IN VITRO ANTIOXIDANT ACTIVITIES OF DICHLOROMETHANE EXTRACTS OF *Strychnos henningsii* G. AND *Ficus sycomorus* L.

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DECLARATION

I duly declare that the work presented in this thesis is my original work and has not been presented for a degree or any other award in any other university or institution

Signature.....**Date** 25/06/19

Supervisors

We hereby confirm that the candidate carried out the work reported in this thesis under our supervision

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DEDICATION

This thesis is dedicated to my parents Mr. Gabriel Wafula and Mrs. Naomi Shikanga for their commitment towards my education.

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ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of variance
CAT	Catalase
DCM	Dichloromethane
DNA	De-oxyribonucleic acid
DPPH	2, 2-diphenlyl-1-picrylhydrazyl
GSH	Glutathione peroxidase
HNE	Hydroxyl -2-noneal
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
ROS	Reactive oxidative species
SEM	Standard error of the mean
SOD	Superoxide dismutase

ABSTRACT

Most of biochemical reactions in the body generates Reactive Oxygen Species (ROS), which are involved in the pathogenesis of oxidative stress-related disorders like diabetes, nephrotoxicity, cancer, cardiovascular disorders, inflammation and neurological disorders when they attack biochemical molecules like proteins, lipids and nucleic acid. Antioxidants are used to protect the cells or tissues against potential attack by ROS. Natural based antioxidants such as catalase, glutathione, superoxide dismutase, vitamin C and E in the body are known to quench free radicals. Most medicinal plants possess a rich source of antioxidants such as flavonoids, phenols, tannins, alkaloids among others. These phytochemiclas are currently pursued as an alternative and complimentary drug. *Strychnos henningsii* and *Ficus sycomorous* are used in Africa as traditional medicine for treatment of various ailments including rheumatism, syphilis, gastro intestinal pain, snake bites, abdominal pain diabetes as well as anaesthology, healing of wounds and as a mouth antiseptic. This study was designed to evaluate *in-vitro* antioxidant activities of DCM leaf extract of *S. henningsii* and stem bark extract of *F. sycomorus* using 1,1-diphenly-2-picrylhydrazyl (DPPH), Hydrogen peroxide and Ferric reducing power assays. The antioxidants activities were assayed against ascorbic acid as a reference drug. Total flavonoids and phenolic compound were determined by Folin–Ciocalteu reaction. The results obtained showed both extracts significantly ($p<0.05$) exhibited antioxidants activities at different concentrations tested. The DCM leaf extract of *S. henningsii* and stem bark extract of *F. sycomorus* scavenge hydrogen peroxide radicals (H_2O_2) in a dose dependent manner. The half maximal percentage inhibition (IC_{50}) of *S. henningsii* and *F. sycomorus* was 0.325 mg/ml and 0.330 mg/ml, respectively. The two extracts also scavenged DPPH in a dose dependent manner. Their IC_{50} value was 0.068mg/ml for *S. henningsii* and 0.062mg/ml for *F. sycomorus*. Both DCM leaf and stem bark extract of *S. henningsii* and *F. sycomorus* were found to have strong ferric reducing power in a dose dependent manner. Both the total phenolic and flavonoid content of DCM stem bark extract of *F. sycomorus* was lower than the leaf extract of *S. henningsii*. Preliminary phytochemical screening showed that both extracts possess saponins, flavonoids, phenols, steroids, alkaloids and cardiac glycoside however terpenoid were found to be absent in *S. henningsii*. Therefore, the results in this study showed both extracts possess secondary metabolites which are associated with antioxidants activities. The present study therefore recommends a further screening to enhance their ultimate application in management of oxidative stress-related disorders.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Oxidative stress is the disparity between production of free radicals and antioxidant defenses in the body (Halliwell, 1995; Sendogdu *et al*., 2006; Halder *et al*., 2010). Free radicals are defined as compounds with unpaired electrons, making them highly reactive molecules that can attack any stable molecules like proteins, carbohydrates and lipids (Agarwal *et al*., 2006). Reactive oxygen species (ROS) are the most common and widely known free radicals. They include super oxide (O_2^-), hydroxyl (HO^-), hydrogen peroxide (H_2O_2), and nitric oxide (NO^-).

Most of biochemical reactions in the body are known to generate ROS, which are potent in damaging important biomolecules like proteins, nucleic acids and lipids if they are not scavenged by antioxidants (Dephour *et al*., 2009). Free radicals are well known to be involved in aging and pathogenesis of stress-related disorders like diabetes, nephrotoxicity, hepatotoxicity, malignancy, cardiovascular disorders, inflammation and neurological disorders (Mondal *et al*., 2006; Pham *et al*., 2008).

An antioxidant is a chemical substance that donates an electron to a free radical and converts it to a harmless molecule. Natural antioxidants such as catalase, superoxide dismutase, Vitamins C and glutathione peroxidase have been reported to be capable of scavenging ROS (Robertson *et al*., 2004; Fatmah *et al*., 2012). Human cells are well protected by antioxidant defense systems against ROS attack however at low

concentrations of antioxidant enzymes some cells have shown to be sensitive to ROS (John, 2007; Tiwari *et al*., 2013). The cellular antioxidant level is used to determines the susceptibility of tissues to oxidative damage. This level normally changes during oxidative stress (Robertson *et al*., 2004).

A wide variety of antioxidants are naturally obtained from plants that constitute our daily diet. Commonly known dietary antioxidants are vitamin C, E and carotenoids (Ahoua, 2012). Consumption of food and fruits rich in antioxidants plays a significant role in augmenting the body's natural resistance to oxidative stress (Shahidi, 2000). Plants also have many other non-nutrient antioxidants such as phenols, flavonoids and alkaloids. These polyphenol compounds have been extensively studied and documented as quenchers of free radicals (Rice, 1995).

Commercially available antioxidant drugs include butyrate hydroxyanisole, butylated hydroxytolune and propyl gallate (Tanaka *et al*., 2002; Gulcin, 2008). However, studies have shown that these synthetic antioxidants have toxic effects (Kulisic *et al*., 2004). This has led to some restrictions being imposed on their use. Researchers now have focused their attention on plant derived antioxidants among others.

Since primordial times, plants have provided a novel source of medicine and they have greatly aided humans to maintain health. It's estimated that 80 percent of the global population actively relies on therapies from herbs for their health needs (Cowan, 1999;

WHO, 2013). The popularity of herbal medicine is due to their better cultural adequacy, availability and less adverse effects (Thevasundari *et al*., 2011).

Globally, more than 1200 plants have been traditionally used for their antioxidant activities (Mohan *et al.*, 2013). Ethno-pharmacological surveys indicate that medicinal plants play a vital role in the management of oxidative stress-related disorders (Keter and Mutiso, 2012). Plant extracts naturally possess phytochemicals such as flavonoids, tannins, phenols and alkaloids, which are well known antioxidants and are currently pursued as alternative and complementary remedies against oxidative stress-related disorders (Dephour *et al*., 2009). Several efficacy studies done on herbal plants have shown that plant based antioxidants are relatively safe, cost efficient and effective in disease management.

The genus *Ficus* is widely known to have strong antioxidant properties due to their richness in phenols and flavonoids (Abdel, 2009). Traditionally, *F. sycomorus* fruits, stem barks and roots have been used as herbal remedies for several ailments such as diarrhea, liver disease, skin infections, stomach disorders, helminthiasis, lactation disorders, epilepsy, tuberculosis, sterility and diabetes mellitus (Igbokwe *et al*., 2010; Daniel and Dluya, 2016).

S. henningsii is another widely distributed evergreen herbs in East Africa. It is known by traditional health practitioners as a cure for rheumatism, snake bite, abdominal pain, gastrointestinal pain, gynecological complaints, malaria and diabetes mellitus (Hutchings, 1989). The crude extracts of *S. henningsii* have been documented to possess significant therapeutic agents against stress-related disorders (Oyedami *et al*., 2010; Piero *et al*., 2011).

It has been successfully used by Mbeere community in Embu County in treatment of diabetes, which is an oxidative stress-related disorders (Piero *et al*., 2011).

In view of this background, the present study seeks to investigate the *in vitro* antioxidant activities of Dichloromethanolic (DCM) leaf extract of *S. henningsii* and stem bark extract of *F. sycomorus.* The study aims to explore and provide preliminary information on *S. henningsii* and *F. sycomorus* as possible bio-resources for generating easily available herbal formulations that are more effective in the treatment and management of oxidative stress-related disorders. The study also aims to reveal relevant research gaps that need to be explored further.

1.2 Statement problem and justification

Oxidative stress has been implicated in pathogenesis and progression of several lifestyle diseases like atherosclerosis, diabetes mellitus, hypertension, ischemic complications, malignancies, cardiovascular diseases, eye disorders, lung, pancreatic and kidney disorders, cancer as well as, ageing and diseases associated with reproductive system (Yoshikawa and Naito, 2002; John, 2007; Rahman *et al*., 2012).

Stress-related disorders have become epidemic in developing and under-developed countries. Conventional therapeutic strategies mostly attempt to relieve the clinical manifestations of these disorders and their complications. However, studies have shown they tend to increase toxicity leading to damage of sensitive organs like liver and brain, they are also suspected to be mutagenic (Kulisic *et al*., 2004). Against this backdrop, the popularity of complementary drugs for oxidative stress-related disorders has increased and

plant based antioxidants therapies are now widely practiced in most of the developing countries (Mohan *et al*., 2013).

Since antioxidants hold a key in preventing oxidative stress-related disorders, many plant extracts and their secondary metabolites are being explored for their antioxidants effects (Gomathi *et al*., 2013). The use of plant based antioxidants, plays an important role in preventing activation of the oxidation induced signaling pathways in our bodies (Joseph *et al.*, 2002). Therefore, the identification of the antioxidants activities of DCM leaf extract of *S. henningsii* and stem bark of *F. sycomorus* are important step in increasing our understanding about their usage in treatment of various stress-related disorders.

1.3 Research questions

i. Do the DCM extracts of *S. henningsii* and *F. sycomorus* have *in vitro* Hydrogen peroxide scavenging activities?

ii. Will the DCM extracts of *S. henningsii* and *F. sycomorus* have *in vitro* DPPH scavenging activities?

iii. Can the Dichloromethane extracts of *S. henningsii* and *F. sycomorus* have *in vitro* ferric reducing power activities?

iv. What are the total phenolic and flavonoid contents of the DCM extracts of *S. henningsii* and *F. sycomorus?*

v. What is the qualitative phytochemical composition of DCM extracts of *S. henningsii* and *F. sycomorus?*

1.4 Objectives

1.4.1 General objective

To determine *in vitro* antioxidant activities of DCM leaf extracts of *S. henningsii* and stem bark extract *F. sycomorus.*

1.4.2 Specific objectives

i) To establish *in vitro* hydrogen peroxide radicals scavenging effect of DCM extracts of *S. henningsii* and *F. sycomorus.*

ii) To evaluate *in vitro* DPPH radicals scavenging effect of DCM extracts of *S. henningsii* and *F. sycomorus.*

iii) To determine *in vitro* antioxidants ferric reducing power of DCM extracts of *S. henningsii* and *F. sycomorus* on ferric cyanide complex.

iv) To establish the total phenolic and total flavonoid contents of DCM extracts of *S. henningsii* and *F. sycomorus.*

v) To determine qualitative phytochemical composition of DCM extracts of *S. henningsii* and *F. sycomorus.*

CHAPTER TWO

LITERATURE REVIEW

2.1 Oxidative stress

Generation of excessive free radicals and their active intermediates in the body is the etiology of oxidative stress. Therefore, creating a situation of higher free radicals than antioxidants levels (Halliwell, 1995; Sendogdu *et al*., 2006; Halder *et al*., 2010). Presently, the concept of oxidative-stress is confined to Reactive Oxygen Species (ROS) like hydroxyl, superoxide and hydrogen peroxide radicals which are capable of attacking biochemical molecules like proteins, carbohydrates and lipids (Garrido *et al*., 2004).

