damage to different cell components induces oxidative stress. Oxidative stress is the contributing factor in the pathogenesis of different disease conditions such as arthritis, heart diseases among others. The various damages that free radicals cause to different cell components and disruption of cell functions accumulate with age. This together with the different diseases accelerate the aging process (Pham- Huy *et al*., 2008; Kumar, 2011).

2.6 Oxidative stress related diseases

2.6.1 Cardiovascular diseases

Cardiovascular diseases such as atherosclerosis, heart disease and hypertension are among main cause of death in the world. Oxidative damage due to high levels of free radicals is taught to be a contributing factor (Bagchi and Puri, 1998).

Atherosclerosis is associated with plaque formation on the walls of blood vessels (Singh *et al*., 2015). Lipid peroxidation mainly affects polyunsaturated fatty acids which are low density lipoproteins, composed of lipids and proteins. Free radicals react with the lipid component forming oxidized LDL which initiates inflammatory reaction (Lobo *et al*., 2010). These oxidized LDL are taken up by white blood cells such as macrophages, which collect in the artery wall leading to foam cell formation and atherosclerotic plaques. The oxidized LDL are chemotactic, they attract other immune cells such as monocytes to the same point. The monocytes later change to macrophages that take up more oxidized LDL further forming more plaques (Mimic-Oka *et al*., 1999). These macrophages attract other immune cells to the same point causing a growth inside the artery which blocks or slows flow of blood to the heart (Lobo *et al*., 2010).

2.6.2 Cancer

This is the uncontrolled growth of cells of different organs in the body, leading to the formation of abnormal cells (cancerous cells). A cancerous cell grows continuously without limitation due to different factors which may be endogenous or exogenous (Sharma, 2014). Oxidative damage to DNA molecules such as DNA repair enzymes, whose function is to regulate the normal cell function, growth and also to repair the

damaged tissues, is a contributing factor to development of cancer. The immune system usually fights back by destroying the abnormal cells before they grow and divide (Lobo *et al*., 2010).

Cancer cells usually divide more meaning that they have higher metabolic rate activities that will produce free radicals. The level of free radicals will be higher in cancerous cells than normal cells, which will deprive the cells of the available antioxidants. This will increase the damage of free radicals in these cells (Phaniendra *et al*., 2015).

Immune cell function can be affected by free radicals therefore reducing immune cell responses, which can allow the abnormal cells to continue growing uncontrollably. Reduced or loss of immune cells responses to abnormal cell growth leads to development of different mutations. If these mutations are carried to next cells, there is development of abnormal cells with abnormal growth and that carry out different functions away from the normal. Gene silencing or activation of genes that modulate cell growth and programmed cell death, also leads to loss or change of function of the affected cells (Shinde *et al*., 2012).

High levels of free radicals also lead to inactivation of tumor suppressor genes and activation of different oncogenes, which will lead to proliferation of cancer cells (Shinde *et al*., 2012). Lipid peroxidation as well lead to formation of different products such Malondialdehyde a carbonyl that affects different cell components away from the

site of production is taught to be mutagenc and carcinogenic, hence can be contributing factor to development of cancer (Zegarac, 2015).

2.6.3 Arthritis

This is an autoimmune disease that is characterized by inflammation at the joints and different tissues surrounding the joints with infiltration of macrophages and activated T cells. It is accompanied by pain at the joint and reduced function of the affected joint (Pham-Huy *et al*., 2008). Reactive oxygen species are produced as defense mechanism at the site of inflammation attracting white blood cells. The inflammation infiltrates accumulate at the synovial membrane causing pressure and pain. In chronic inflammation the activated white blood cells can undergo respiratory burst producing more free radicals, which can cause more damage to the joints. The levels of antioxidants in the sites affected reduces due to the increased free radicals creating an imbalance, thus inducing oxidative damage to the cells (Rahman *et al*., 2012).

2.6.4 Cataracts

Oxidation of the lens proteins in the eye leads to production of free radicals. These radicals are acted upon by the antioxidant defense system in the eye, but in excess they deprive these antioxidants leading to an imbalance that initiates oxidative damage. Also accumulation of oxidized lens components is thought to be the contributing factor. This leads to formation of different products that block the passage of light through the lens. Oxidative damage to gap junctions in the lens alters intercellular communication. This

affects normal functioning which may contribute to cataract formation (Pham-Huy *et al*., 2008; Rahman *et al*., 2012).

2.6.5 Ageing

Ageing can be defined as the continuous decrease in the physiological functions of an organism (Valko *et al*., 2007). This can arise from different effects caused by free radicals. Damage to different molecules such as proteins (DNA repair enzymes) leading to loss or reduced function, lipid peroxidation that affects membrane fluidity hence decreased function. DNA damage as well leads to formation of abnormal cells that have lost the normal physiological functions (Shinde *et al*., 2012).

Free radicals interfere with genes that regulate cell growth and programmed cell death (apoptosis) leading to loss of equilibrium between cell proliferation and cell death. This may lead to increased cell death, a key feature in aging. These damages to different cellular components induces ageing. In some situations accumulation of products of damage and intense damages may lead to cell death (Valko *et al*., 2006). Reactive oxygen species also may activate a number of chain reactions that lead to early apoptosis. These free radicals damages, reduced antioxidant defense system due to free radical effects encourages ageing (Agarwal and Allamaneni, 2004).

2.6.6 Neurological diseases

These are conditions that affect the central nervous system. They involve diseases like Parkinson's disease, Alzheimer disease among others. Parkinson's disease is a

neurodegenerative condition that arises due to loss of neurons in the substantia nigra pars compacta located in the midbrain. These neurons contain dopamine an important neurotransmitter used for communication between these neurons and other cells in the brain (Hwang, 2013). This condition is characterized with postural imbalance, resting rigidity and muscular rigidity among others (Nikolova, 2012; Hwang, 2013).

Oxidative stress due to free radicals is thought to be one of the contributing factors that lead to Parkinson's disease (Kumar *et al*., 2012). Neurons rely on aerobic respiration that occurs during oxidative phosphorylation in the mitochondria. Any mitochondria alterations or dysfunction leads to low levels of ATP production, incomplete reduction of oxygen leading to production of radicals such as superoxide radical, hydroxyl and peroxynitrate. Besides, this induces mitochondrial DNA damage. Accumulation of mitochondrial DNA damage initiates aging and cell death (Kumar *et al*., 2012; Nikolova, 2012; Hwang, 2013).

Microglia are immune cells that occur in the central nervous system, they are involved in elimination of foreign particles from the brain (Kumar *et al*., 2012). When activated the microglia produce free radicals that aid them in elimination of invading or foreign particle. These radicals in excess cause oxidative damage to different cells in the central nervous system. They also deprive the system the antioxidants that offer defense (Koutsilieri *et al*., 2002).

The neurotransmitter dopamine is susceptible to auto-oxidation when it is excess in the cytosol leading to formation of products such as dopamine-quinone capable of covalently modifying different cell molecules. In the process of auto-oxidation different free radicals such superoxide and hydrogen peroxide are produced (Nikolova, 2012; Koutsilieri *et al*., 2002).

2.6.7 Diabetes mellitus

This is a metabolic disorder that occurs due to defects in insulin secretion or reduced insulin action. It is characterized by hyperglycemia, glycosuria and polyuria. There are two types of diabetes, type I (insulin-dependent) and type II (non-insulin dependent). Type I occurs due destruction of cells responsible for insulin production, the pancreatic ß-cells to while type II occurs due to reduced glucose uptake in muscles and adipose tissue leading to high levels of glucose in blood circulation (Jakus, 2000; Omotayo, 2012).

Exposure of cells to high levels of glucose leads to cell dysfunction and enzyme destruction including the antioxidant enzymes due to glucose toxicity (Singh *et al*., 2015). The high level of glucose activates activities such as oxidative phosphorylation, glucose auto-oxidation and lipogenesis among others. All these processes lead to free radical production. Due to reduced or loss of function of antioxidant enzymes, the radicals will accumulate and cause oxidative damage to different cells including the ß-cells thus affecting insulin secretion and function (Rahman *et al*., 2012; Singh *et al*., 2015).

Free radicals in right levels are involved in regulation of insulin signals, in high levels they negatively regulate insulin signaling which may later lead to insulin resistance a characteristic feature in diabetes type II (Asmat *et al*., 2015).

The pancreatic ß-cells are sensitive to free radicals because their natural antioxidant defense system are lower compared to other cells, hence they are susceptible to free radical attack that will lead to oxidative damage (Omotayo, 2012; Asmat *et al*., 2015).

2.7 Antioxidants

Antioxidants are the substances that protect cells from the damage caused by free radicals through interacting with free radicals and neutralizing them (Shinde *et al*., 2012). Antioxidants can protect the body or improve the body's response against different disease conditions. Antioxidants can act by different methods; by preventing chain reactions (chain breakers), inhibiting oxidation of different molecules by free radicals, as metals chelators , as reducing agents and by inhibiting enzymes that lead to production of free radicals (Carocho and Ferreira, 2013). For a substance to act as an antioxidant it has to be effective at low concentration, non-toxic at its working concentration, neutral, stable and should not be very reactive. Antioxidants can be classified as synthetic or natural antioxidants (Hamid *et al*., 2010; Sarma *et al*., 2010).

2.7.1 Natural antioxidants

These are antioxidants found in the body or obtained from diets. They act as chain breakers by reacting with lipid radicals and converting them into more stable products. They are mainly phenolic in structure (Hamid *et al*., 2010).

Natural antioxidants are also divided into enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants involves different enzymes in the body that offer antioxidant activity such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GRx) (Stanczyk *et al*., 2005).

Non-enymatic antioxidants include those from diets such as ascorbic acid (vitamin C), vitamin E and carotenoids among others. Also metal binding proteins, such as ferritin and minerals such as copper, selenium, Zinc and Manganese that catalyze different reactions fall in this group (Hamid *et al*., 2010).

2.7.1.1 Superoxide dismutase (SOD)

This is one of the major known enzymatic antioxidants that catalyzes the transformation of superoxide radical to hydrogen peroxide (Kumar, 2011).

$$O_2^{\cdot} \longrightarrow H_2O_2 + H_2O$$

There are different types of SOD depending on the metals they require as co-factors to function. Type one that binds Copper and zinc, type two that binds to either iron or manganese and type three that binds nickel. In the cytosol the co-factors for superoxide

dismutase are zinc and copper while in the mitochondria it is manganese and zinc (Lobo *et al*., 2010; Kabel, 2014).

Superoxide dismutase is an inducible enzyme in that certain conditions will result in increase or decrease of its concentration (increasing or decreasing its activity) such as in the case where there are high levels of oxygen in the cells there is an increase in the concentration of SOD (McDowell *et al*., 2007).