Involvement of oxidative stress in the pathogenesis and progression of several lifestyle diseases like atherosclerosis, diabetes mellitus, hypertension, ischemic complications has been significantly highlighted (Yoshikawa *et al*., 2002). They are also known to damage and oxidize cellular components like nucleic acids, protein and lipids which leads to cardiovascular diseases, eye disorders, lung, pancreatic and kidney disorders as well as cancer, ageing and diseases associated with reproductive system (John, 2007; Rahman *et al*., 2012). There is increasing evidence that link ROS to cellular damage through the activation of stress sensitive signaling pathways that are used to regulate gene expression (Joseph *et al*., 2002).

2.2 Genesis of reactive oxidative species (ROS)

Generation of reactive oxygen species (ROS) involve normal intracellular metabolism in mitochondria, peroxisomes and several cytosolic enzyme systems. It is also known that a number of external agents can initiate ROS production (Rahman *et al*., 2012). Biologically,

ROS are generated in the body during oxidation process, and they are removed via an elaborate antioxidants defense systems (Yoshikawa *et al*., 2002).

Generally, the formation of ROS in a cell is majorly through enzymatic pathways. Possible enzymatic reactions capable of synthesizing ROS includes respiratory pathways, those involve in phagocytosis, prostaglandins synthesis and cytochrome P450 system (Pham *et al*., 2008). Table 2.1 below summarizes the common ROS, sources and their effects.

Table 2.1: Types of reactive oxygen species (ROS), sources and their effects

ROS Radicals	Formula	Sources	Effects
Hydroxyl	OH^-	Fenton reaction	Attack on most cellular component and damage them
Superoxide	O_2^-	Auto-oxidation reactions and by the ETS	Release of Fe^{2+} from iron sulphur proteins and ferretin
Peroxyl	RO^-	Radical reactions with cellular component	Causes Lipid peroxidation and DNA damages
Alkoxyl	RO^-	Radical reactions with cellular component	Causes Lipid peroxidation and DNA damages
Hydroperoxyl	RO_2^-	Radical reactions with cellular component	Causes Lipid peroxidation and DNA damages

Source: Rahman *et al*., (2012)

Under normal physiological condition, Reactive oxygen species (ROS) generated do not pose any threat. However, when they are excessively generated and they are not scavenged by available antioxidants they result into oxidative stress. From therapeutic viewpoint,

oxygen-derived radicals have been attracting attention because of their ability to react with molecules in biological tissues and membranes. Therefore, they are responsible for inducing various oxidative stress-related disorders. ROS tends to affects signals of several genes which encode transcription factors required for differentiation, and development of cells as well as stimulating cell-cell adhesion. They are also useful in bringing out cell apoptosis in birth canal during parturition and strengthening of biological defense mechanisms during physical exercise and increased expression of antioxidant enzymes (Yoshikawa *et al*., 2002). Some ROS produced by neutrophils and macrophages are used to eliminate antigens produced by pathogens (Tiwari *et al.*, 2013).

2.3 Endogenous antioxidants defense systems

Antioxidants provide protection living cells against the damage from free radicals. They react and inhibit free radicals produced during metabolic reactions, thereby, preventing damage to tissue or cells (Prior *et al.*, 2005). These compounds function as free radical scavengers, metal chelators, as well as reducing agents and quenchers of reactive oxygen species (Mohamed *et al*., 2010). Therefore, antioxidants have a significant role in the overall prevention of oxidative damage related diseases.

Cell damage is mediated through the oxidation chemical process which involves transfer of electrons from compounds that generate free radicals. Antioxidants compounds arrests oxidative stress by inhibition of the oxidation chain reactions (Velioglu *et al*., 1998). These antioxidants remove free radical intermediates and inhibiting other oxidation reactions hence terminating chain of cell damaging reactions (Lopez *et al.*, 2007; Tiwari *et al.*, 2013).

To survive oxidative stress, mammalian cells have established an array of endogenous antioxidants defense mechanisms. These comprises of enzymes based antioxidants like superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase and non-enzyme based antioxidants like, ascorbate, α-tocopherol, thioredoxin, cysteine, polyphenols, glutathione, caratenoid and vitamins (A, C and E) as shown in figure 2.1 (Robertson *et al*., 2004; Fatmah *et al*., 2012; Rahman *et al*., 2012; Tiwari *et al.*, 2013).

Glutathione is among the most abundant antioxidant enzyme present in all mammalian tissues at a concentration of 1–10mM, with the liver having the highest concentration. The enzyme is a common biomarker of redox imbalance. Glutathione enzyme participates in many cellular reactions as scavengers of free radicals. Deficiency in glutathione tend to contributes to oxidative stress which will ultimately leads to stress-related disorders (Fang *et al*., 2002; Tiwari *et al.,* 2013).

Another common antioxidant enzyme found in peroxisome of living cells is Catalase. Catalase regulates hydrogen peroxide metabolism. Hydrogen peroxide is a highly reactive molecule produced as a byproduct from energy production pathways (Halliwel, 1995). Catalase is responsible for the removal of hydrogen peroxide (H_2O_2) by converting it to water and oxygen. Studies have shown that a decrease in the enzyme catalase leads to increase in oxidative damage in the liver due to toxic effect of hydrogen peroxide radicals (Tiwari *et al.,* 2013). Therefore, catalase is a key enzyme against oxidative stress generated by highly dangerous peroxide radicals.

Superoxide dismutase (SOD) catalyzes the dismutation of superoxide into molecular oxygen and hydrogen peroxide. The enzyme plays a key role in cell protection against the toxic superoxide radical produced during aerobic respiration. All mammalian tissues are known to contain three forms of metal based Superoxide dismutase which are Manganese-SOD, Copper-Zinc-SOD, and extracellular SOD (Rahman *et al*., 2012).

Vitamins E, C and β-carotene are the principal vitamins-based antioxidants, used against free-radicals (Nakamura and Omaye, 2005). Vitamin E serves as the first line of defense against peroxidation of phospholipids, as a chain-interrupter antioxidant, neutralizer of free radicals and inhibitor of lipid peroxidation (McDowell, 2000). Vitamin C is a potent antioxidant which is highly soluble in water and exist in the aqueous state in cells. It contributes to radical scavenging via electrons donations, therefore, brings about the stability in ROS produced (Schaffer *et al*., 2005). Normally, cells use their endogenous antioxidants defense systems to neutralize ROS. This is an important process in maintaining proper cellular function (Matough *et al*., 2012). When this system fails to provide enough compensatory response for restoration of cellular redox balance, then, reduced glutathione (GSH) levels fall and oxidative stress ensues. GSH is the major cellular antioxidant which is regenerated by glutathione reductase and reduced Nicotinamide Adenine Dinucleotide Phosphate (NADPH) as shown in Figure 2.1 (Joseph *et al*., 2002).

It has been noted that an over expression of the antioxidant enzymes tends to be advantageous to cells since they offer an extra protection against the deleterious effects of ROS (Joseph *et al.*, 2002). The cellular antioxidant status is used as a determinant to show

susceptibility of tissues to oxidative damages. This status is usually altered in response to oxidative stress (Robertson *et al*., 2004). Antioxidants whether exogenously, endogenously, synthetically or naturally produced they are effective in preventing oxidative stress (Kalekar *et al.,* 2013).

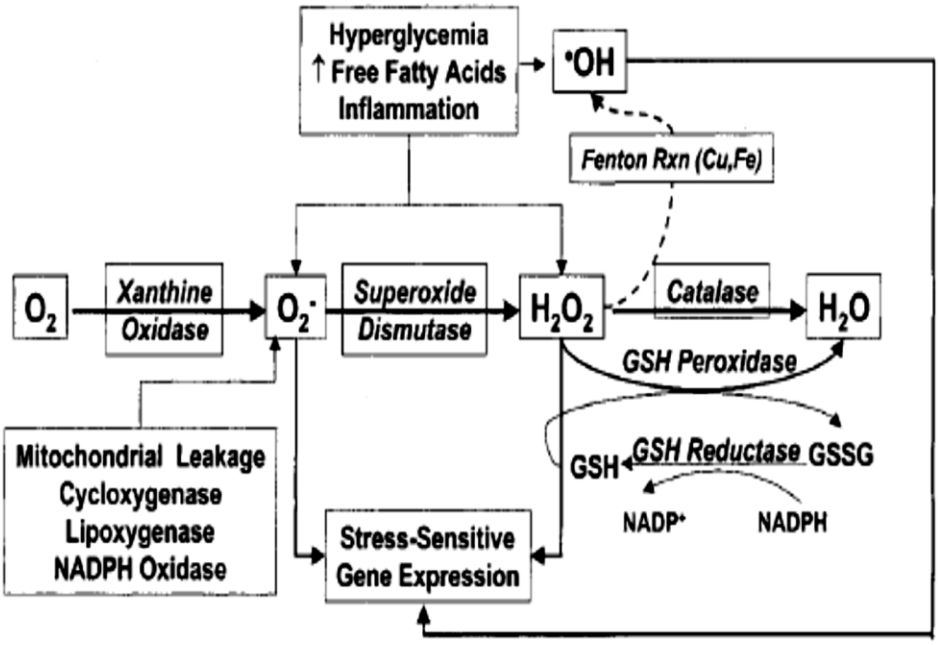

Figure 2.1. Endogenous antioxidant defense mechanisms. Source: (Joseph *et al*., 2002)

2.4 Exogenous Source of Oxidative stress

These are external factors that can induce oxidative stress including; smokes from cigarette, ozone exposure, hyperoxia, and heavy metal ions. Inhalation of the cigarette smoke can activate accumulation of neutrophils and macrophage which increases the oxidant injury of the tissue. The smoke also contains superoxide and nitric oxide which are free radicals that causes oxidative stress (Birben *et al*., 2012).

Lipid peroxidation and influx of neutrophils into the epithelial cells lining the breathing system is usually caused by long term exposure to ozone. On the other hand, production of

inflammatory mediators such as cationic proteins, lactate dehydrogenase, eosinophil and albumin is caused by little exposure to ozone. This elicits inflammation of epithelial cells lining the air ways (Hiltermann *et al*., 1999).

Differential higher oxygen levels than the normal partial pressure of oxygen in the lungs or other body tissues causes hyperoxia. Higher levels of oxygen tend to increase the production of reactive oxygen due to availability of excess oxygen for oxidation (Comhair *et al*., 2000). Hydrogen peroxide can be produced by conversion of hydroxyl, superoxide and other organic radicals by ionizing radiation and this reaction occurs in presence of oxygen molecules. The hydrogen peroxide produced then reacts with redox active metal ions like Cu^{2+} and Fe^{2+} in the Fenton reactions leading to oxidative stress (Chiu *et al*., 1993).

Biosynthesis of reactive radicals is induced by heavy metal ions such as iron, nickel, cadmium, copper, mercury, lead and arsenic. They also cause cellular damage via lipid peroxidation, reacting with nuclear proteins and DNA molecules during Fenton reactions. Arsenic compounds indirectly induce ROS formation by activating radical producing system in the cell (Birben *et al*., 2012).

2.5 Oxidative stress-related disorders

2.5.1 Neurodegenerative diseases

Parkinson's and Alzheimer's are two widely known neurodegenerative oxidative stress disorders. Alzheimer's disease, occurs when the amyloid β-peptide induces oxidative stress via lipid peroxidation. Clinical test has shown that Alzheimer patients tend to have high

levels of 4-hydroxy-2-nonenal (HNE), in their cerebrospinal fluid. HNE is a secondary product of lipid peroxidation, HNE molecules when they accumulate in the cell cytoplasm modifies amyloid β-peptides which then inhibits the formation of proteasome leading to neuro-degeneration (Butterfield *et al*., 2013). Moreover, HNE modify membranes leading to impairment of Na^{+}/Ca^{2+} pumps which disrupts glucose and glutamate transporters along the membrane by interfering with ionic balances hence disrupting the cell signals (Keller *et al*., 1997).

In the case of Parkinson's disease, production of ROS causes the dysfunction of mitochondria and degeneration of dopamine cells (Zarkovic, 2003). *In vitro* studies have shown that there is a decrease in dopamine uptake when the dopaminergic neuron is incubated with HNE (Morel *et al*., 1998). In most cases the end products of lipid peroxidation have been shown to enhance pathogenesis of Parkinson disease (Morel *et al*., 1998).

2.5.2 Cardiovascular diseases

Hypertension, smoking, hypercholesterolaemia, diabetes, stress, poor diet and physical inactivity are some of the multiple etiologies of cardiovascular diseases (Bahorun *et al*., 2006). ROS associated with pathogenesis of most cardiovascular diseases such as atherosclerosis, cardiomyopathy, ischemia, high blood pressure, cardiac hypertrophy and congestive heart failure (Ceriello *et al*., 2008). For instance, in the case of atherosclerosis, ROS from vascular cells oxidize the plasma low-density lipoproteins (LDLs) leading to formation of HNE and other aldehydes. The HNE formed attacks the scavenger receptors on the surface of the smooth muscle cells cause development of foam cells. The buildup of

foam cells forms plaque which causes fibrogenesis (Vindis *et al*., 2006). Diabetes manifest itself when the islets of pancreas are damaged by ROS leading to decline in insulin secretions by alpha cells of the (Haydent and Tyagi, 2002).

2.5.3 Cancer

The pathogenesis of cancer in human being is a complicated process which involves changes at cellular and molecular levels. These changes are brought about by diverse endogenous and exogenous stimuli. During carcinogenesis, ROS production tend to increases when oncogenes are activated, energy metabolism is modified as mitochondrial dysfunction ensues (Barrera, 2012). Oxidative DNA damage leads to development of various malignancies in the body (Valko *et al*., 2004). Consumption of fats has been associated with development of cancer such as leukemia, ovary, breast, rectum among elderly people (Limbers and Young, 2015). This could be caused by lipid peroxidation as a result of ROS (Limbers and Young, 2015). Infection by *Helicobacter pylori* is clinically linked to the development of gastric cancer due to its ability to increase the production of ROS in human stomach (Vasavidevi *et al*., 2006).

2.5.4 Rheumatoid arthritis

Chronic inflammation at the joints and tissue around the joints is the manifestation of rheumatoid arthritis (DeGroot *et al*., 2011). Accumulation of free radicals at the site of inflammation is associated with pathogenesis of this disease. Increased levels of isoprostanes and prostaglandins in both the serum and synovial fluid has been associated with various rheumatic diseases (Mahajan and Tandon, 2004). ROS have been reported to cause osteoarthritis by damaging the bone cartilage (Yudoh *et al*., 2005).

2.5.5 Nephropathy

Renal disorders like glomerulonephritis, chronic renal failure, proteinuria, uremia and nephritis are also associated with oxidative stress (Galle *et al*., 2001). Nephrotoxicity of some drugs such as gentamycin, vinblastine, cyclosporine and bleomycin is mediated by oxidative stress through lipid peroxidation (Massicot *et al*., 2007). In high altitudes, acute mountain sickness (AMS), high altitude pulmonary edema (HAPE) and high altitude cerebral edema (HACE) may be as a result of increased in ROS level (Rahman *et al*., 2012).

2.5.6 Ocular disease

Oxidative stress is also implicated to some age-related eye disorder like cataract, corneal disease diabetic retinopathy among others (Santosa and Jones, 2005). Studies by Dogru *et al.* (2009), showed the role played by oxidative stress in damaging the cells of the anterior eye leading to disorders like dry eyes, conjuctivochalasis and tobacco smoke-induced ocular epithelial surface.

2.6 Conventional management of oxidative stress

The natural antioxidants such as superoxide dismutase, catalase, glutathione peroxidase and both Vitamins C and E have been documented as a protector of cells against lipid peroxidation, as scavengers of free radicals and defense against oxidative cellular damage and injury (Robertson *et al*., 2004; Fatmah *et al*., 2012; Mohan *et al*., 2013). High concentration of vitamin C is known to improve endothelial dysfunction in diabetes conditions (Mohora *et al*., 2007). Lipoic acid, on the other hand, leads to a decrease in the severity of neuropathy by maintaining GSH levels, improvement in endothelial function and improvement in insulin sensitivity (Joseph *et al*., 2002; Matough *et al*., 2012).

Commercially available antioxidant drugs include, butyrate hydroxyanisole (BHA), butylated hydroxytolune (BHT) and propyl gallate (PG) (Tanaka *et al.*, 2002; Gulcin, 2008). Complex diseases such as atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer have been prevented and managed using antioxidant-based drug formulations (Gomathi *et al*., 2013). Due to adverse effects of synthetic antioxidants, high costs, unavailability and increase incidences of stress related disorder (Kibiti and Afolayan, 2015). There is a clear need for development of alternative herbal remedies which are inexpensive with minimal side effects.

2.7 Herbal management of oxidative stress

Use of natural antioxidants from plants has considerable increase (Shyur *et al*., 2005). Plants extracts are widely believed to be safe, cost effective and readily available. There is also a common belief that plant derivatives have been in practice from time immemorial to cure and treat human ailments (Shyur *et al*., 2005; Kambli *et al.,* 2014). Recently, considerably efforts have been directed in search of natural antioxidants from plants due to their ability to protect the cells against free radicals' damage (Kibiti and Afolayan, 2015).

The chief sources of natural antioxidants are spices and herbs which are generally grouped as vitamins, polyphenols (flavonoids and phenolic acids), and volatile compounds (Sakanaka and Ishihara**,** 2008). Normally, therapeutic plants contain different constituents of phytochemicals which are bioactive showing significant therapeutic effects. Phenols, Saponins, flavonoids, tannins and alkaloids are among the well-known secondary metabolites possessing antioxidant activities (Daniel and Dluya, 2016).

The two main phytochemicals which have been documented in several medicinal studies as potent antioxidants are flavonoids and phenols. Flavonoids plays a key role as an antioxidants scavenger of ROS, delaying or inhibiting the oxidation of biomolecules and inhibition of lipid peroxidation by free radicals (Kumawat *et al.*, 2012; Kambli *et al.*, .2014). Flavonoids exert their antioxidant activities through inactivation of the ferric ions, chelation and suppression of superoxide-driven Fenton reaction (Kibiti and Afolayan, 2015).

Phenols are also common in diet and are divided into two classes, the benzoic derivatives like gallic acid and cinnamic derivatives like caffeic acids. The mechanism of action of phenolic compounds as antioxidants activity is through inactivation of lipid free radicals or by preventing the decomposition of hydroperoxides into free radicals (Dai and Mumper, 2010).

Alkaloids express antioxidants ability by removing the effects of hydrogen peroxide-induced oxidative damage via reduction of electrons or hydrogen atoms (Kim *et al*., 2004). Saponins shows their ability as antioxidant through scavenging hydrogen peroxide. On the other hand, terpenoids are active antioxidants against the enzyme based reactions (Joshi *et al*., 2008), while steroids, are believed to possess both antioxidants and analgesic properties (Argal and Pathak, 2006). Tannins achieve their antioxidants ability through chelating metal ions such as $Fe^{3+}$and destabilize some key steps in the Fenton reaction (Kibiti and Afolayan, 2015).

A variety of medicinal plants contain large amounts of polyphenols which have been shown to be therapeutic to several stress-related disorders in human being. Thousands of plants with medicinal values have been identified and used to treat different ailments (Farnsworth and Soejarto, 1991). Various species of gingers in tropical countries have been shown to exhibit strong antioxidants activities (Sushi *et al*., 2008; Mohamed *et al*., 2011).

Ludwigia octovalvis and *Vitis thunbergii* extracts exhibited strong antioxidant activities as a chemoprotector against cancer (Shyur *et al.*, 2005). *Acacia nilotica* has been shown to have pharmacological activities such as antibacterial, antidiabetic, insect repellent, antioxidant and antiviral properties (Bachaya *et al*., 2009). Traditionally the stem bark, leaves, flowers and pods of many plants have been used to treat ailments like cough, dysentery, fever, gallbladder, hemorrhoid, sclerosis, smallpox, bleeding piles, leucoderma, tuberculosis, leprosy and menstrual problems (Vadivel and Biesalski, 2012).

Proteins from soy beans have been attributed to a number of beneficial effects on human health which includes prevention of obesity lowering of cholesterol levels and have also been linked to possible prevention of cancer, menopausal disorders, osteoporosis and cardiovascular diseases (Mitra *et al*., 2014). Other plants used to promote antioxidant effects include *Phyllanthus emblica*, *Curcuma longa*, *Tinospora cordifolia*, *Momordica charantia* (Bitter melon), *Trigonella foenum* (Fenugreek seeds) and *Gymnema sylvestre* (Hui *et al*., 2009; Kalekar *et al*., 2013).

Although there are great advancements in regards to our recent understanding of the way oxidative stress induced by various cellular based reactions ultimately leads to tissue damage, there is limited knowledge about the effective therapeutic strategies aimed at preventing or delaying the development of this tissue damages Thus, there is a need to develop a therapeutic agent that can prevent or delay the development of these tissue damages. The use of plant antioxidants can be useful in preventing activation of the oxidative induced signaling pathways since they all have a common biochemical basis (Joseph *et al*., 2002).

2.8 *Ficus sycomorus*

2.8.1 Description

Ficus sycomorus (Linn) is a member of mulberry family; Moraceae, which consists of about 40 genera and over 1400 species (Igbokwe *et al*., 2010). *Ficus* is one of the most common tree with diverse local names like Bamba (Amharic), Sabula (Arabic) (Khemira and Mars, 2015), wild fig, strangler (English), Katema (Lozi), Barda (Somali), Sicomoro (Spanish), Mukuyu (Embu), Makuyu (Kamba) Omukhuyu (Luhya) Figuier (French) and Mukunyu (Luganda). The species *sycomorus* comes from the Greek word 'sykamorea' (Orwa *et al*., 2009)**.** Sycamore was the tree that Zacchaeus climbed to see Jesus according to biblical accounts (Magness, 2012).