2.7.1.2 Catalase

It is an iron containing enzyme that occurs in the peroxisomes of aerobic cells but small amounts are also found in the heart mitochondria of mammalian cells. It catalyzes the conversion of hydrogen peroxide to water and molecular oxygen as shown in the equation below. Cells that have reduced or lack catalase activity are sensitive to the effects of hydrogen peroxide (Wu and Cederbaum, 2003; Kabel, 2014).

$$H_2O_2 \longrightarrow H_2O \text{ and } O_2$$

2.7.1.3 Glutathione peroxidase

There are two forms of this enzyme; the selenium-independent (glutathione-S-transferase, GST) form and the selenium-dependent (GPx) which differ on the number of subunits and how it binds to selenium. It occurs in tissues where it is coupled to cellular membranes. In presence of reduced glutathione (GSH), (GPx) catalyzes the conversion of hydrogen peroxide to water and has high affinity for hydrogen peroxide than SOD (Stanczyk *et al*., 2005).

$$2GSH + H_2O_2 \xrightarrow{GPx} GSSG + 2H_2O$$

Glutathione-S- transferase, helps in removing certain drugs, chemicals and reactive molecules from the cells. Glutathione peroxidase reduces lipid peroxides (ROOH) formed during lipid peroxidation, which is the oxidation of polyunsaturated fatty acids, to a stable hydroxyl fatty acid (ROH). Glutathione peroxidase works with phospholipase to convert phospholipids hydroperoxides (PL-OOH) to phospholipids hydroxide (PL-OH) (Kumar, 2011). It works together with glutathione reductase that helps to regenerate reduced glutathione from its oxidized form. Regeneration of reduced glutathione requires reduced nicotinamide adenosine dinucleotide phosphate (NADPH) (Wu and Cederbaum, 2003).

2.7.1.4 Glutathione

Glutathione is synthesized from three amino acids which are glutamate, glycine and cysteine, hence it is not required in the diet. It has a thiol group in its cysteine moiety, which act as a reducing agent hence its antioxidant activity. It helps in xenobiotic metabolism (source of ROS) where it is utilized for conjugation and it quenches ROS and lipid peroxides (Percival, 1998; Lobo *et al*., 2010). Also it acts as a co-factor of different detoxifying enzymes such as glutathione peroxidase and helps in regeneration of vitamins A and E that function as antioxidants (Valko *et al*., 2007).

2.7.1.5 Vitamin E (tocopherol)

It is lipid soluble and functions as a membrane bound antioxidant in breaking chain reactions during lipid peroxidation, by reacting with lipid peroxyl radicals and forming

stable radicals in cell membrane and lipid molecules. It also protects the thymus and circulating white blood cells from damage by free radicals. It occurs in avocados, nuts, seeds and whole grains (Devasagayam *et al*., 2004; McDowell *et al*., 2007; Birben *et al*., 2012).

2.7.1.6 Vitamin C (ascorbic acid)

It is a water soluble antioxidant that occurs in the cytosol. It is a radical scavenger that protects membranes against oxidation. It also regenerates vitamin E in cell membranes and maintains low density lipoproteins (LDL) integrity. It acts as an antioxidant, anti-carcinogenic and as an immunomodulator. They occur in oranges, mangoes, broccoli and spinach (Knight, 2000; Valko *et al*., 2007).

2.7.1.7 Carotenoids

These are a group of highly liphophilic compounds due to possession of long unsaturated alkyl chains. Its members are carotenes (β-carotene and lycopene) and xanthophylls (lutein and zeaxanthin). Foods rich in carotenoids include carrots, spinach, mangoes and sweet potatoes (Eboh, 2014). Carotenoids protect the different cell membranes and lipid lipoproteins against the peroxy radical by reacting with it to form carbon-centered radical adducts that are stable. β-carotene helps in quenching of the singlet oxygen radical (Pham-Huy *et al*., 2008).

2.7.1.8 Phenols

Phenols (polyphenols) are one of the major plants' secondary metabolites with flavonoids and phenolic acids as the major classes in this group. They taught to have

antioxidant activity through different mechanisms such as hydrogen donation and radical scavenging (Eboh, 2014).

Flavonoids are a group of polyphenolic compounds that occur mostly as glucosylated derivatives in plants. Members of this group include flavanols, flavanones, flavones, isoflavones, catechins and anthocyanin. Plants differ in the combination of different derivatives of flavonoids hence the difference in their antioxidant activity (Pham-Huy *et al*., 2008).

Flavonoids show different activities such as antitumoural by preventing DNA damage caused by hydroxyl radicals, anti-inflammatory, and anti-hepatotoxic. They also inhibit a number of enzymes such as xanthine oxidase, lipogenase and NADH oxidase. They can obtained from diets such as green tea, berries, soyabeans, broccoli, fruits, vegetables and onions (Singh *et al*., 2004).

2.7.1.9 Minerals

They also help in provision of antioxidant activity though not directly. Selenium is a trace element and a critical co-factor for different enzymes in the body such as the enzyme glutathione peroxidase which catalyzes the oxidation of hydrogen peroxide to water and superoxide dismutase that catalyzes the conversion of superoxide radical to hydrogen peroxide (Stanczyk *et al*., 2005).

Zinc and copper act as co-factors of the enzyme superoxide dismutase. Zinc prevents production of hydroxyl radicals through competition with copper thus preventing its binding to the cell membrane. Copper can also act as an antioxidant protein (in celuroplasmin or water phase), that helps to prevent copper and iron from participating in oxidation reactions (Carocho and Ferreira, 2013).

2.8 Secondary or synthetic antioxidants

These are antioxidants that have been developed for different functions in food industry such as to prolong product shelf life and to enhance stability of therapeutic agents susceptible to oxidation in pharmaceutical industries. They are also used in comparison with natural antioxidants during different studies (Shebis *et al*., 2013). They capture free radicals and stop chain reactions. Some these compounds include; butylated hydroxyl anisole (BHA), butylated hydroxyrotoluene (BHT), propyl gallate (PG) and nordihydro guaretic acid (NDGA) which are mainly used as food antioxidants while tertiary butyl hydroquinone (TBHQ) is used to stabilize and preserve animal food products so as to maintain their colour, flavor and nutritive value (Carocho and Ferreira, 2013).

2.9 Management of oxidative stress

2.9.1 Conventional management

There are several synthetic antioxidants classified as phenolic compounds that capture free radicals preventing them from initiating a number of chain reactions. These include Butylated hydroxyl anisole (BHA), Butylated hydroxyrotoluene (BHT), Propyl gallate (PG), N-acetylcysteine, alpha-lipoic acid and metal chelating agent (MCA), Tertiary butyl hydroquinone (TBHQ) and Nordihydro-guaretic acid (NDGA) (Hamid *et al*.,

2010). These have been analyzed to reduce free radical production to amounts that are not harmful to the body. However, these antioxidants are thought to be carcinogenic and can cause liver damage. Also they are expensive, costly and have different side effects. (Moulisha *et al*., 2010).

2.9.2 Alternative and complementary management

Oxidative stress can be managed by taking of foods rich in antioxidants (Wilhelmina, 2005), which protect the body cells from the damage caused by free radicals by donating electrons to the radicals without destabilizing themselves thus making them stable. This helps prevent the damage that these radicals could have caused (Hamid *et al*., 2010).

Diets rich in foods such as fruits and vegetables, nuts and organic green tea, provide a source of mineral antioxidants (Selenium, Copper, Zinc and iron) and vitamins needed for most metabolic processes (vitamin B, C and E). Examples of antioxidants from dietary sources include vitamin A, C, E, lycopene, beta-carotene, resveratrol and others (Hamid *et al.*, 2010; Asif, 2015). Reduction of stress and having sufficient sleep helps to reduce the risk of increase in oxidation activities that will produce more oxidants (Anu *et al*., 2014).

Use of medicinal plants, which contain different phytochemicals such as flavonoids and phenols that contribute to high antioxidant activity (Lacine *et al*., 2013; Kuang-hung *et al*., 2014). These will react with lipid radicals making them more stable, thus reducing

their reactivity. Examples of herbs include rosemary, ginger, milk thistle and green tea polyphenols (Anu *et al*., 2014). Studies that have been carried out on *Strychnos henningsii* indicates that, it has good antioxidant activity, in East Africa its bark has been documented to be used in wound healing and as an antiseptic for the mouth. *S. henningsii* has been found to contain alkaloids, brucine, bitter glycosides and curarine. (Oyedemi *et al.*, 2010).

Advantages of the complimentary means are, they are easier to carry out as opposed to conventional means that are tedious and need a lot of care and time. It involves use of products that are naturally available hence not very expensive compared to the synthetic antioxidants. They are also low in toxicity and easily available (Asif, 2015). And have high acceptability and biomedical benefits (Haile *et al*., 2008).

Different herbal medicines have been used in the management of oxidative stress related conditions. Through research, the antioxidant activity of most herbal medicines has been attributed to active components such as phenolic acids, tannins and flavonoids (Ebrahimzadeh *et al*., 2010). *Acacia catechu* (L.F.) wild is a herb in the Mimosaceae family. Traditionally, it has been used to manage conditions such as diarrhea, dysentery, skin eruptions and cough among other uses. Its antioxidant activity has been linked to its active components catechin and quercetrine. Catechin provides antioxidant activity by enhancing the activities of catalase and superoxide dismutase enzymatic antioxidants (Sati *et al*., 2010).

Saraca indica bark is among the most widely used herbal medicines in India. Traditionally it is used to manage skin infections, and problems of the genital-urinary tract and central nervous system (Panchawat and Sisodia, 2010). According to studies by Panchawat and Sisodia (2010), it has been shown to possess antioxidant activity attributed to its high levels of phenolic compounds which inhibit oxidation of different cellular components such lipids and proteins by donation of an hydrogen atom to the radical molecule (Singh *et al*., 2013).

Melanthera scandens (schum.and Thonn) is an herb widely distributed in the tropical Africa. Traditionally, a decoction of its leaves has been used to manage cough and sore throat. In Nigeria, malcerated or decocted leaves are applied to wound and cuts to enhance healing. Through studies it has been found to possess antioxidant activity linked to its active components phenolic compounds (Adesegun *et al*., 2010).

2.10 Plants used in the study

2.10.1 *Clutia abyssinica*

2.10.1.1 Description and distribution

Clutia abyssinica Jaub. and Spach. is a shrub that belongs to the Euphorbiaceae family. It is commonly known as lightning bush or smooth fruited clutia. It is concisely of about 2m high with sparsely branched glabrous twigs. Leaves are alternate and simple with an obtuse apex that is narrowed at the base. It has pinnate leaves with 5-12 pairs of lateral veins. Stipules are absent. Three varieties have been described in *Clutia abyssinica* based on the differences in the hairness of the plant, the length of pedicel and the shape of the leaves (Schmelzer and Gurib-fakim, 2008). It occurs in high elevations and in dry

grassland. It is native in Tanzania, Kenya, South Africa, Angola, Ethiopia and Zimbambwe (Mergia *et al*., 2014).

Figure 2.10.1 Shows a photo of *clutia abyssinica* (Matu, 2008)

2.10.1.2 Medicinal uses

A root extract of is used to treat intestinal worms, influenza, colds and fever, and as a remedy for indigestion. The sap from leafy twigs is drunk to treat chest pains, side pains and shortness of breath. An infusion made from leafy twigs or leaves is drunk or eaten as ash to treat skin problems, elephantiasis, diarrhoea and tachycardia (Mergia *et al*., 2014). Roots are ground and applied as an enema to treat gonorrhoea. Boiled roots are made into soup which is taken as a remedy for enlarged spleen, also to treat kidney problems, headache, stomachache and malaria. In Rwanda a leaf extract is drunk to induce labour during childbirth. In Tanzania and Kenya the roots are boiled with food to

add flavour (Schmelzer and Gurib-fakim, 2008). An oral decoction made from leaves is used in treatment of hepatitis, sores and as an anti-inflammatory. Root extracts are also used in the management of habitual miscarriages (Iwu, 2010).

2.10.2 *Maytenus obscura*

2.10.2.1 Description and distribution

Maytenus obscura (A. Rich.) Cufod belongs to the genus *Maytenus* and family of *Celastraceae.* It is commonly known as black seed oil, climbing staff tree and intellect tree (Neha and Shashi, 2012). It is a shrub that grows up to 10m, with ling slender branches that are reddish brown in colour. Its leaves are simple, broad and ovate with toothed margins. The stem grows up to 23 cm in diameter and it has lenticels (Deodhar and Shinde, 2015).

Maytenus obscura is native in India, but is also grows in Australia, China, Taiwan, Cambodia, Indonesia, Laos, Malaysia, Myanmar, Nepal, Sri-Lank, Africa, Brazil, Paraguay, Uruguay and Argentina and in southern regions of Saudi Arabia (Neha and Shashi, 2012).

Many plants from this genus *Maytenus* are rich in triterpenes, diterpenes, sesquiterpene alkaloids and spermidine alkaloids. Flavonoids, phenolic glucoside agarofurans, and flavonoid glycosides occur in some species of the genus *Maytenus* (Mohamed and Perwez, 2014).

Figure 2.10.2 Shows a photo of *Maytenus obscura* courtesy of Christian P. (African plant database).

2.10.2.2 Medicinal uses

Species belonging to this genus are becoming attractive to researchers due to their different phytochemical constituents that make it to be widely used as traditional medicine. It is used as an antiseptic, antiasthmatic, antitumor, antiulcer and in fertility-regulation (Maina *et al.,* 2015). Its root decoction is used as a laxative for cleaning the digestive system, juice from the leaves are given in opium poisoning as a de-addiction aid. It is also used in treatment of rheumatism, gout, leucoderma, paralysis, asthma and as a stimulant in nerve tonic (Deodhar and Shinde, 2015).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Collection and preparation of plant materials

Fresh leaves of *Clutia abyssinica* were collected in September, 2015 from Uasin Gishu County of Kenya, while fresh *Maytenus obscura* leaves were collected in October, 2015 from Makunguru village, Mbeere North Subcounty, Embu County of Kenya. The plant materials were collected from their geo-ecological habitats based on their traditional use as was obtained from traditional medical practitioners from the areas. They were taxonomically identified by an acknowledged taxonomist at the department of plant and microbial sciences, Kenyatta University, Kenya. Voucher numbers 001/2015 and 002/2015 were assigned to *Clutia abyssinica* and *Maytenus obscura* respectively. The voucher specimens were deposited in Kenyatta university herbarium for future reference.

The plant samples were harvested while still fresh, cut into small portions and dried under shade until dry. They were then ground to powder using a mechanical grinder and stored in closed, dry plastic bags at room temperature away from direct sunlight awaiting extraction.

3.2 Extraction procedure

Four hundred grams of dried powder of each plant was soaked for 24 hours in a one litre of dichloromethane and methanol mixture in the ratio of 1:1. The mixture was then decanted into a clean dry conical flask and filtered with a filter paper into another

conical flask. The filtrate was then vacuum dried using a rotary evaporator at a temperature of 64^{o}C to yield a solid residue and stored at 4^{o}C awaiting use in bioassays.

3.3 Determination of DPPH radical scavenging activity

This was done according to the method described by Ayoola *et al.* (2008) with some modifications. Where concentrations of the extracts (0.0625, 0.125, 0.25, 0.5 and 1 mg/ml) were prepared in methanol. The same concentrations were also prepared for ascorbic acid, which was used as a standard. The extract (1 ml) was placed in a test tube and 1 ml of methanol added followed by 0.5ml of 0.1 M DPPH. The mixture was shaken vigorously and left to stand for 5 minutes. A blank solution was prepared containing the same amount of methanol and DPPH. The absorbance of the resulting solution was measured at 517 nm using a spectrophotometer. The experiment was repeated three times. The DPPH radical scavenging activity was estimated based on the percentage of DPPH radical scavenged using the following equation:

$$\text{DPPH scavenging activity } (\%) = \frac{\text{Ac} - \text{As}}{\text{Ac}} \times 100$$

Where: A_S = absorbance of the sample

A_C = absorbance of the control

A curve was generated based on the percentage of DPPH radical scavenging activity using linear regression and it was used to calculate the half maximal inhibitory concentration (IC_{50}), which indicated the concentration of the extract at which 50% of the radical is scavenged.

3.4 Determination of total reducing power

The total reducing power of the extracts was determined using the method described by Oyaizu (1986) with some modifications. Five different concentrations of the extracts (0.2, 0.4 0.6 0.8 and 1 mg/ml in distilled water) were prepared, mixed with 2 ml phosphate buffer (0.2 M, pH 6.6) and 2 ml of 1% Potassium Ferricyanide [$K_3Fe(CN)_6$]. The mixture was then incubated at 50°C for 20 minutes, after which 2 ml of 10% Trichloroacetic acid (TCA) was added to the mixture. The mixture was then centrifuged for 10 minutes at 300 rpm. The upper layer of solution (2 ml) was pipetted into a clean test tude, mixed with 2 ml of distilled water following which 0.5 ml of $FeCl_3$ (0.1%) was added and the reaction was allowed to stand for 10 minutes. The same procedure was done for ascorbic acid, which was used as a standard. The experiment was done in triplicates and absorbance was then measured at 700 nm using a spectrophotometer.

3.5 Determination of hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging activities of the extracts was determined according to the method described by Ruch *et al.* (1989). The plant extracts were prepared in distilled water at five different concentrations (0.00625, 0.0125, 0.025, 0.05, and 0.1 mg/ml), then mixed with 1 ml of 40 mM H_2O_2 solution prepared in phosphate buffer (0.1 M, PH 7.4) and left to stand for 10 minutes at room temperature. This procedure was also done for ascorbic acid, which was used as a standard. The experiment was done in triplicates and the absorbance of the solution was taken at 560 nm against blank solution containing phosphate buffer without H_2O_2. The hydrogen peroxide scavenging activity of the extracts was calculated using the following equation:

$$\text{Hydrogen peroxide scavenging activity } (\%) = \frac{\text{Ac} - \text{As}}{\text{Ac}} \times 100$$

Where: A_S = absorbance of the sample

A_C = absorbance of the control

3.6. Determination of total phenolic contents

The total phenolic contents of the extracts were done according to Folin-Ciocalteu method with some modifications Duarte-Almeida *et al*. (2006). The extract (1 ml) was mixed with 2 ml of Folin-Ciocalteu reagent which was prepared by dilution with distilled water in a ratio of 1:10 v/v, after which 1 ml of 20% Sodium Carbonate (Na_2CO_3) was added. The mixture was shaken for 20 seconds and incubated at 40^0C for 30 minutes. Absorbance was measured at 765 nm. Gallic acid was used for the generation of the standard curve. This was done by measuring the absorbance of gallic acid at different concentrations mixed with the different reagents as described above. These were then used to generate a curve using linear regression. The total phenolic content was expressed as mg of gallic acid equivalents (GAE) per gram (g) of the dried sample.

3.7 Qualitative phytochemical screening

Qualitative phytochemical screening was carried out to screen for the presence of secondary metabolites such as alkaloids, flavonoids, sterols and steroids, saponins and tannins in the plant extracts as described by Harbone (1998) and Kotake (2000). These secondary metabolites are associated with antioxidant activities.

3.7.1 Phenolics

To 2 ml aqueous methanol extract, 1 ml of ferric chloride solution was added. The formation of blue to green colour indicated the presence of phenolics.

3.7.2 Alkaloids

To test for alkaloids, 1 ml of hydrochloric acid (HCL), was added to 5 ml aqueous methanol extract to acidify it. The acidic solution was then heated in a water bath for some few minutes and treated with few drops of Dragendruff's reagent. The formation of an orange reddish brown precipitate showed the presence of alkaloids.

3.7.3 Tannins

To 1 ml of the aqueous methanol extract, 1 ml of freshly prepared 10% potassium hydroxide (KOH) was added. A dirty white precipitate showed the presence of tannins.

3.7.4 Steroids

To 1 ml of aqueous methanol extract in a test tube, 5 drops of concentrated sulphuric acid was added (Salkowki test). Red colouration indicated presence of steroids.

3.7.5 Phlobatanins

A few drops (3-4) of 1% HCL (hydrochloric acid) were added to 1 ml of aqueous methanol extract in a test tube. A Red precipitate showed presence of phlobatanins.

3.7.6 Saponins

To 0.5g of extract, 5ml of distilled water was added. Frothing test was done in which 2 ml of the extract in test tube was shaken vigorously for 2 minutes. Frothing observed, indicating presence of saponins.

3.7.7 Cardiac glycosides

To 0.5g of the extract, 2ml of glacial acetic acid containing four drops of 10% ferric chloride ($FeCl_3$) solution was added and under-layered with 1ml of concentrated sulphuric acid. The formation of a violet, greenish or a brown ring at the interphase indicated the presence of cardiac glycosides.

3.7.8 Terpenoids

To 0.5g of the extracts, 1ml of ethyl acetate was added and then mixed into 2ml of chloroform, 3ml of concentrated sulphuric acid was carefully added alongside. Formation of a reddish brown coloration indicated presence of terpenoids.

3.7.9 Flavonoids

To 3 ml of the plant extract in a test tube, 1 ml of 10% sodium hydroxide (NaOH) was added. Yellow colouration indicated presence of flavonoids.

3.8 Data management and statistical analysis

Data obtained from DPPH scavenging activities, hydrogen peroxide scavenging activities and reducing powers obtained was entered into broad spreadsheets and expressed by descriptive statistics as mean ± standard error of the mean (SEM). The

data was further analyzed by one way- analysis of variance (ANOVA) followed by Tukey's post *hoc* test. Data from total phenols was analyzed by unpaired t-test. A value of $P< 0.05$ was considered statistically significant. To compute the IC_{50} values, linear regression analysis was used.

CHAPTER FOUR

RESULTS

4.1 DPPH scavenging activities

The DCM: MeOH leaf extracts of *C. abyssinica* and *M. obscura* showed a remarkable *in vitro* DPPH scavenging activity in a dose dependent manner (Table 4.1). At concentrations of 0.0625, 0.125, 0.25 and 1 mg/ml, the DPPH scavenging activity of the DCM: MeOH leaf extracts of *M. obscura* was significantly higher than that of *C. abyssinica* leaf extracts ($p<0.05$; Table 4.1). However, at the extract concentration of 0.5 mg/ml, *M. obscura* was as effective as *C. abyssinica* ($p>0.05$; Table 4.1).