F. sycomorus is a large, semi-deciduous plant with maximum height of 46 metres. The stems are buttressed while young stems have pale green barks with a soft powdery covering but when they become older, they turn grey-green which are fairly smooth (Sirisha *et al*., 2010). A grey scale appears scattered but where scales have fallen off a pale brown patches

are formed. The stem when cut exudes pale pink heavy latex flow which is oxidized to brown. Leaves are broad with a rounded apex with margin which is entire. Flowers are unisexual and greenish. The fruits are either solitary or paired, normally born on old part or end of a stem. They appear yellow-red to reddish-purple when ripe (Olusesan *et al*., 2010), figure 2.2

Fig 2.2 *Ficus sycomorus Source:* (*Pikiwiki Israel 5476 fruits of sycamore tree.jpg***)**

2.8.2 Distribution

F. sycomorus is a common savannah tree that grows along water bodies. The species is adversely affected by frost although it can survive cold weather (Orwa *et al.*, 2009). Common habitat includes rain forest, riverside forest, woodland, along edges and in bush land; sometimes they appear as single trees in farmland and they are also found as single trees on rocky outcrops. Their altitude ranges from 0-2000 metres, the tree does well in deep well-drained loam to clay. Sandy soils may also be suitable if they have shallow ground water (Orwa *et al.*, 2009). Documented species distribution in their native range

shows greater population distribution all over Africa and Middle East countries (Sirisha *et al*., 2010; Sarg *et al.*, 2011).

2.8.3 Cultural use

The tree has a wide cultural application. Mature fruits can be eaten while fresh, stewed, or dried and stored for later use (Orwa *et al.*, 2009). They are also used to make alcoholic beverage. Leaves are added to soups and groundnut dishes for spicing. In Ghana, the bark is added to kola nut, while the wood ash is usually used as a salt (Nkafamiya *et al.*, 2010; Auda, 2012). In semi-arid areas they are valuable fodder with fairly high nutritive value. Shed leaves form excellent humus after decay that improves soil qualities such as nutrient levels, infiltration rate and water retaining capacity of the soil (Orwa *et al.*, 2009; Adoum *et al*., 2012).

The tree is used for timber, beehives, doors, dugout canoes, stools, drums, carvings, mortals, pestles, firewood, charcoal or as the base block to make fire by the friction method (Orwa *et al*., 2009). Wild *Ficus* trees in crop husbandry are used to control sand-dune fixation and riverbank stabilization (Orwa *et al.*, 2009). The tree forms an excellent shade and shelter (Orwa *et al.*, 2009). It has been traditionally used as an ornamental tree near temples, wells, roadsides and community places (Orwa *et al.*, 2009; Thagriki *et al.*, 2015). In agriculture, it is usually intercropped with bananas. The sycamore tree is highly valued for spiritual and sacred devotions as mentioned in the Holy Bible and in ancient Egypt (Orwa *et al.*, 2009; Magness, 2012; Olusesan *et al*., 2010).

2.8.4 Ethnomedicinal use

Various members of the genus *Ficus* are used in traditionally medicine for therapeutic purposes. Plants parts like leaves stem and roots have been shown to possess medicinal value (Igbokwe *et al*., 2010). The grounded bark is used to treat ailments like, coughs, throat, ringworm and chest diseases, lactation disorders, while the milky latex is used for treatment of ringworm, dysentery, abdominal pain, infertility (Orwa *et al.*, 2009). Leaves have shown effectiveness against jaundice, snakebite, epilepsy and tuberculosis (Orwa *et al.*, 2009). Finally, the roots have both laxative and anti-helmintic properties (Orwa *et al.*, 2009; Sirisha *et al*., 2010; Kambli *et al.*, 2014).

2.9 *Strychnos henningsii*

2.9.1 Description

Strychnos henningsii (Gilg) is a member of Logoniaceae family. The genus *Strychnos* has about 190 species mainly found in the tropics (Oyedemi *et al*., 2010). Some local names across Kenya tribes includes: Muthumbi (Mbeere-Embu) Muteta (Kikuyu/Kamba), Maset (Kipsigis), Muchimbi (Meru), Kapkamkam (Pokot), Entuyesi (Maasai), Nchipilikwa (Samburu), Turukukwa (Tugen) and Yapolis (Turkana) Red bitter berry (English) (Kipkemoi *et al*., 2013).

Strychnos is a small-large, erect, greatly branched and evergreen tree, with a height of about 2-12metres. It is one of an important indigenous medicinal plant, but in Kenya it is threatened to become extinct due to indiscriminate and unsustainable harvesting by the herbalists, hotels and restaurants owners (Kipkemoi *et al*., 2013). Leaves are dark green with a characteristic aromatic-pungent smell, leaf margin is entire, and the leaves are

oppositely arranged on stem. The stem bark is green-reddish while the twigs have waxy skins. Flowers are scented, yellowish-green but turn orange when they become old. They are of cymes types and are borne on flat clusters. Ovary is a globule while style is short. Their fruits are drupe and appear broad and roundish. The fruit changes to red, brown or orange when ripened (Sparg *et al*., 2000), figure 2.3.

Fig 2.3. *Strychnos henningsii* Source: (www.prota4u.org)

2.9.2 Distribution

S. henningsii is a highly diversified species occupying a wide range of habitat from dry to moist forests, wooded hillsides, thickets, on rocky hills, coastal forests to stream banks. The plant can thrive at an altitude range of 340-2 000 metres above the sea level. Documented species distribution shows a native range across East and South Africa countries of Angola, Kenya, Mozambique, South Africa, Swaziland, Tanzania and Uganda. Exotic species are also available, thus making it one of the most widely distributed species in east and southern Africa. The species have been often associated with *Olea* and *Podocarpus* species (Oyedemi *et al*., 2010).

2.9.3 Cultural use

It has several cultural uses depending on the communities. In Mbeere community of Embu County, fruits are used to flavor beer. In most pastoralist communities of East Africa, S. *henningsii* is used in the preparation of fatty-meat and milk soups. The bark contains a poisonous bitter alkaloid that causes paralysis. In highland areas, it is used to control soil erosion. Due to its vast branches it provides an excellent shade and shelter. It is also an ornamental plant due to its shiny foliage, pleasant shade and fragrant flowers (Daniel and Dluya, 2016). It is also used as a live fence while its pole is good for fencing and making tool handle. It produces a valuable timber which appear brown to dark grey, is hard, heavy, and resistant to termites therefore durable (Maphosa and Masika, 2010).

2.9.4 Ethnomedicinal use

S. henningsii has been used in traditional medicine to treat various complications including syphilis, gastrointestinal pain, rheumatism, snake bites, abdominal pain and malaria, as an anaesthology due to its muscle relaxing effects, healing wounds, mouth antiseptic and diabetes mellitus. The bark powder has been used as a mouth antiseptic and applied to wounds in cattle and horses for healing. *S. henningsii* is a potential plant for development of new antinociceptive and antispasmodic drugs (Orwa *et al*., 2009; Oyedemi *et al*., 2010; Kipkemoi *et al*., 2013).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection of medicinal plants

S. henningsii leaves and stem barks of *F. sycomorus* were collected from their natural habitats from Makunguru village, Mbeere North sub-county, Embu County, Kenya. An acknowledged authority authenticated their botanical identities and voucher specimens (*Strychnos S.* 001/10/21015 and *Ficus S.* 001/10/2015) were deposited in the Kenyatta University Herbarium for future reference. Sample materials were carefully sorted, packed in a sealed bags and transported to the Department of Biochemistry and Biotechnology, Kenyatta University where the study was undertaken.

3.2 Preliminary processing and extraction

The fresh plant materials were air dried at room temperature under a shade for a week. After drying they were milled into fine powder by use of an electric mill. The powdered plant materials were sieved using mesh pore of 0.5 mm and packed in closed, dry sealed bags and stored awaiting extraction. For extraction, 250 g of each powdered plant material was soaked in 1litre of Dichloromethane (DCM) for 24 hours. The resultant extract was poured into a clean dry conical flask and then filtered using Whatman's No.1 filter papers. To obtained dried extra the filtrate was then concentrated under reduced pressure and vacuum using a rotary evaporator at a temperature of 40 ^{o}C. The concentrates were placed in airtight containers weighed and stored at -4 ^{0}C awaiting use in bioassays.

3.3 Determination of *In vitro* hydrogen peroxide scavenging activity

The *in vitro* hydrogen peroxide scavenging potential of DCM extracts of *S. henningsii* and *F. sycomorus* were determined following the protocol described by Ruch *et al.* (1989).

Briefly, 50Mm, pH 7.4 of phosphate buffer solution was used to prepare 250 ml solution of hydrogen peroxide (40mM). Hydrogen peroxide solution at a volume of 0.6 ml was added to1 ml of varying concentration (0.1-0.5 mg/ml) of the plant extract and ascorbic acid (standard). The mixture was left to stand for 10 minutes after which, absorbance was determined at 560 nm using UV spectrophotometer. Blank solution containing phosphate buffer was used as the negative control. This was done in three replicates. The percentage radical scavenging activity of the extract and ascorbic acid was determined as follows;

$$\% \text{ Hydrogen scavenging activity} = \frac{\text{Abs. of control-Abs. of sample/standard}}{\text{Abs. of control}} \text{ x } 100$$

Where *abs*. is absorbance.

3.4 Determination of *In vitro* Diphenyl- 2-picrylhydrazyl (DPPH) radical scavenging activity

The abilities of the DCM extracts of *S. henningsii* and *F. sycomorus* to scavenge DPPH radicals *in-vitro* were determined based on the method documented by Mamta *et al.* (2015). Following this method, 2.66 mg of DPPH were dissolved in 50 ml of ethanol to form a concentration of 0.135 mM. Various dilutions namely 0.2, 0.1, 0.05, 0.025, and 0.0125 mg/ml of the plant extracts and Ascorbic acid (standard) were prepared. One mililiter of the DPPH solution dissolved in methanol was mixed with 1ml of each diluted plant extract and Ascorbic acid (reference drug). The mixtures were then agitated thoroughly and left in a dark room for 30 minutes at room temperature. Three replicates of the assays were prepared. The absorbance of the mixture was then measured at 517 nm using a spectrophotometer. The actual decrease in absorbance was measured against that of the control. The negative control was a blank solution containing ethanol without H_2O_2. The

percentage DPPH scavenging abilities of the plant extracts were then derived using the equation below:

$$\% \text{ DPPH scavenging activities} = \frac{\text{Abs. of control-Abs. of sample/standard}}{\text{Abs. of control}} \times 100$$

Where *abs*. is absorbance.

3.5 Calculation of half maximal Inhibitory Concentrations (IC_{50}) in hydrogen peroxide and DPPH radicals

The half maximal inhibitory concentration (IC_{50}) of DCM extracts of *S.henningsii, F. sycomorus* and the Ascorbic acid (standard) were analysed using linear regression analysis in MS excel. The IC_{50} which represents the concentration at which 50% of the radicals were scavenged by test samples, was determined from a graph of percentage scavenging activity against the concentration of the test sample.

3.6 *In vitro* Ferric reducing power assay

The *in-vitro* ferric reducing power of the DCM extracts of *S. henningsii* and *F. sycomorus* alongside ascorbic acid (positive control) were established according to the protocol described by Oyaizu (1986). In brief, various concentrations (0.2-1mg/ml) of 1 ml of the plant extracts and ascorbic acid were added to 2.5 ml of 0.2 M phosphate buffer of pH 7. The resulting solution was then mixed with 2.5ml of potassium ferricyanide and incubated at a 50 ^{0}C for 20 minutes. Afterwards, 2.5 ml of trichloroacetic acid (10%) was then put to the mixture and centrifuged at 10 minutes at 3000 rpm. Then, 2.5 ml was drawn from the upper layer of the solution then added to 2.5ml of distilled water and 0.5 ml freshly prepared ferric chloride ($FeCl_3$) solution) (1%) was added. The assay was done in triplicates. The absorbance of the extracts and ascorbic acid was determined at 700 nm using spectrophotometer.

3.7 Determination of total phenolic contents

Folin- Ciocalteu reagent was used to determine the total phenolic levels of the plant extracts as described by Spanos and Wrolstad (1990) and modified by Lister and Wilson (2010). Briefly, 2 ml of each plant extract, 2.5ml of 10% dilution of Folin-Ciocalteu reagent and 2 ml of Na_2CO_3 (7.5%, w/v) were mixed and left to stand for 15minutes at 45 °C. The absorbance of all the treatments was determined at 765 nm spectrophotometrically. Gallic acid was used as the reference to derive the calibration curve. The total phenolic content was determined using the linear equation based on the calibration curve and contents expressed as milligrams of gallic equivalent per gram of dry weight (mg GAE/g dw) (Medini *et al.,* 2014).

3.8 Determination of total flavonoid contents

The methodology described by Lamaison and Carnet (1990) was used to determine the total flavonoid contents of the extracts. Briefly, volume of 1.5 ml of the extracts was mixed with an equivalent volume of 2% $AlCl_3.6H_2O$ (2 g in 100 ml methanol) solution. The solution was vigorously shaken to mix then incubated for 10 minutes and absorbance read at 430 nm using spectrophotometer. Rutin was used as the referenced to generate the calibration curve. The total flavonoid content was expressed as milligrams of rutin equivalent per gram of dry weight (mg RE/g dw) based on the calibration curve (Medini *et al.,* 2014).

3.9 Qualitative phytochemical screening

Qualitative phytochemical screening of DCM extracts of *S. henningsi* and *F. sycomorus* was done to determine presence or absence of selected plant secondary metabolites using standard methods described by Harbone (1998) and Kotake (2000). Secondary metabolites

screened for includes flavonoids, cardiac glycosides, saponins, alkaloids, sterols, phenolics and terpenoids. These phytochemicals are associated with antioxidants activities.

3.9.1 Saponins (Froth test)

To test for saponins, 2 ml of sodium bicarbonate solution were added to an equal volume of the plant extract then mixed vigorously. The mixture was incubated for 15-20 minutes at room temperature and was classified for saponin content as follows:

a) Absence of froth indicated absence of saponins
b) Less than 1cm froth indicated weak presence of saponins
c) Froth of 1.2 cm high indicated normal/moderate positive presence of saponins
d) Froth greater than 2cm it shows high levels of saponins

3.9.2 Alkaloids

For alkaloids, 5 ml of each extract was acidified using 1 ml of 1M HCl. The acidic medium was heated and then treated with Dragendroff‘s reagent. The formation of an orange or reddish brown precipitate was indicative of the presence of alkaloids.

3.9.3 Terpenoids (Salkowski test)

To determine presence terpenoids in the plants, 0.5 g of each of the extract was mixed with 1ml of ethyl acetate and 2ml of chloroform. Drop-wise, 3 ml of 2 M sulphuric acid (H_2SO_4) was added to the mixture to form a layer. Formation of a reddish brown colouration at the interface indicated presence of terpenoids.

3.9.4 Flavonoids (Sodium hydroxide test)

Extracts were tested for flavonoids by mixing 2 ml of each extract with 2 ml of 5 M sodium hydroxide (NaOH). An intense/golden yellow precipitate indicated positive results.

3.9.5 Cardiac glycosides (Keller-Kilian test)

To test for cardiac glycosides, 2 ml glacial acetic acid containing 2 drop of 10% ferric chloride ($FeCl_3$) solution were mixed with 0.5 g of the extracts. This was followed by addition of 1ml of concentrated sulphuric acid. Appearance of a brown, violet or greenish ring at the interphase shows presence of deoxysugar characterizing cardenolides.

3.9.6 Steroids

To screen for steroids, 0.5 g of each of the extract was dissolved in 2 ml of chloroform. Three mililiter of 2 M H_2SO_4 was carefully added by the sides of the test tube to form a layer. A reddish brown colour at the interface indicates the presence of steroidal ring.

3.9.7 Phenols

The extracts were screened for phenols by adding 1 ml of ferric chloride solution to 2 ml of each extract. Formation of blue to green colour indicated the presence of phenolics.

3.10 Data management and statistical analysis

The data for the *In vitro* antioxidant activities of the extracts was obtained from the concentrated solutions (stock solution) of the extract in different dilutions and replicates. The data were entered onto broad sheet in Ms Excel program. They were then subjected to descriptive statistics using Minitab statistical software17.0 (State College, Pennsylvania) and expressed as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) was done to determine the statistical significant difference among treatments. Tukey's tests was done for pairwise comparison of means. Unpaired student t-test was done comparison of mean total phenolic and flavonoid contents of DCM leaf extract of *S. henningsii* and stem bark of *F. sycomorus*. The values of $p \leq 0.05$ were considered to be significantly different. The data obtained were presented in a tabular form. The

phytochemical screening was done qualitatively and the result obtained (positive/ negative) for each test was recorded in a table.

CHAPTER FOUR

RESULTS

4.1 *In vitro* Hydrogen Peroxide Radical Scavenging Activities of DCM Extracts of *S. henningsii* and *F. sycomorus*

The *in-vitro* hydrogen peroxide scavenging potential of the DCM leaf extract of *S. henningsii* and stem bark extract of *F. sycomorus* were measured. Generally, both extracts of *S. henningsii* and *F. sycomorus* showed remarkable *in vitro* hydrogen peroxide scavenging activity at all the concentrations (0.1, 0.2, 0.3, 0.4 and 0.5 mg/ml). The extracts showed H_2O_2 scavenging activities in a dose related manner The H_2O_2 scavenging activities of both extracts were significantly lower than that of the standard (ascorbic acid) ($p \leq 0.05$; Table 4.1; Appendix I). However, there was no significant difference on the hydrogen peroxide scavenging activities of *S. henningsii* and *F. sycomorus* at all the tested concentration ($p > 0.05$; Table 4.1).

The DCM leaf extract of *S. henningsii* and *F. sycomorus* stem bark extract showed IC_{50} values of 0.330 mg/ml and 0.325mg/ml respectively, while that of the standard (ascorbic acid) was found to be 0.245 mg/ ml (Table 4.1).

Table 4.1: *In vitro* Hydrogen Peroxide Radical Scavenging Activities of DCM Extract of *S. henningsii* and *F. sycomorus*

	Concentration					
Groups	**0.1mg/ml**	**0.2mg/ml**	**0.3mg/ml**	**0.4mg/ml**	**0.5mg/ml**	**IC_{50}value**
Ascorbic Acid	27.72±2.38[a]	42.25±2.27[a]	60.38±1.77[a]	74.24±2.68[a]	88.05±2.55[a]	**0.245mg/ml**
F. sycomorus	15.71±1.47[b]	31.07±4.15[b]	47.24±2.38[b]	61.41±2.81[b]	75.85±1.66[b]	**0.330mg/ml**
S. henningsii	14.22±2.69[b]	30.45±2.64[b]	48.27±2.50[b]	61.57±2.92[b]	75.22±2.12[b]	**0.325mg/ml**

Values are expressed as Mean ± SEM for three replicates. Column means with the same superscript are not significantly different by ANOVA followed by Tukey's post hoc test ($p > 0.05$).

4.2 In *vitro* DPPH Radical Scavenging Activities of DCM Extracts of *S. henningsii* and *F. sycomorus*

As shown in table 4.2, both extracts of *S. henningsii* and *F. sycomorus* as well as the ascorbic acid demonstrated dose-dependent scavenging of DPPH radicals. At all the tested doses (0.00125-0.2 mg/ml), the two extracts showed lower DPPH scavenging activities than the standard (ascorbic acid) (Table 4.2; Appendix II). The extracts activities were found not to be significantly different at all the tested concentrations ($p>0.05$; Table 4.2). The IC_{50} of *S. henningsii* and *F. sycomorus* was found to be 0.062 mg/ml and 0.068mg/ml respectively while that of the ascorbic acid was found to be 0.025 mg/ml (Table 4.2).

Table 4.2: *In vitro* DPPH Radical Scavenging Activities of DCM Extracts of *S. henningsii* and *F. sycomorus*

	Concentration					
Groups	**0.0125mg/ml**	**0.025mg/ml**	**0.05mg/ml**	**0.1mg/ml**	**0.2mg/ml**	**IC_{50} value**
Ascorbic acid	42.84± 1.05[a]	50.00± 1.85[a]	64.06± 1.75[a]	80.13± 2.06[a]	91.39± 1.38[a]	**0.025mg/ml**
S. henningsii	30.38± 1.61[b]	34.93± 2.42[b]	46.60± 1.49[b]	61.71± 1.69[b]	78.33± 1.65[b]	**0.068mg/ml**
F. sycomorus	29.73± 2.21[b]	37.94± 1.15[b]	46. 45± 2.74[b]	63.81± 2.27[b]	76.38± 1.92[b]	**0.062mg/ml**

Values are expressed as mean ±SEM of the three replicates. Column means followed by the same superscript letters are not significantly different (p>0.05) by one way ANOVA followed by Tukey's post hoc test

4.3 *In vitro* Ferric Reducing Power Activities of DCM Extracts of *S. henningsii* and *F. sycomorus*

In this assay, the extracts were tested for their ability to reduce Fe^{3+} to Fe^{2+} via electron donation. The results showed a dose related ferric reduction by the DCM extracts of *S. henningsii* and *F. sycomorus* (Table 4.3; Appendix III).

The *in vitro* ferric reducing power activity of the leaf extract of *S. henningsii* was comparable to that of standard ascorbic acid ($p>0.05$; Table 4.3) at the lowest concentrations of 0.2 mg/ml. However, at this concentration, the stem bark extract of *F. sycomorus* had significantly lower ferric reducing power than ascorbic acid ($p\leq 0.05$; Table 4.3). At all the other tested concentrations (0.4-1 mg/ml), the ferric reducing power activities of the two extracts had no significant difference ($p >0.05$; Table 4.3). However, they were significantly different from that of standard (ascorbic acid) ($p\leq 0.05$; Table 4.3).

Table 4.3: *In vitro* Ferric Reducing Power Activities of the DCM Extracts of *S. henningsii* and *F. sycomorus*

	Concentration				
Groups	**0.2mg/ml**	**0.4mg/ml**	**0.6mg/ml**	**0.8mg/ml**	**1.0mg/ml**
Ascorbic Acid	0.427 ± 0.02^{a}	0.612 ± 0.02^{a}	0.705 ± 0.04^{a}	0.864 ± 0.02^{a}	0.915 ± 0.02^{a}
S. henningsii	0.317 ± 0.04^{ab}	0.452 ± 0.02^{b}	0.524 ± 0.02^{b}	0.643 ± 0.02^{b}	0.730 ± 0.02^{b}
F. sycomorus	0.217 ± 0.04^{b}	0.316 ± 0.04^{b}	0.486 ± 0.02^{b}	0.603 ± 0.02^{b}	0.703 ± 0.02^{b}

Values are expressed as mean ±SEM of the three replicates. Column means followed by the same superscript letters are not significantly different ($p>0.05$) by one way ANOVA followed by Tukey's post hoc test

4.4 Total Phenolic Content of DCM Extracts of *S. henningsii* and *F. sycomorus*

The total phenolic concentration in the leaf extract of *S. henningsii* and stem bark extract of *F. sycomorus* were quantified and expressed as milligrams of Garlic acid equivalent per gram of dry weight (mg GAE/g dw) (table 4.4) using a standard Gallic acid calibration curve ($y = 1.52x + 0.234$; $R^2 = 0.9782$; Appendix IV). Generally, as the concentration of the extract increased the total phenolic content increased. Overall, at all the concentrations tested (0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml), the two extracts had significantly different phenolic contents ($p \leq 0.05$), with *S. henningsii* extract having the highest phenolic content (Table 4.4).