At all the tested concentrations, the *in vitro* DPPH radical scavenging activity of the standard (ascorbic acid) was significantly higher than that of the two studied plant extracts ($p<0.05$; Table 4.1). The IC_{50} values for the DCM: MeOH extracts of *C. abyssinica, M. obscura* and the standard were 0.087, 0.065 and 0.044 respectively (Table 4.1).

Table 4.1: *In vitro* DPPH scavenging activities of *C. abyssinica* and *M. obscura* leaf extracts

	DPPH scavenging activity (% inhibition)					
Extract concentration (mg/ml)	**0.0625**	**0.125**	**0.25**	**0.5**	**1**	**IC_{50}**
Ascorbic acid	50.52±0.24[a]	66.94±0.36[a]	81.01±0.23[a]	83.82±0.48[a]	91.77±0.24[a]	0.044
DCM: MeOH extract of *C. abyssinica*	46.91±0.27[c]	54.31±0.37[c]	60.77±0.11[c]	71.32±0.25[b]	80.00±0.11[c]	0.087
DCM: MeOH extract of *M. obscura*	49.33±0.16[b]	58.90±0.27[b]	64.59±0.30[b]	72.45±0.22[b]	82.57±0.16[b]	0.065

Values expressed as Mean ± SEM of the experiment triplicates in each concentration. Values with the same superscript letters are not significantly different by one way ANOVA followed by Tukey's post hoc test ($p>0.05$)

4.2 Total ferric reducing power

The total ferric reducing powers of the DCM: MeOH leaf extracts of *C. abyssinica* and *M. obscura* were demonstrated by a dose dependent increase in absorbance at 700 nm (Table 4.2). The DCM: MeOH leaf extracts of *M. Obscura* largely produced significantly higher total ferric reducing power than *C. abyssinica* ($p<0.05$; Table 4.2). However, at extract concentration of 0.6 mg/ml, both plant extracts were equally effective ($p>0.05$; Table 4.2).

It was also observed that the standard (ascorbic acid) exhibited significantly higher total ferric reducing power than both plant extracts at all the tested concentrations ($p<0.05$; Table 4.2).

Table 4.2: Total ferric reducing power of *C. abyssinica* and *M. obscura* leaf extracts

	Absorbance at 700 nm				
Concentration (mg/ml)	**0.2**	**0.4**	**0.6**	**0.8**	**1**
Ascorbic acid	0.2533±0.005[a]	0.4130±0.003[a]	0.5037±0.003[a]	0.6027±0.004[a]	0.6207±0.006[a]
DCM: MeOH extract of *C. abyssinica*	0.0547±0.003[c]	0.1067±0.002[c]	0.1623±0.005[b]	0.1713±0.004[c]	0.2177±0.010[c]
DCM: MeOH extract of *M. obscura*	0.0917±0.01[b]	0.1660±0.012[b]	0.2133±0.023[b]	0.2877±0.02[b]	0.3667±0.02[b]

Values expressed as Mean ± SEM of the experiment triplicates in each concentration. Valuses with the same superscript letters are not significantly different by one way ANOVA followed by Tukey's post hoc test ($p>0.05$)

4.3 Hydrogen peroxide scavenging activities

As table 4.3 shows, the DCM: MeOH leaf extracts of *C. abyssinica* and *M. obscura* exhibited remarkable hydrogen peroxide scavenging activities. Noticeably, the hydrogen peroxide scavenging effects of the two studied plant extracts decreased with increasing extracts concentration (Table 4.3).

At all the tested extract concentrations, the DCM: MeOH leaf extracts of *M. obscura* was significantly more effective than the DCM: MeOH leaf extracts of *C. abyssinica* ($p<0.05$; Table 4.3). However, the effects of the two plant extracts were significantly lower than that of the standard (ascorbic acid) at all the tested concentrations ($p<0.05$; Table 4.3). The IC_{50} values for the DCM: MeOH leaf extracts of *C. abyssinica*, *M. obscura* and ascorbic acid were 0.0350, 0.0446 and 0.1104 respectively (Table 4.3).

Table 4.3 Hydrogen peroxide scavenging activities of DCM: MeOH leaf extracts of *C. abyssinica* and *M. obscura*

	Hydrogen peroxide scavenging activity (%)					
Extract concentration (mg/ml)	**0.00625**	**0.0125**	**0.025**	**0.05**	**0.1**	**IC_{50}**
Ascorbic acid	90.18±0.28[a]	83.33±0.28[a]	73.35±0.09[a]	64.11±0.18[a]	56.58±0.16[a]	0.1104
DCM: MeOH extract of *C. abyssinica*	75.50±0.30[c]	68.03±0.27[c]	54.34±0.23[c]	42.01±0.33[c]	36.99±0.16[c]	0.0350
DCM: MeOH extract of *M. Obscura*	79.73±0.38[b]	70.69±0.18[b]	57.16±0.32[b]	46.03±0.41[b]	39.92±0.26[b]	0.0446

Values expressed as Mean ± SEM of the experiment triplicates in each concentration. Values with the same superscript letters are not significantly different by one way ANOVA followed by Tukey's post hoc test ($p>0.05$)

4.4 Total phenolic contents

Determination of the total phenolic contents of the DCM: MeOH leaf extracts of *M. obscura* and *C. abyssinica* showed that *M. obscura* contained total phenolic content of 78.38±1.03 expressed as mg of GAE/g (Table 4.4). On the other hand, it was observed that *C. abyssinica* had a total phenolic content of 19.82±0.597 mg of GAE/g (Table 4.4). The total phenolic content of *M. obscura* was significantly higher than that of *C. abyssinica* ($p<0.05$; Table 4.4).

Table 4.4: Total phenolic contents of DCM: MeOH leaf extracts of *C. abyssinica* and *M. obscura*

Sample	TPC (mg GAE/g)
Maytenus obscura	78.38 ± 1.03^{a}
Clutia abyssinica	19.82 ± 0.597^{b}

Values expressed as mean ± SEM of the experiment triplicates. Values with different superscript letters are significantly different by unpaired student t-test.

4.5 Qualitative phytochemical composition

The qualitative phytochemical screening of the DCM: MeOH leaf extracts of *M. obscura* and *C. abyssinica* showed the presence of alkaloids, cardiac glycosides, flavonoids, phenolics, saponin, steroids, tannins and terpenoids (Table 4.5). However, phlobatanins were absent in both plant extracts (Table 4.5).

Table 4.5: Qualitative phytochemical composition of DCM: MeOH leaf extracts of *C. abyssinica and M. obscura*

Phytochemical	*M. obscura*	*C. abyssinica*
Phenols	+	+
Flavonoids	+	+
Alkaloids	+	+
Phlobatanins	-	-
Tannins	+	+
Saponins	+	+
Terpernoids	+	+
Steroids	+	+
Cardiac glycosides	+	+

A positive sign (+) denotes presence and negative sign (-) denotes absence of that given phytochemical.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

Free radicals are a great threat to human life due to the different diseases that arise as a result of their accumulation (Rahman *et al*., 2012). Different efforts are being made to manage different conditions arising from oxidative damage. Synthetic antioxidants such as BHA, BHT and Propryl gallate are used in the management of oxidative stress (Shebis *et al*., 2013). They are added to foods as supplements in food industry to reduce the susceptibility of the foods to oxidation. BHT and BHA work by reducing oxygen radicals as well as interfering with the propagation step of oxidation, thereby reducing the levels of free radicals that arise due to oxidation (Shebis *et al*., 2013). However, the use of different synthetic drugs to manage such conditions is associated with unaffordability, unavailability and varied side effects (Moulisha *et al*., 2010).

Different medicinal plants have traditionally been used in the management of different disease conditions, most of which arise due to oxidative stress. Research has shown that foods rich in antioxidants help in reducing different conditions that rise due to oxidative stress (Sen *et al*., 2010). Besides, research has shifted to natural antioxidants because they are considered to be safe and of higher therapeutic value (Sreeramulu *et al*., 2013).

The main aim of this study was to determine the *in vitro* antioxidant activities of DCM: MeOH extracts of *Clutia abyssinica* and *Maytenus obscura*. There are different methods of determining antioxidant activities. These methods are based on electron transfer and hydrogen transfer (Prior *et al*., 2005; Badarinath *et al*., 2010; Moharram and Youssef,

2014). Based on electron transfer, the methods include ferric reducing antioxidant power, copper reduction assay and total phenols by Folin-Ciocalteu. Hydrogen transfer methods include oxygen radical absorbance capacity (ORAC), lipid peroxidation inhibition capacity (LPIC), hydrogen peroxide radical scavenging, hydroxyl radical scavenging and superoxide radical scavenging (Moharram and Youssef, 2014). Besides, there are other methods that work through both electron and hydrogen transfer mechanisms. These are DPPH free radical scavenging and Trolox equivalent antioxidant capacity (TEAC) decolouration assays (Prior *et al*., 2005).

Different solvents are usually used in the extraction process. This is due to the different polarities of different antioxidant compounds that may be present in the medicinal plant. The most commonly used solvents include methanol, ethanol, acetone and dichloromethane (Lacine *et al*., 2013). In this study, methanol and dichloromethane were used in the extraction process, so as to extract both the polar and non-polar antioxidant compounds.

Antioxidant activity of a given plant extract or compound cannot be accurately concluded based on a single method since the response of antioxidant compounds may differ depending on the radical involved (Saumya and Mahaboob, 2011). There is, therefore, need to employ different methods in determination of antioxidant activity. In this study, the antioxidant activities were determined using DPPH free radical scavenging, hydrogen peroxide radical scavenging and reducing power activities along with total phenol content determination by Folin-Ciocalteu assay.

DPPH (1,1- diphenyl-2-picryl-hydrazyl) is a stable free radical that is crystalline deep violet powder in colour, with a maximum absorption at about 520 nm. When made into solution and mixed with a substance that can donate a hydrogen atom, it is reduced and turns colour from violet to pale yellow or colourless. Substances with the ability to reduce DPPH are regarded as antioxidants. Antioxidants react with DPPH and reduce it to DPPH-H (Equation 5.1) (Lacine *et al*., 2013).

DPPH + H-A ⟶ DPPH-H + (A). (Equation 5.1)

DPPH is commonly used in *in vitro* antioxidant assays and in electron paramagnetic resonance signals (Molyneux, 2004). DPPH free radical scavenging is among the most commonly used method in determination of *in vitro* antioxidant activities, because it is simple, fast, reliable and only requires a spectrophotometer to work (Prior *et al*., 2005).

Generally, the DCM: MeOH leaf extracts of *C. abyssinica* and *M. obscura* demonstrated significant DPPH free radical scavenging activities in dose dependent fashions. These findings were similar to work carried out by Moulisha *et al*. (2010), where they reported an increase in the DPPH scavenging effects of petroleum ether fruit extracts of *Dregea volubilis* with increase in extract concentration. Another study by Rabia *et al*. (2015) showed that the methanolic leaf extracts of *Zanthoxylum armatum* demonstrated a dose dependent increase in DPPH scavenging activity. Oyedemi *et al*. (2010) reported a dose dependent increase in the DPPH scavenging effects of aqueous bark extracts of *Strychnos henningsii* Gilg. This implies that higher concentrations are associated with stronger free radical scavenging potential (Rabia *et al*., 2015).