Table 4.4: Total Phenolic Contents of DCM Extracts of *S. henningsii* and *F.sycomorus*

	Mass in mg/g Gallic acid equivalent				
Groups	**0.1mg/g**	**0.2mg/g**	**0.3mg/g**	**0.4mg/g**	**0.5mg/g**
S. henningsii	0.043±0.01[a]	0.107±0.01[a]	0.167±0.01[a]	0.22±0.01[a]	0.29±0.01[a]
F. sycomorus	0.023±0.01[b]	0.05±0.01[b]	0.097±0.01[b]	0.16±0.01[b]	0.23±0.015[b]

Values expressed as mean ±SEM of the three replicates. Column means followed by the same superscript letters are not significantly different (p>0.05) by un paired student t-test

4.5 Total Flavonoid Contents of DCM Extracts of *S. henningsii* and *F. sycomorus*

The total flavonoid contents of the DCM leaf and stem bark extracts of *S. henningsii and F. sycomorus* were calculated from the standard Rutin calibration curve ($y = 2.535x - 0.047$; $R^2 = 0.9778$; Appendix V). The leaf extract of *S. henningsii* had significantly higher flavonoid concentrations than *F. sycomorus* stem bark extract ($P \leq 0.05$; Table 4.5). However, the Rutin equivalence at the lowest concentration of 0.1mg/ml for both extracts was not significantly different ($P > 0.05$; Table 4.5).

Table 4.5: Total Flavonoid Content of DCM Extracts of *S. henningsii* and *F. sycomorus*

	Mass in mg/g rutin equivalent				
Groups	**0.2mg/g**	**0.4mg/g**	**0.6mg/g**	**0.8mg/g**	**1.0mg/g**
S. henningsii	0.157 ± 0.00^{a}	0.227 ± 0.00^{a}	0.377 ± 0.00^{a}	0.443 ± 0.00^{a}	0.53 ± 0.00^{a}
F. sycomorus	0.103 ± 0.01^{a}	0.16 ± 0.01^{b}	0.237 ± 0.01^{b}	0.343 ± 0.00^{b}	0.383 ± 0.00^{b}

Values expressed as mean ±SEM of the three replicates. Column means followed by the same superscript letters are not significantly different ($p>0.05$)) by un paired student t-test

4.6 Qualitative Phytochemical Screening of DCM Extracts of *S. henningsii* and *F. sycomorus*

The phytochemistry of the leaf extract of *S. henningsii* revealed presence of alkaloids, phenols, saponins, steroids cardiac glycosides and flavonoids (Table 4.6) terpenoids were found to be absent. On the other hand, *F. sycomorus* contained saponins, flavonoids, alkaloids, steroids, phenols, cardiac glycosides and terpenoids. The presence of different phytochemicals in the extract was detected by the intensity of the colour formed, while absence of the colour indicated absence of the screened phytochemical.

Table 4.6: Phytochemical screening of DCM leaf extracts of *S. henningsii* and stem bark of *F. sycomorus*.

Composition	*F. sycomorus*	*S. henningsii*
Saponins	+	+
Alkaloids	+	+
Terpenoids	+	-
Flavonoids	+	+
Steroids	+	+
Phenolics	+	+
Cardiac glycosides	+	+

Key (+) present and (-) absence

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

The interest in search of natural antioxidants has grown rapidly amongst clinical and medical practitioners. This is because reactive oxygen species (ROS) and oxidative stress have been clinically linked to pathogenesis of several diseases like diabetes, cancer, cardiovascular disorders, as well as kidney, lung and liver damages, among others. Researchers have also indicated that free radicals are involved in pathogenesis of some autoimmune disorders like rheumatoid arthritis (Halliwell, 1997; Beckman and Ames**,** 1998). On the other hand, the use of synthetic antioxidants has been linked to toxicity, high cost and side effects (Kibiti and Afolayan, 2015). Therefore, arguably, plants offer better alternatives as antioxidants. Plant leaves, stems, flowers, fruits and roots have been known for centuries to possess therapeutic value and, therefore, have been extensively studied to provide alternative answers to the diverse stress-related disorders.

According to Gong *et al*. (2012), the yield and the antioxidant activity of a plant extract depend on the selected extraction solvent. Different solvents are employed to isolate antioxidant compounds due to disparities in polarities. Widely used solvents for extraction processes include methanol, ethanol, dichloromethane (DCM), water and acetone. Solvents for extraction of polar compounds such as phenolic compounds and flavonoids includes ethanol, methanol and water due to their good polarities. Non-polar solvents for example ether, acetone and dichloromethane are preferred for extraction of non-polar compounds (Teh *et al*., 2013). In this study, DCM was used as a solvent to extract non-polar antioxidant compounds from the two studied medicinal plants.

Several methodologies to determine *in vitro* antioxidant activities of medicinal plants have been developed. These include DPPH radical scavenging assay, hydroxyl radicals assay, hydrogen peroxide radical scavenging assay, ferric reducing power and total determination of flavonoid and phenolic contents, among others (Shimada *et al*., 1992). The broad aim of this study was to determine the *in vitro* antioxidants potential of the DCM leaf extract of *S. henningsii* and stem bark extract of *F. sycomorus*, using standard *in vitro* antioxidants assays against Hydrogen peroxide (H_2O_2), Diphenyl-2-picrylhydrazyl (DPPH) radicals as well as Ferric reducing power assays. Total phenolic and flavonoid contents were also determined in this study. In addition, the phytochemical screening of the extract was done to establish the presence of some phytochemicals commonly associated with antioxidants effects.

Literature has shown that both the *in vitro* and *in vivo* antioxidants activities of *S. henningsii* was done by Oyedami *et al*. (2010). Their studies, however, were done in Nigeria using aqueous stem bark extract. In this study, *in vitro* DCM leaf extract of *S. henningsii* was used.

Hydrogen peroxide (H_2O_2) is a harmless and less reactive molecule, which becomes harmful, toxic and reactive to the cell when it is converted to hydroxyl radical (Halliwell, 1991; Gulcin *et al*., 2003). Hydroxyl compounds are among the most deleterious ROS produced by mitochondria, causing oxidative damage and are clinically linked to causes of various stress-related disorders. Thus, removal of H_2O_2 is a critical step for maintaining a functional antioxidant defense system in cells or food systems (Keser *et al*., 2012).

DCM leaf extract of *S. henningsii* and stem bark extract of *F. sycomorus* showed significant antioxidant activity against hydrogen peroxide in a dose associated trend. The dose dependent activities of both extracts showed that increase of concentrations of the extract increased the levels of bioactive antioxidants compounds. Sirisha *et al*. (2010) demonstrated similar *in vitro* antioxidant activity while working on methanolic leaf extract of *F. carcia.* They showed that the inhibition of hydrogen peroxide was dose dependent. Other studies done on different *Ficus* species have reported that the species exhibit strong hydrogen peroxide antioxidant activity which occur in a dose dependent manner (Ahoua *et al*., 2012).

The half maximal percentage inhibition (IC_{50}) value is widely used as a quantitative measure of extracts, antioxidant potential. It is used to indicate the amount of substance required to inhibit a given biological process by half. In pharmacokinetics IC_{50} represent the concentration of a drug that is required for 50% inhibition. Literature shows that the lower the IC_{50} value, the better the antioxidant activity of the plant extract (Daniel and Dluya, 2016).

The findings of this study showed that both S. *henningsii* and *F. sycomorus* extracts had low IC_{50} of 0.330 mg/ml and 0.325 mg/ml respectively against the H_2O_2 radicals. Thus, the low IC_{50} values obtained from the study indicates that the two extracts have strong H_2O_2 scavenging activities. Thus supports their therapeutic use against stress-related disorders. The IC_{50} value obtained for *F. sycomorus* was similar to one obtained by Deo *et al.* (2016) on some selected herbal extracts inhibitory properties against protein glycation and

angiotensin enzyme linked to type II diabetes. Additionally, *P. amarus* and *L. pumila* var. *alata* medicinal plants have been shown to possessed potent radical inhibiting properties with low IC_{50} values of 3.4 and 5.7 µg/ml, respectively (Saputri and Jantan, 2011).

The reduction of DPPH radical is one of the popular and simpler ways to measure antioxidant activities of medicinal plants. The potential of plant extracts to inhibit DPPH radical is strongly linked to their ability to donate electrons to the radical (Daniel and Dluya, 2016). Normally, DPPH radical is stable in various solvents including methanol, ethanol and water. Therefore, the radical is usually prepared in a solution of either ethanol or methanol (Shimada *et al*., 1992; Fukumoto and Mazza, 2000). In the present study, the DPPH radical was prepared in ethanol. The *in vitro* DPPH scavenging assay was preferred in this study because it is rapid, easy, reliable and less expensive since it does not require specialized device and methods.

In vitro radical scavenging activities of the extracts against DPPH were monitored at a wavelength of 517 nm. Due to its ability to bind hydrogen atom or receive an electron, DPPH radical can be reduced to non-reactive state (Soare *et al.,* 1997). Conversion of DPPH to DPPH-H (diphenylhydrazine) in the presence of an antioxidant agent, changes the solution colour from purple to colorless. The level of discoloration increases with decrease in the amount of DPPH radicals in the solution. The change in colour of the DPPH is indicative of radical inhibiting Properties of the extract (Molyneux, 2004; Guo *et al.,* 2007).

The results obtained in this study showed dose dependent DPPH scavenging activities of the two extracts. It was, however, noted that the DCM leaf extract of *S. henningsii* and *F. sycomorus* stem bark extract had lower DPPH scavenging abilities than ascorbic acid. This could be due to the crude nature of extract as compared to the refined standard drug. This result corresponded with the observations of Igbinosa *et al*. (2011), who found that *Jatropha curcas* had lower DPPH activities than ascorbic acid (standard).

The results of stem bark extract of *F. sycomorus* agrees with a study by Kambli *et al.* (2014), who found that the DPPH scavenging activity of *F. racemosa* was considerable but not higher than that of the standard drug. The good antioxidant property of *F. sycomorus* stem bark extract against DPPH, corroborate well with findings of Degollado *et al*. (2014), who noted that *F. odorata* had a good antioxidant activity against DPPH radicals. The DCM leaf extract of *S. hennigsii* also showed a good antioxidant activity against DPPH. However, studies done by Oyedemi *et al*. (2010), while working on the stem bark extract of *S. hennigsii*, reported weaker antioxidant activities against DPPH, which they attributed to low levels of flavonoids in the stem bark extract compared to the leaf. The findings in this study indicates that the extracts of these plants had compounds that were relatively strong scavengers of radicals, which is why they are strongly used as antioxidant supplements.

The IC_{50} values of the leaf extract of *S. henningsii* and stem bark extract of *F. sycomorus* against DPPH free radical were 0.068 mg/ml and 0.062 mg/ml respectively. The low IC_{50} values obtained from this study, showed that both extracts had excellent antioxidants activities against DPPH radicals. This argument is in line with studies by Mbaebie *et al*.