In this study, dose ranges of 1 to 0.0625 mg/ml were used in determination of DPPH scavenging activities of the DCM: MeOH leaf extracts of *C. abyssinica* and *M. obscura.* These were similar to those used by Oloyede (2012) and Onocha *et al*. (2011) while determining the antioxidant activities of seed extracts of *Ricinus communis* and leaf extracts of *Acalypha torta* respectively.

The IC_{50} values for the DCM: MeOH extracts of *C. abyssiniva, M. obscura* and the standard (ascorbic acid) DPPH scavenging activities were 0.087, 0.065 and 0.044 respectively (Table 4.1). The DCM: MeOH leaf extracts of *M. obscura* showed a slightly lower IC_{50} value than the DCM: MeOH leaf extracts of *C. abyssinica*. This means that a lower concentration of *M. obscura* was needed to cause DPPH scavenging activity than *C. abyssinica,* which would require a slightly higher concentration.

In this study, total ferric reducing power assay for the DCM: MeOH leaf extracts of *C. abyssinica* and *M. obscura* was also done. Ferric reducing power assay is a method based on the principle of reduction of Potassium ferricyanide ($Fe^{3+\cdot}$) to Potassium ferrocyanide (Fe^{2+}), which reacts with Iron (III) Chloride reducing it to Iron (II) chloride (Jayanthi and Lalitha, 2011). Reduction of iron (III) Chloride to iron (II) chloride in a reaction mixture is an indicator of an electron-donation activity (Esmaeili *et al*., 2009). The formation of Iron (II) complex is then monitored at 700 nm. Increasing absorbance indicates increase in reducing power. Reducing power is used as an indicator of antioxidant activity (Nabavi *et al*., 2009).

This study showed significant total ferric reducing power of DCM: MeOH leaf extracts of *C. abyssinica* and *M. obscura* (Table 4.2). These results were consistent with other *in vitro* antioxidant studies that have been done. A study by Patel *et al*. (2012) indicated that aqueous extracts of *Withania somnifera* and *Aloe vera* produced ferric reducing power. Methanolic aerial parts extract of *Salvia glutinosa* produced remarkable ferric reducing power (Esmaeli *et al.*, 2009). Besides, studies by Ebrahimzadeh *et al*. (2010) indicated that methanolic extracts of *Leonurus cardiac subsp. Persicus, Grammosciadium platycarpum* and *Onosma demawendicum* demonstrated ferric reducing power.

The DCM: MeOH leaf extracts of *C. abyssinica* and *M. obscura* produced dose dependent increase in ferric reducing power. Studies by Jayanthi and Lalitha (2011) indicated that the ferric reducing power of different solvent extracts of *Eicnornia crassipes (mart.) solms* increased with increase in extract concentration. Besides, Kibiti and Afolayan (2015) observed a dose dependent increase in ferric reducing power of acetone and aqueous extracts of *Bulbine abyssinica* while determining its antioxidant activities. This implies that as the extract concentration increases, the amount of antioxidant compounds present in the extract increases, thus the increase in ferric reducing power, which shows an increase in antioxidant potential.

The present study used a dose range of 0.2 to 1 mg/ml to determine the ferric reducing power of the DCM: MeOH leaf extracts of *C. abyssinica* and *M. obscura*. Arya and Yadav (2011) used similar dose ranges in determination of the ferric reducing power of

leaf extracts of *Cassia occidentalis L.* and *C. tora L.* Another study by Zulkefli *et al*. (2013) on the antioxidant activity of *Tinospora crispa* and *Tabernaemontana corymbosa* used similar dose ranges. However, in determination of the antioxidant activities of leaf extracts of *Pterocarpus marsupium roxb* (fabaceae), Kumaravel *et al*. (2013) used dose ranges of 20 to 100µg/ml.

In vitro hydrogen peroxide scavenging assay was also undertaken for the DCM: MeOH leaf extracts of *C. abyssinica* and *M. obscura*. Hydrogen peroxide is a radical produced during oxidative metabolism through a dismutation reaction in the body, or it can be inhaled from the environment (Alam *et al*., 2013; Eboh, 2014). It is mostly used as an oxidizing agent in different chemical reactions. It is commonly used as a ripening, bleaching and anti-infective agent. It is unstable and it easily decomposes to hydrogen and water (Alam *et al*., 2013). It can easily penetrate through membranes and decompose into hydroxyl radical that is highly toxic (Oyedemi *et al*., 2010). Hydrogen peroxide scavenging assay is based on the ability of the extracts to scavenge the hydrogen peroxide and reduce it to water. It is among the preferred methods because it is easier and faster to execute.

In this study, it was observed that the DCM: MeOH leaf extracts of *C. abyssinica* and *M. obscura* demonstrated significant hydrogen peroxide scavenging activities (Table 4.3). These findings are consistent with other *in vitro* antioxidant assays. Kibiti and Afolayan (2015) indicated that both acetone and aqueous extracts of *Bulbine abyssinica* had remarkable hydrogen peroxide scavenging activities. Methanolic leaf extracts of

Vernonia amygdalina Del also were found to demonstrate significant hydrogen peroxide scavenging activity (Adesanoye and Farombi, 2014).

There was a dose dependent reduction in the hydrogen peroxide scavenging activities of the DCM: MeOH leaf extracts of *C. abyssinica* and *M. obscura* (Table 4.3). A dose dependent decrease in hydrogen peroxide scavenging activity was also demonstrated by both acetone and aqueous whole plant extracts of *Bulbine abyssinica* (Kibiti and Afolayan, 2015). However, in a study carried out by Oloyede *et al*. (2014), hexane extracts of *Cyperus esculentus* produced a dose dependent increase in hydrogen peroxide scavenging activities, while its methanolic extract produced a dose dependent decrease in hydrogen peroxide scavenging activity. Moreover, Oloyode and Abimbade (2014) found that the hydrogen peroxide scavenging effects of cocoa oil and cake increased with increase in concentration. Dose-dependent decrease in hydrogen scavenging activity implies that low concentrations had high scavenging activities hence high antioxidant abilities.

This trend in the hydrogen peroxide scavenging activity could have been due to saturation of reactive centers of hydrogen peroxide by the high extract concentrations leading to low activities, compared to dilute concentrations that ensure easier and rapid reaction, leading to high activity.

The IC_{50} values for the DCM: MeOH extracts of *C. abyssinica*, *M. obscura* and the standard (ascorbic acid) were 0.0350, 0.0446 and 0.1104 respectively. This means that

the potential of the extracts to scavenge hydrogen peroxide decreased with increase in concentration.

Determination of total phenolic content of the two studied plant extracts was also done in this study. Phenols are the major secondary metabolites that occur in many different plant species. They contain hydroxyl groups in their structure, which gives them their antioxidant properties. Their antioxidant properties include acting as free radical scavengers by donation of hydrogen atom and protection of antioxidant enzymes (Lacine *et al*., 2013; Nimse and Pal, 2015).

In this study, total phenols were determined using the Folin-Ciolcalteu method. Folin-Ciocalteu's phenol reagent is a colourless compound that consists of phosphomolybdic and phosphotungstic acids. It is used for phenol and polyphenol assays. This method is simple, precise and sensitive (Prior *et al*., 2005). When it reacts with a solution containing reductants, it forms molybdenum and tungsten blue, which are responsible for the colour change to blue (Agbor *et al*., 2014).

The DCM: MeOH leaf extracts of *C. abyssinica* and *M. obscura* demonstrated a significant amount of total phenolic content (Table 4.4). However, the phenolic content of the DCM: MeOH leaf extracts of *M. obscura* was significantly higher than that of *C. abyssinica* (Table 4.4). This explains why the tested antioxidant activities of DCM: MeOH leaf extracts of *M. obscura* were consistently higher than those of DCM: MeOH leaf extracts of *C. abyssinica*. Phenols are linked to antioxidant activity due to their

ability to quench and neutralize free radicals by donation of hydrogen atom or electrons (Nimse and Pal, 2015). A study by Ahmed *et al*. (2013) linked the strong antioxidant activity of *Dicliptera roxburghiana* to the high total phenolic content of the plant extract.

The observed *in vitro* antioxidant activities of the two studied plant extracts could have been due to the presence of different bioactive secondary metabolites. The qualitative phytochemical screening of DCM: MeOH leaf extracts of *C. abyssinica* showed the presence of phenols, alkaloids, flavonoids, tannins, steroids, terpernoids, cardiac glycosides and saponins, while the leaf extracts of *M. obscura* showed the presence of phenolics, flavonoids, alkaloids, steroids, saponins, terpernoids, cardiac glycosides and tannins (Table 4.5).

Phenols are the major secondary metabolites that are associated with antioxidant activity. They include compounds such as flavonoids, tannins and athocyanides (Sen *et al*., 2010). For instance, Rao *et al*. (2013) showed that phenols contributed to the antioxidant activity of *Gnidia glauca* (fresen.) Gilg. Ngonda (2013) demonstrated that the antioxidant activity of *Trichodesma zeylanicumm (burm. f)* was due to its flavonoid and phenolic constituents. Polyphenols act as antioxidant by inhibiting the activity of enzymes such as lipoxygenases, xanthine oxidases and cyclooxygenases that are involved in activities that lead to free radical generation. Polyphenols increase the activities of antioxidant enzymes, act as free radical scavengers and aid in protection of antioxidant enzymes (Ali and Neda, 2011).

Flavonoids have a polyphenolic structure and include groups like flavonols, flavones, isoflavones, flavanone and athocyanidins. They occur mostly in tea, vegetables, fruits, seeds and nuts (Ali and Neda, 2011; Saxena *et al*., 2012; Banjarnahor and Artanti, 2014). Studies by Ebrahimzadeh *et al*. (2009) attributed the antioxidant activities of *Vicia caescece* to its flavonoid constituents. They have the ability to act as free radicals scavengers, suppressors of the activities of free radicals production enzymes and stimulators of antioxidant enzymes. They can also act as electron donors and metal chelating molecules (Pietta, 2000; Banjarnahor and Artanti, 2014; Nimse and Pal, 2015).

Tannins are water soluble phenolic compounds. On the basis of their structure, tannins fall into four classes; condensed, complex, Gallotannins and Ellagitannins. They occur in grapes, legumes, sorghum and corn (Saxena *et al*., 2013).. In a study by Sasikala *et al*. (2011), tannins have been linked to the antioxidant activity of *Passiofiora foetida L*. In different *in vitro* assays, tannic acid was found to be an effective antioxidant molecule (Gulcin *et al*., 2010). Tannins act as antioxidants by donating hydrogen atom or electrons to other molecules thus stabilizing them. They also act as metal chelators (Amarowicz, 2007).

Alkaloids are secondary metabolites with heterocyclic nitrogen atoms. Due to differences in their heterocyclic ring system, they fall into different categories. These include indoles, imidazole, pyrrolidine, quinolizidine and purine (Saxena *et al*., 2013; Kaur and Arora, 2015). Alkaloids from *Catharanthus roseus* (L.) G. Don, demonstrated

antioxidant activity at low doses (Tiong *et al*., 2013). Alkaloids provide antioxidant activity by acting as metal chalators and free radical scavengers. They also act as electron or hydrogen donating molecules (Kaur and Arora, 2015).