(2012), who worked on stem bark extract of *S. latifolia* against DPPH radical, and obtained an IC_{50} value of 0.126mg/ml thereby strongly recommending the plant to be used as an antioxidant supplement.

The potential of the extract sample to reduce Fe^{3+} to Fe^{2+} via an electron donation was determined. The level of Fe^{2+} complex generated was measured in terms of the absorbance of the Perl's Prussian blue colour at 700 nm wavelength. Change of yellow colour of the test solution to various shades of green and blue indicated the reducing power of the extract.

It was evident that increased extracts concentrations increased their ferric reducing power. Basically as a results of more Fe^{3+} being reduced to Fe^{2+} as more electrons were being donated by antioxidant components (Ganu *et al*., 2010; Mitra *et al*., 2014). High absorbance was evidence of increased in reducing ability of the extract (Dehpour *et al*., 2009). Both extracts were, however, found to have a lower reducing capacity at all the tested concentrations compared to ascorbic acid, the reference compound. This observation correlates with the findings of Ahoua *et al*. (2012), who studied antioxidants activities of eight different species of *Ficus* and they noted that majority of them had significantly lower reducing power against the ascorbic acid. The observation in this study could be explained by the fact that the extracts used were in crude form as compared to the refined form of the standard drugs. The crude form tends to have fewer bioactive compound per concentration as compared to the refined one.

The results on ferric reducing power activities of the two studied extracts suggest that the two extracts are reductones. Reductones are involved in varied antioxidant mechanism

including prevention of chain initiation, binding of metal ions, decomposition of peroxides and radical scavenging (Ganu *et al*., 2010; Mitra and Uddin, 2014). The phytochemicals in the extracts either directly bind the metal ions or indirectly suppress their chelating reactivities by occupying their coordination sites (Edeoga *et al*., 2005; Njoku and Obi, 2009). The ultimate outcome of the reduction reaction in the antioxidant defense system is to terminate the radical chain reactions, which may otherwise be very detrimental to tissues. The determination of total flavonoid and phenolic contents of extracts is one of key *in vitro* antioxidants assays. This is because several studies have reported that there is a correlation between the overall antioxidant activities and the total phenolic and flavonoid contents. These two polyphenols are considered to be the most bioactive substances for various antioxidant activities of plant extracts. After analyzing the antioxidant activities of phenolic compounds, Heim *et al*. (2002) and Fukumoto *et al.* (2000) concluded that an increase in scavenging activity is directly related to increases in number of hydroxyl groups in polyphenols and the decrease in glycosylated groups. Therefore, phenolic and flavonoid contents of plant extracts directly contribute to their antioxidant activities. In addition, some researchers like Arnous *et al*. (2002) and Lee *et al*. (2003) have suggested that the antioxidant activity expressed by plant extract is as a result of the synergism between different phytochemicals in plants.

In most plant extracts, there is a positive correlation between antioxidant activity and the amount of polyphenolic compounds (Hudah *et al.,* 2009; Li *et al*., 2009). However, some studies have reported that there is no positive relationship between the polyphenolics compounds and their antioxidants activities (Rafat *et al*., 2010; Hesam *et al*., 2012). In this study, a positive correlation was observed between the total antioxidant status of the plant

extracts and their composition of phenolic and flavonoids components. The potential antioxidants activities of the DCM extract of *S. henningsii* and *F. sycomorus* can be positively related to their higher total phenolic and flavonoid compounds as shown by this study.

Flavonoids and phenols naturally exhibit strong scavenging abilities for free radicals due to their hydroxyl groups (Mohamed *et al.,* 2010), which are attached to their aromatic ring structures and help to quench the radicals either by donating their electrons and thus neutralizing them or via the electron delocalization over all three ring system achieved by ortho-dihydroxyl of the B-ring and 4-oxo group of the ring C of the flavonoid, which actively reduce radicals like DPPH and Fe^{3+} to Fe^{2+} ions (Jin *et al*., 2010). Proanthocyanidins, which is a class of flavonoid family exert their activity through inactivation of the ferric ions or by chelation or suppression of the superoxide-driven Fenton reaction (Kibiti and Afolayan, 2015). The Fenton reaction is one of the major sources of reactive oxygen species formed in cells and tissues.

Phenols are common in diet and are divided into two classes; the benzoic derivatives like gallic acid and cinnamic derivatives like caffeic acids. Gallic acid is the best known polyphenol working efficiently in polar medium, the compound has greater ability of scavenging hydroxyl free radicals through deprotonation. Gallic acid is a prolific scavenger, with a greater ability to deactivate variety of ROS and RNS mainly via electron movement through the cellular physiological pH (Mareno *et al*., 2014). The mechanism of antioxidant action of phenolic compounds is through inactivation of lipid free radicals or

by preventing the decomposition of hydroperoxides into free radicals (Jin *et al*., 2010; Kibiti and Afolayan, 2015).

In this study, the phtytochemical screening of *S. henningsii* and *F. sycomorus* extracts showed that in addition to flavonoids and phenols, the extracts contained saponins, terpenoids, alkaloids, steroids and cardiac gylcosides, which are helpful in protecting cells against oxidative stress. Saponins are surface active group of complex compounds largely found in plants but also to some extent, in marine organisms (Yoshika *et al*., 1998). The structure of saponins, consists of two parts; a sugar oligosaccharide and a steroid (Akinpelu *et al*., 2014). Saponins have been shown to have an increasing use in traditional and industrial applications as anti-inflammatory, molluscicidal, antimicrobial, antispasmodic, antidiabetic, antitumor and as well as an antioxidant (Chen *et al*., 2014). There is also growing evidence linking saponins to strong antioxidant activities through electron donation (Tapondjou *et al*., 2011). They are also widely used in beverage, cosmetic and confectionary industries due to their antimicrobial effects (Bi *et al.*, 2012).

Alkaloids are widely distributed among different plants families. Some reports have indicated that they have a strong antioxidant property. They express their antioxidant activities by removing the effects of hydrogen peroxide-induced oxidation, inhibit lipid peroxidation by inhibiting the cyclooxygenase enzyme, and protect neurons against toxic effects of dopamine and glutamate. Besides, other studies have shown that alkaloids exert protective effects on oxidative neuronal damage through ROS scavenging (Kim *et al*., 2004). In addition, Moura *et al*. (2007) demonstrated significant antioxidant effects of alkaloids on yeast.

Condensed or hydrolysable tannins are naturally occurring plant polyphenol compounds composed of a central glucose molecule with galloy residues. They are found in several beverages and green leaves (Chung *et al*., 1998). Horikawa *et al*. (1994) found that tannins have anti-mutagenic and anti-carcinogenic properties. Bouchet *et al*. (1998) reported that tannins have antiradical and potent antioxidant properties. They are also known to inhibit lipid peroxidation by inhibiting the enzyme cyclooxygenase (Chen *et al*., 2014). Tannins achieve their antioxidant properties through chelating metal ions such as Fe^{3+} and they are also known to destabilize some key steps in the Fenton reaction, thereby slowing down the oxidation process (Andrade *et al*., 2005).

Although the *in vivo* analysis of the two plants extracts are yet to be done, results obtained in this study strongly point out that the two tested extracts exhibit strong *in vitro* antioxidant activities. No wonder these plants have been in use in African traditional medical practices in treatment and management of diseases like rheumatism, diabetes, abdominal pain, healing wounds and mouth antiseptic which are all oxidative stress-related disorders.

5.2 Conclusions

From this study, it is concluded that:

i. The DCM extracts of *S. henningsii* and *F. sycomorus* showed good hydrogen peroxide scavengers.
ii. The DCM extracts of *S. henningsii* and *F. sycomorus* showed potent DPPH radical scavenging activities.
iii. The DCM extracts of both *S. henningsii* and *F. sycomorus* showed significant ferric reducing power abilities.

iv. Both DCM extracts of *S. henningsii* and *F. sycomorus* had appreciable amounts of total phenolic and flavonoid contents, which are related to antioxidant activities.

v. DCM extracts of *S. henningsii* and *F. sycomorus* contain other phytochemicals which are rich in antioxidant activities.

5.3 Recommendations

This study recommends that the Dichloromethanolic leaf extract of *S. henningsii* and stem bark extract of *F.sycomorus* can be potentially used for alternative and complementary antioxidants.

5.4 Suggestions for further research

From this study, further research suggestions include;

i. Bioassay-guided fractionation and isolation of active antioxidant compounds.

ii. *In vivo* antioxidant assays to confirm the *in vitro* assay results.

iii. *In vivo* toxicity assays to establish the safety of the extracts.

iv. The use of other solvents to extract the phytochemicals

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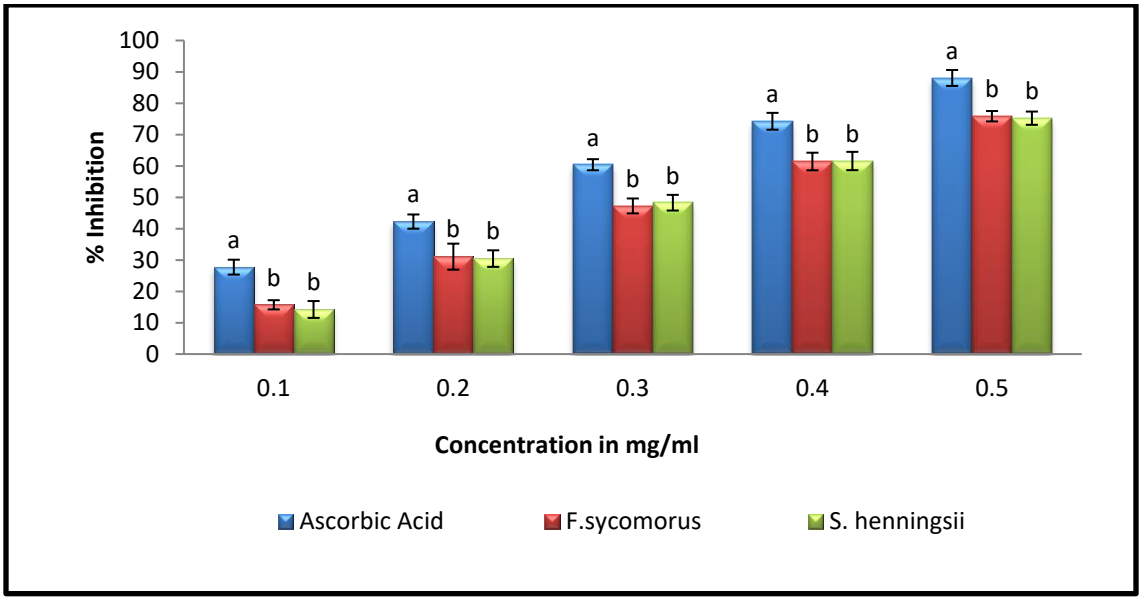
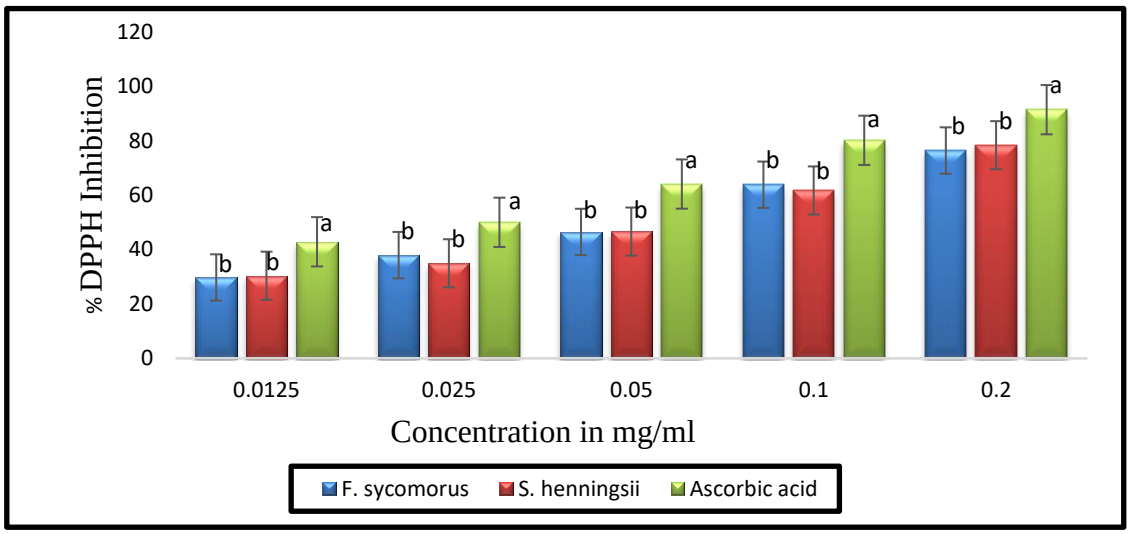
APPENDICES