Terpenoids are natural products divided into different classes based on their carbon skeleton. These classes include monoterpenes, sesquiterpenes, diterpene, triterpenes, tetraterpene and polyterpene (Grassmann, 2005). Terpernoids from *Nigella sativa* have been found to possess antioxidant activity (Bourgou *et al*., 2012). For instance, tetraterpenes, mainly carotenoids provide antioxidant activity by quenching of singlet oxygen and scavenging of peroxyl radical (Grassmann, 2005).

It is, therefore, not surprising that the two plants exhibited strong in vitro antioxidant activities. Results of this study provides a convincing basis of the use of the two plants in the traditional management of oxidative stress-related disorders.

5.2 Conclusions

From this study, it can be concluded that;

i) The DCM: MeOH extracts of *C. abyssinica* and *M. obscura* have *in vitro* DPPH free radical scavenging activities.

ii) The DCM: MeOH leaf extracts of *C. abyssinica* and *M. obscura* have *in vitro* ferric reducing power activities.

iii) The DCM: MeOH leaf extracts of *C. abyssinica* and *M. obscura* have *in vitro* hydrogen peroxide scavenging activities.

iv) The DCM: MeOH extracts of *C. abyssinica* and *M. obscura* have significant amounts of total phenolic compounds.

v) The DCM: MeOH leaf extracts of *M. obscura* and *C. abyssinica* have phytochemicals associated with antioxidant effects.

Therefore, the research questions of this study have been affirmatively answered.

5.3 Recommendations

This study recommends that,

1) The DCM: MeOH leaf extracts of *C. abyssinica* and *M. obscura* can be used as dietary supplements to manage oxidative stress, subject to more studies such as toxicity tests.

5.4 Suggestions for further studies

1) Further studies are necessary to find out the mechanism of action of these herbal plants, this could serve as an opening ground for more research.
2) The antioxidant activity of the aqueous extracts can be done, to find out how it compares with organic extracts.
3) *In vivo* antioxidant studies to be carried out so as to provide more information which can be compared with the *in vitro* studies.

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APPENDICES

Appendix I: Analysis of DPPH scavenging activity of DCM: MeOH extracts of *C. abyssinica* and *M. obscura*

Descriptive Statistics: CA0.0625, CA0.125, CA0.25, CA0.5, CA1, MO0.0625, MO0.125, MO0.25, …

Variable	Mean	SE Mean
CA0.0625	46.911	0.272
CA0.125	54.312	0.371
CA0.25	60.765	0.110
CA0.5	71.315	0.250
CA1	80.000	0.106
MO0.0625	49.327	0.162
MO0.125	58.899	0.265
MO0.25	64.587	0.295
MO0.5	72.446	0.221
MO1	82.569	0.159
AS0.0625	50.520	0.239
AS0.125	66.942	0.361
AS0.25	81.009	0.231
AS0.5	83.823	0.478
AS1	91.774	0.239

One-way ANOVA: CA0.0625, MO0.0625, AS0.0625

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	CA0.0625, MO0.0625, AS0.0625

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	20.2807	10.1404	64.54	0.000
Error	6	0.9427	0.1571		
Total	8	21.2234			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.396376	95.56%	94.08%	90.01%

Means

Factor	N	Mean	StDev	95% CI
CA0.0625	3	46.911	0.471	(46.351, 47.471)
MO0.0625	3	49.327	0.280	(48.767, 49.887)
AS0.0625	3	50.520	0.414	(49.960, 51.080)

Pooled StDev = 0.396376

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
AS0.0625	3	50.520	A
MO0.0625	3	49.327	B
CA0.0625	3	46.911	C

Means that do not share a letter are significantly different.

One-way ANOVA: CA0.125, MO0.125, AS0.125

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	CA0.125, MO0.125, AS0.125

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	245.245	122.622	363.21	0.000
Error	6	2.026	0.338		
Total	8	247.271			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.581040	99.18%	98.91%	98.16%

Means

Factor	N	Mean	StDev	95% CI
CA0.125	3	54.312	0.642	(53.491, 55.133)
MO0.125	3	58.899	0.459	(58.078, 59.720)

```
AS0.125  3  66.942  0.624  (66.121, 67.763)

Pooled StDev = 0.581040
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Factor   N    Mean  Grouping
AS0.125  3  66.942  A
MO0.125  3  58.899    B
CA0.125  3  54.312      C

Means that do not share a letter are significantly different.
```

One-way ANOVA: CA0.25, MO0.25, AS0.25

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Factor       3  CA0.25, MO0.25, AS0.25


Analysis of Variance

Source  DF   Adj SS   Adj MS  F-Value  P-Value
Factor   2  694.141  347.070  2276.80    0.000
Error    6    0.915    0.152
Total    8  695.056


Model Summary

       S    R-sq  R-sq(adj)  R-sq(pred)
0.390433  99.87%     99.82%      99.70%


Means

Factor  N    Mean  StDev       95% CI
CA0.25  3  60.765  0.191  (60.213, 61.316)
MO0.25  3  64.587  0.511  (64.036, 65.139)
AS0.25  3  81.009  0.400  (80.458, 81.561)

Pooled StDev = 0.390433
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Factor  N    Mean  Grouping
AS0.25  3  81.009  A
MO0.25  3  64.587    B
CA0.25  3  60.765      C

Means that do not share a letter are significantly different.
```

One-way ANOVA: CA0.5, MO0.5, AS0.5

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Factor       3  CA0.5, MO0.5, AS0.5


Analysis of Variance

Source  DF   Adj SS   Adj MS  F-Value  P-Value
Factor   2  287.138  143.569   422.91    0.000
Error    6    2.037    0.339
Total    8  289.175


Model Summary

       S    R-sq  R-sq(adj)  R-sq(pred)
0.582647  99.30%     99.06%      98.42%


Means

Factor  N    Mean  StDev       95% CI
CA0.5   3  71.315  0.434  (70.492, 72.138)
MO0.5   3  72.446  0.382  (71.623, 73.270)
AS0.5   3  83.823  0.827  (83.000, 84.646)

Pooled StDev = 0.582647
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Factor  N    Mean  Grouping
```

```
AS0.5   3  83.823  A
MO0.5   3  72.446    B
CA0.5   3  71.315    B

Means that do not share a letter are significantly different.
```

One-way ANOVA: CA1, MO1, AS1

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Factor       3  CA1, MO1, AS1


Analysis of Variance

Source  DF   Adj SS   Adj MS  F-Value  P-Value
Factor   2  229.949  114.974  1229.41    0.000
Error    6    0.561    0.094
Total    8  230.510


Model Summary

       S    R-sq  R-sq(adj)  R-sq(pred)
0.305810  99.76%     99.68%      99.45%


Means

Factor  N    Mean  StDev       95% CI
CA1     3  80.000  0.183  (79.568, 80.432)
MO1     3  82.569  0.275  (82.137, 83.001)
AS1     3  91.774  0.414  (91.342, 92.206)

Pooled StDev = 0.305810
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Factor  N    Mean  Grouping
AS1     3  91.774  A
MO1     3  82.569    B
CA1     3  80.000      C

Means that do not share a letter are significantly different.
```

Appendix II: Analysis of total ferric reducing power of DCM: MeOH extracts of *C. abyssinica* and *M. obscura.*

Descriptive Statistics: MO 0.2, MO 0.4, MO 0.6, MO 0.8, MO 1, CA 0.2, CA 0.4, CA 0.6, ...

Variable	Mean	SE Mean
MO 0.2	0.0917	0.0129
MO 0.4	0.1660	0.0120
MO 0.6	0.2133	0.0225
MO 0.8	0.2877	0.0237
MO 1	0.3667	0.0204
CA 0.2	0.05467	0.00318
CA 0.4	0.10667	0.00145
CA 0.6	0.16233	0.00463
CA 0.8	0.17133	0.00371
CA 1	0.21767	0.00994
AS 0.2	0.25333	0.00491
AS 0.4	0.41300	0.00265
AS 0.6	0.50367	0.00273
AS 0.8	0.60267	0.00441
AS 1	0.62067	0.00549

One-way ANOVA: MO 0.2, CA 0.2, AS 0.2

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	MO 0.2, CA 0.2, AS 0.2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0.066974	0.033487	166.60	0.000
Error	6	0.001206	0.000201		
Total	8	0.068180			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0141774	98.23%	97.64%	96.02%

Means

Factor	N	Mean	StDev	95% CI
MO 0.2	3	0.0917	0.0224	(0.0716, 0.1117)
CA 0.2	3	0.05467	0.00551	(0.03464, 0.07470)
AS 0.2	3	0.25333	0.00850	(0.23330, 0.27336)

Pooled StDev = 0.0141774

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
AS 0.2	3	0.25333	A
MO 0.2	3	0.0917	B
CA 0.2	3	0.05467	C

Means that do not share a letter are significantly different.

One-way ANOVA: MO 0.4, CA 0.4, AS 0.4

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	MO 0.4, CA 0.4, AS 0.4

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0.158370	0.079185	517.17	0.000
Error	6	0.000919	0.000153		
Total	8	0.159288			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0123738	99.42%	99.23%	98.70%

Means

Factor	N	Mean	StDev	95% CI
MO 0.4	3	0.1660	0.0208	(0.1485, 0.1835)
CA 0.4	3	0.10667	0.00252	(0.08919, 0.12415)
AS 0.4	3	0.41300	0.00458	(0.39552, 0.43048)

Pooled StDev = 0.0123738

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
AS 0.4	3	0.41300	A
MO 0.4	3	0.1660	B
CA 0.4	3	0.10667	C

Means that do not share a letter are significantly different.

One-way ANOVA: MO 0.6, CA 0.6, AS 0.6

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	MO 0.6, CA 0.6, AS 0.6

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0.203403	0.101701	190.81	0.000
Error	6	0.003198	0.000533		
Total	8	0.206601			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0230868	98.45%	97.94%	96.52%

Means

Factor	N	Mean	StDev	95% CI
MO 0.6	3	0.2133	0.0389	(0.1807, 0.2459)
CA 0.6	3	0.16233	0.00802	(0.12972, 0.19495)
AS 0.6	3	0.50367	0.00473	(0.47105, 0.53628)

Pooled StDev = 0.0230868

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
AS 0.6	3	0.50367	A
MO 0.6	3	0.2133	B
CA 0.6	3	0.16233	B

Means that do not share a letter are significantly different.

One-way ANOVA: MO 0.8, CA 0.8, AS 0.8

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	MO 0.8, CA 0.8, AS 0.8

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0.298807	0.149403	251.52	0.000
Error	6	0.003564	0.000594		
Total	8	0.302371			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0243721	98.82%	98.43%	97.35%

Means

Factor	N	Mean	StDev	95% CI
MO 0.8	3	0.2877	0.0410	(0.2532, 0.3221)
CA 0.8	3	0.17133	0.00643	(0.13690, 0.20576)
AS 0.8	3	0.60267	0.00764	(0.56824, 0.63710)

Pooled StDev = 0.0243721

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping

```
AS 0.8  3  0.60267  A
MO 0.8  3   0.2877    B
CA 0.8  3  0.17133      C

Means that do not share a letter are significantly different.
```

One-way ANOVA: MO 1, CA 1, AS 1

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Factor       3  MO 1, CA 1, AS 1


Analysis of Variance

Source  DF    Adj SS    Adj MS  F-Value  P-Value
Factor   2  0.249126  0.124563   228.28    0.000
Error    6  0.003274  0.000546
Total    8  0.252400


Model Summary

S            R-sq   R-sq(adj)     R-sq(pred)
0.0233595  98.70%     98.27%      97.08%


Means

Factor  N     Mean    StDev        95% CI
MO 1    3   0.3667   0.0354  ( 0.3337,  0.3997)
CA 1    3  0.21767  0.01721  (0.18467, 0.25067)
AS 1    3  0.62067  0.00950  (0.58767, 0.65367)

Pooled StDev = 0.0233595
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Factor  N     Mean  Grouping
AS 1    3  0.62067  A
MO 1    3   0.3667    B
CA 1    3  0.21767      C

Means that do not share a letter are significantly different.
```

Appendix III: Analysis of hydrogen peroxide scavenging activity of DCM: MeOH extracts of *C. abyssinica* and *M. obscura*

Descriptive Statistics: CA0.00625, CA0.0125, CA0.025, CA0.05, CA0.1, MO0.00625, MO0.0125, …

Variable	Mean	SE Mean
CA0.00625	75.496	0.291
CA0.0125	68.025	0.271
CA0.025	54.336	0.228
CA0.05	42.006	0.326
CA0.1	36.991	0.157
MO0.00625	79.728	0.377
MO0.0125	70.690	0.181
MO0.025	57.158	0.318
MO0.05	46.029	0.408
MO0.1	39.916	0.261
AS0.00625	90.178	0.276
AS0.0125	83.333	0.276
AS0.025	73.354	0.0905
AS0.05	64.107	0.181
AS0.1	56.583	0.157

One-way ANOVA: CA0.00625, MO0.00625, AS0.00625

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	CA0.00625, MO0.00625, AS0.00625

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	342.638	171.319	565.41	0.000
Error	6	1.818	0.303		
Total	8	344.456			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.550452	99.47%	99.30%	98.81%

Means

Factor	N	Mean	StDev	95% CI
CA0.00625	3	75.496	0.504	(74.719, 76.274)
MO0.00625	3	79.728	0.653	(78.951, 80.506)
AS0.00625	3	90.178	0.479	(89.400, 90.955)

Pooled StDev = 0.550452

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
AS0.00625	3	90.178	A
MO0.00625	3	79.728	B
CA0.00625	3	75.496	C

Means that do not share a letter are significantly different.

One-way ANOVA: CA0.0125, MO0.0125, AS0.0125

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	CA0.0125, MO0.0125, AS0.0125

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	401.305	200.653	1097.12	0.000
Error	6	1.097	0.183		
Total	8	402.403			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.427657	99.73%	99.64%	99.39%

Means

Factor	N	Mean	StDev	95% CI
CA0.0125	3	68.025	0.470	(67.421, 68.629)
MO0.0125	3	70.690	0.313	(70.085, 71.294)
AS0.0125	3	83.333	0.479	(82.729, 83.937)

Pooled StDev = 0.427657

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
AS0.0125	3	83.333	A
MO0.0125	3	70.690	B
CA0.0125	3	68.025	C

Means that do not share a letter are significantly different.

One-way ANOVA: CA0.025, MO0.025, AS0.025

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	CA0.025, MO0.025, AS0.025

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	631.960	315.980	1961.97	0.000
Error	6	0.966	0.161		
Total	8	632.926			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.401314	99.85%	99.80%	99.66%

Means

Factor	N	Mean	StDev	95% CI
CA0.025	3	54.336	0.394	(53.770, 54.903)
MO0.025	3	57.158	0.550	(56.591, 57.725)
AS0.025	3	73.3542	0.1567	(72.7873, 73.9212)

Pooled StDev = 0.401314

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Factor   N     Mean  Grouping
AS0.025  3  73.3542  A
MO0.025  3   57.158    B
CA0.025  3   54.336      C

Means that do not share a letter are significantly different.
```

One-way ANOVA: CA0.05, MO0.05, AS0.05

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Factor       3  CA0.05, MO0.05, AS0.05


Analysis of Variance

Source  DF   Adj SS   Adj MS  F-Value  P-Value
Factor   2  831.398  415.699  1359.71    0.000
Error    6    1.834    0.306
Total    8  833.232


Model Summary

       S    R-sq  R-sq(adj)  R-sq(pred)
0.552926  99.78%     99.71%      99.50%


Means

Factor  N    Mean  StDev       95% CI
CA0.05  3  42.006  0.565  (41.225, 42.787)
MO0.05  3  46.029  0.707  (45.248, 46.810)
AS0.05  3  64.107  0.313  (63.325, 64.888)

Pooled StDev = 0.552926
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Factor  N    Mean  Grouping
```

```
AS0.05  3  64.107  A
MO0.05  3  46.029    B
CA0.05  3  42.006      C

Means that do not share a letter are significantly different.
```

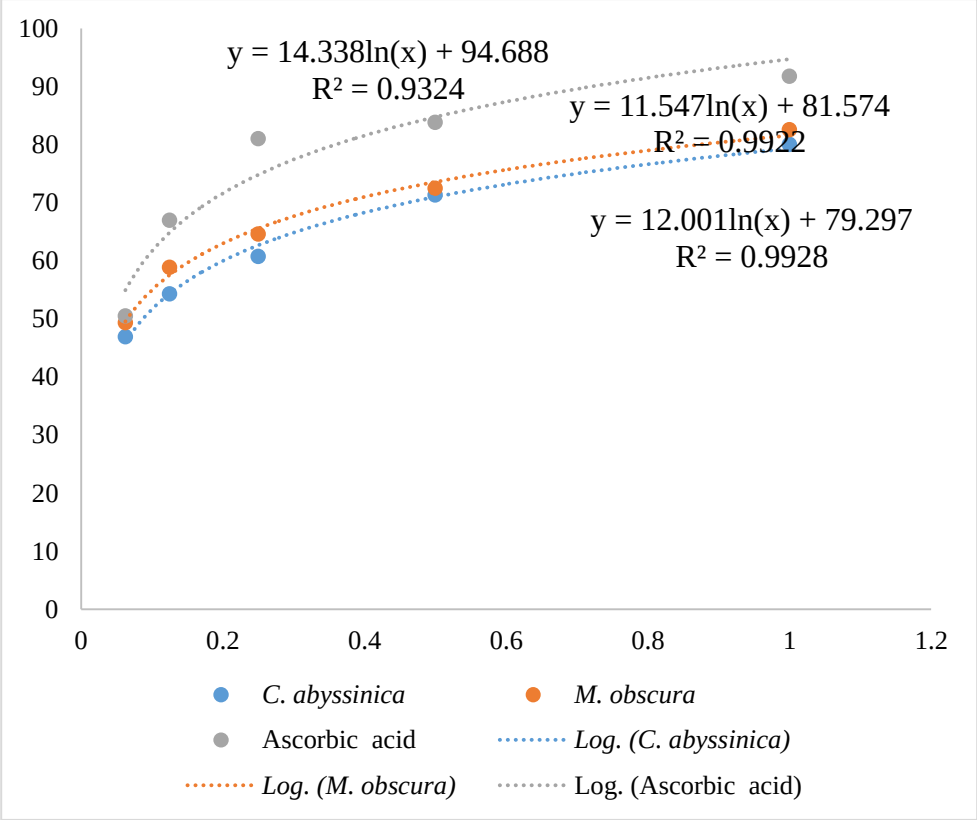
One-way ANOVA: CA0.1, MO0.1, AS0.1

```
Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Factor Information

Factor  Levels  Values
Factor       3  CA0.1, MO0.1, AS0.1


Analysis of Variance

Source  DF   Adj SS   Adj MS  F-Value  P-Value
Factor   2  670.203  335.102  2854.91    0.000
Error    6    0.704    0.117
Total    8  670.908


Model Summary

       S    R-sq  R-sq(adj)  R-sq(pred)
0.342604  99.90%     99.86%      99.76%


Means

Factor  N    Mean  StDev       95% CI
CA0.1   3  36.991  0.271  (36.507, 37.475)
MO0.1   3  39.916  0.452  (39.432, 40.400)
AS0.1   3  56.583  0.271  (56.099, 57.067)

Pooled StDev = 0.342604
```

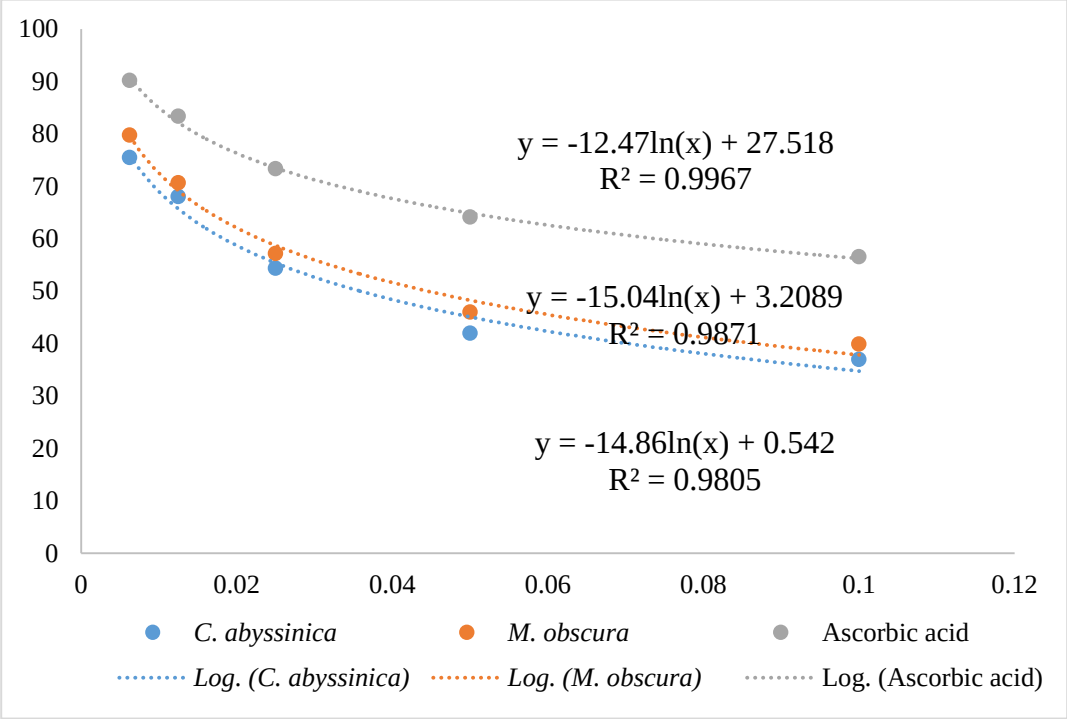
Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

Factor  N    Mean  Grouping
AS0.1   3  56.583  A
MO0.1   3  39.916    B
CA0.1   3  36.991      C

Means that do not share a letter are significantly different.
```

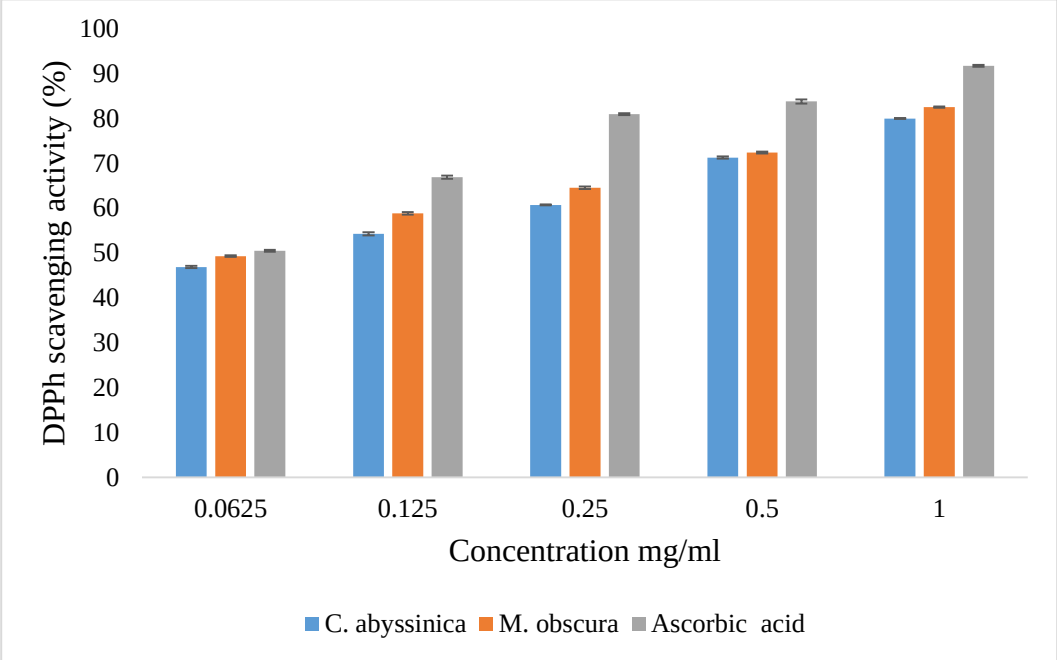
Appendix IV: Standard curve used to calculate/compute IC_{50} of DPPH radical scavenging activities



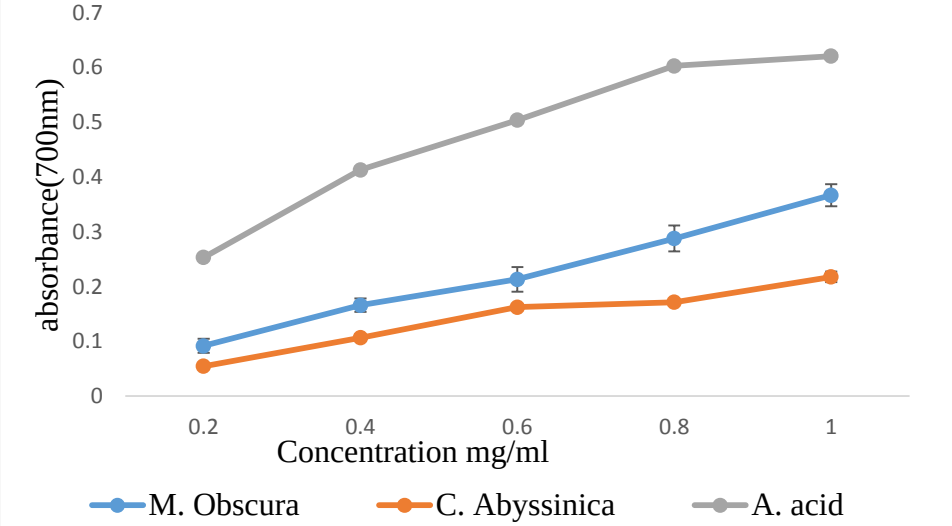
Appendix V: Standard curve used to calculate/compute IC_{50} of hydrogen peroxide scavenging activities



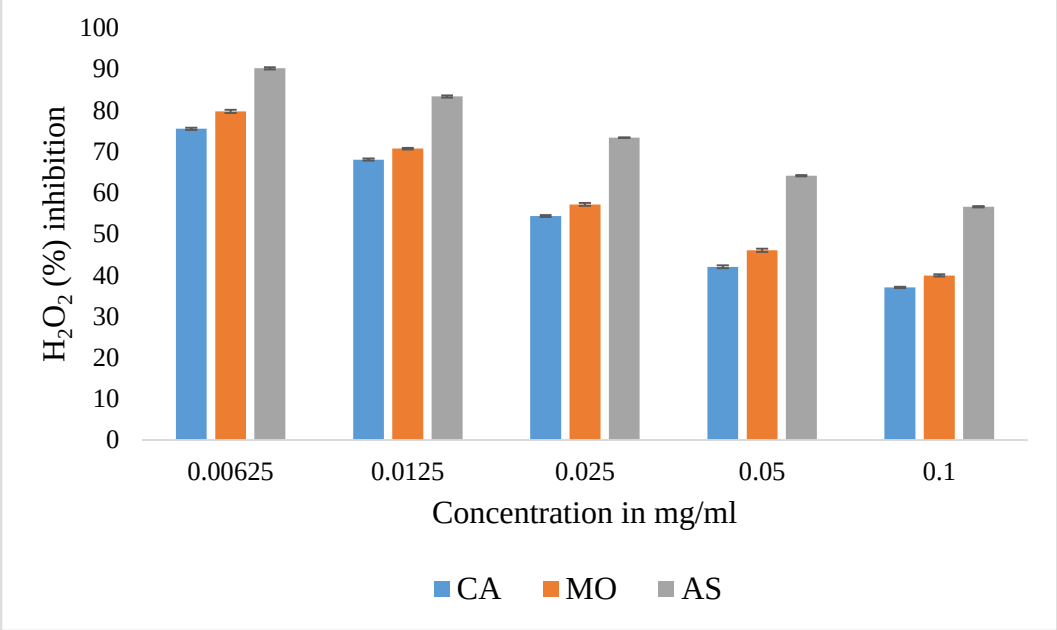
Appendix VI: Figure showing the DPPH radical scavenging activities



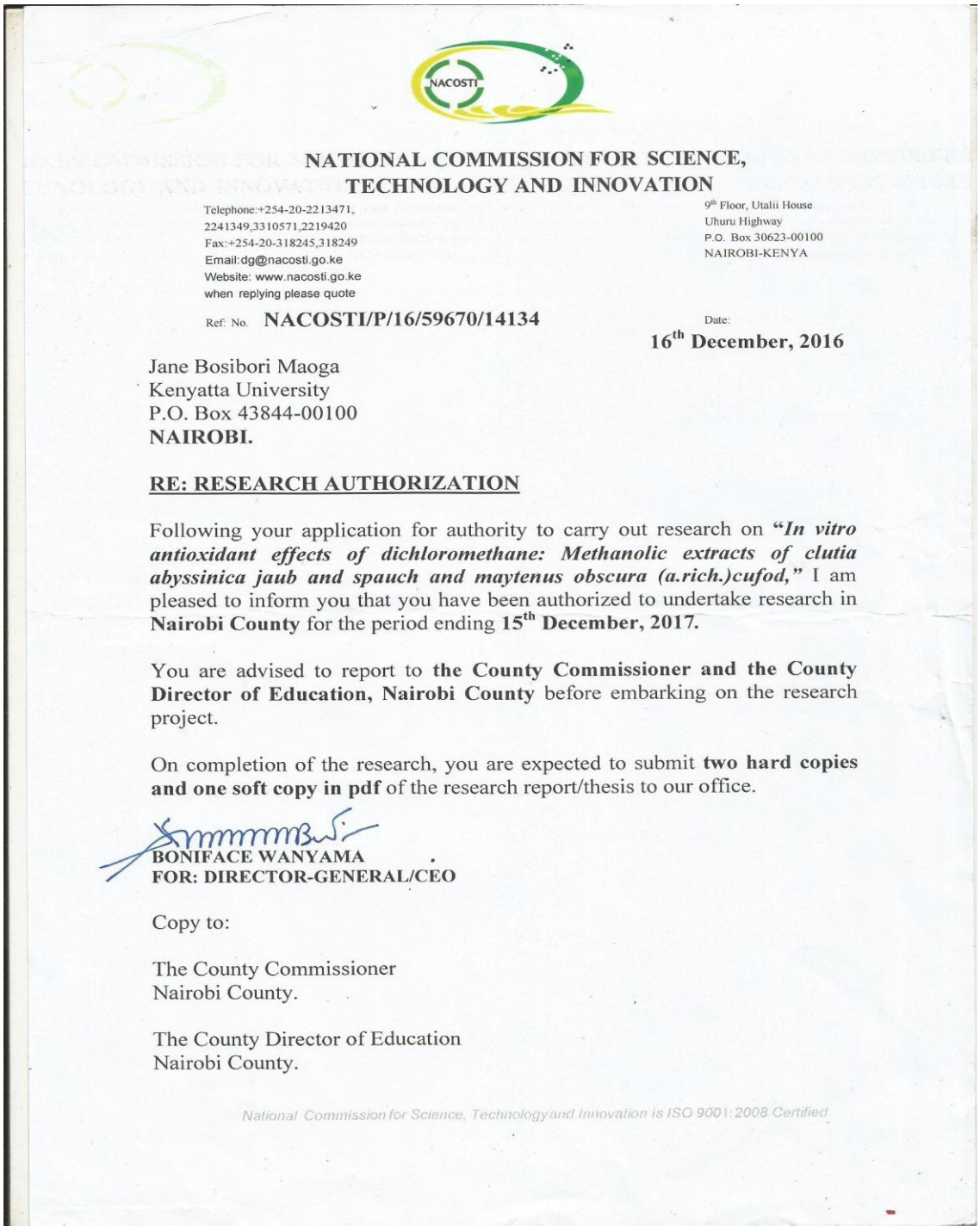
Appendix VII: Figure showing the total ferric reducing powers



Appendix VIII: Figure showing the hydrogen peroxide scavenging activities



Appendix IX: NACOSTI Research Authorization



NATIONAL COMMISSION FOR SCIENCE,
TECHNOLOGY AND INNOVATION

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Ref: No. **NACOSTI/P/16/59670/14134**

Date:
16th December, 2016

Jane Bosibori Maoga
Kenyatta University
P.O. Box 43844-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"In vitro antioxidant effects of dichloromethane: Methanolic extracts of clutia abyssinica jaub and spauch and maytenus obscura (a.rich.)cufod,"*** I am pleased to inform you that you have been authorized to undertake research in **Nairobi County** for the period ending **15th December, 2017.**

You are advised to report to **the County Commissioner and the County Director of Education, Nairobi County** before embarking on the research project.

On completion of the research, you are expected to submit **two hard copies and one soft copy in pdf** of the research report/thesis to our office.

BONIFACE WANYAMA
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Nairobi County.

The County Director of Education
Nairobi County.

National Commission for Science, Technology and Innovation is ISO 9001:2008 Certified