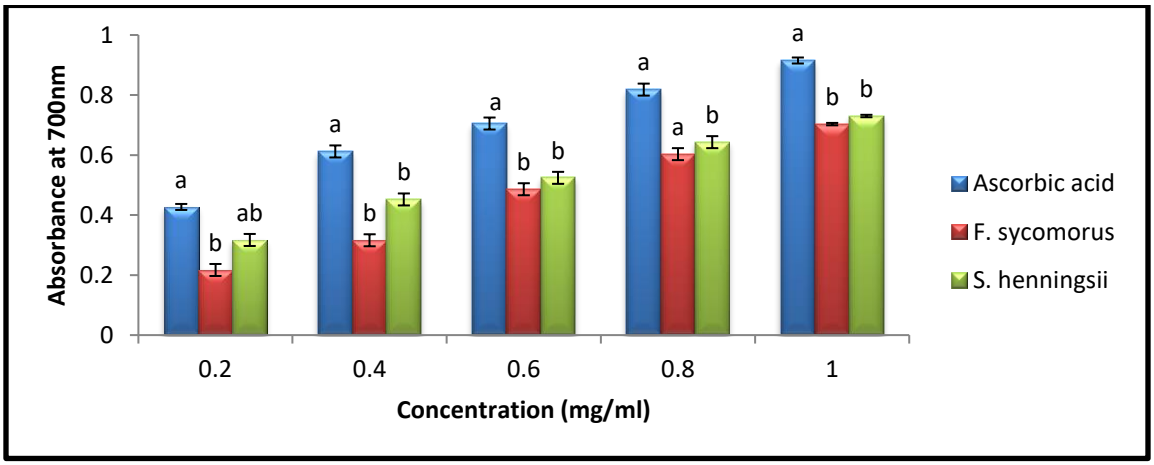
Appendix I: Percentage Hydrogen peroxide inhibition of DCM leaf extract of *S. hennningsii*, stem bark extract of *F. sycomorous*, and standard ascorbic acid.



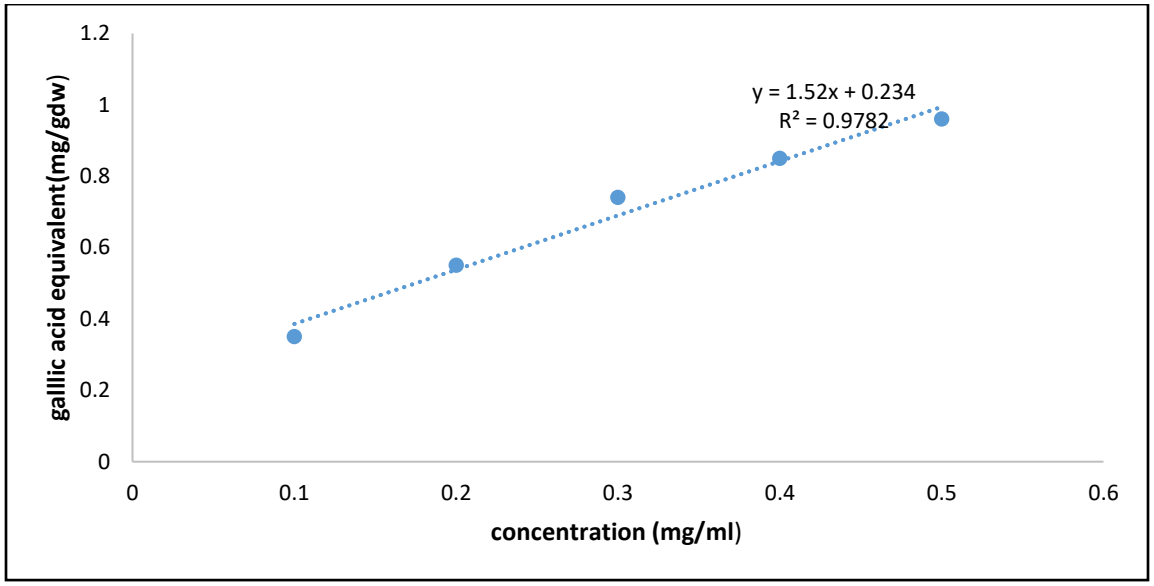
Appendix II: Percentage DPPH scavenging activities of *S. henningsii*, *F. sycomorus* and standard ascorbic acid.



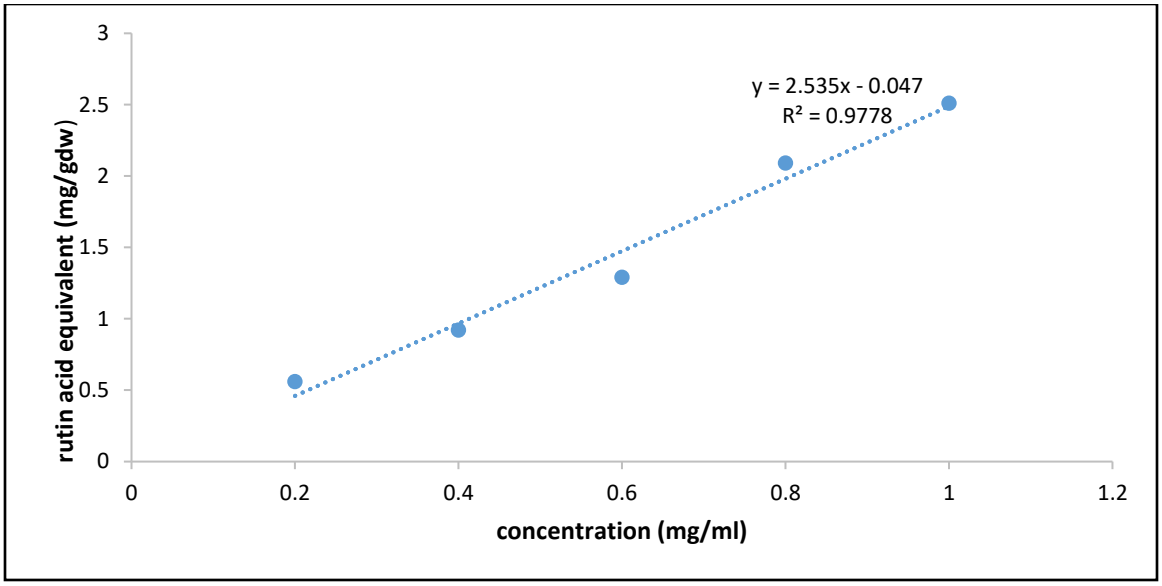
Appendix III: Analysis of Ferric reducing power of *S. hennningsii*, *F. sycomorus* and standard ascorbic acid.



Appendix IV: Calibration curve for Gallic acid equivalent for total phenolic.



Appendix V: Calibration curve for Rutin acid equivalent for total flavonoid.



Appendix VI: Statistical analysis of DPPH radical scavenging activities

Descriptive Statistics: F0.0125mg/ml, S0.0125mg/ml, SA0.0125mg/ml

Variable	Mean	SE Mean	StDev
F0.0125mg/ml	29.73	2.21	3.83
S0.0125mg/ml	30.38	1.61	2.79
SA0.0125mg/ml	42.84	1.05	1.81

Descriptive Statistics: F0.025mg/ml, S0.025mg/ml, SA0.025mg/ml

Variable	Mean	SE Mean	StDev
F0.025mg/ml	37.94	1.15	2.00
S0.025mg/ml	34.93	2.42	4.20
SA0.025mg/ml	50.50	1.85	3.21

Descriptive Statistics: F0.05mg/ml, S0.05mg/ml, SA0.05mg/ml

Variable	Mean	SE Mean	StDev
F0.05mg/ml	46.45	2.74	4.74
S0.05mg/ml	46.60	1.49	2.59
SA0.05mg/ml	64.06	1.75	3.02

Descriptive Statistics: F0.1mg/ml, S0.1mg/ml, SA0.1mg/ml

Variable	Mean	SE Mean	StDev
F0.1mg/ml	63.81	2.27	3.94
S0.1mg/ml	61.71	1.69	2.93
SA0.1mg/ml	80.13	2.06	3.56

Descriptive Statistics: F0.2mg/ml, S0.2mg/ml, SA0.2mg/ml

Variable	Mean	SE Mean	StDev
F0.2mg/ml	76.38	1.92	3.33
S0.2mg/ml	78.33	1.65	2.86
SA0.2mg/ml	91.39	1.38	2.39

One-way ANOVA: F0.0125mg/ml, S0.0125mg/ml, SA0.0125mg/ml

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.
Means

Factor	N	Mean	StDev	95% CI
F0.0125mg/ml	3	29.73	3.83	(25.59, 33.87)
S0.0125mg/ml	3	30.38	2.79	(26.24, 34.52)
SA0.0125mg/ml	3	42.84	1.81	(38.70, 46.98)

Pooled StDev = 2.92910

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
SA0.0125mg/ml	3	42.84	A
S0.0125mg/ml	3	30.38	B
F0.0125mg/ml	3	29.73	B

Means that do not share a letter are significantly different.

One-way ANOVA: F0.025mg/ml, S0.025mg/ml, SA0.025mg/ml

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.
Means

Factor	N	Mean	StDev	95% CI
F0.025mg/ml	3	37.94	2.00	(33.33, 42.55)
S0.025mg/ml	3	34.93	4.20	(30.32, 39.55)
SA0.025mg/ml	3	50.50	3.21	(45.89, 55.11)

Pooled StDev = 3.26325

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
SA0.025mg/ml	3	50.50	A
F0.025mg/ml	3	37.94	B
S0.025mg/ml	3	34.93	B

Means that do not share a letter are significantly different.

One-way ANOVA: F0.05mg/ml, S0.05mg/ml, SA0.05mg/ml

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	F0.05mg/ml, S0.05mg/ml, and SA0.05mg/ml

Analysis of Variance

```
Source DF Adj SS Adj MS F-Value P-Value
Factor   2 615.52 307.76    24.10    0.001
Error    6   76.61   12.77
Total    8 692.12

Model Summary
 S    R-sq R-sq(adj)  R-sq(pred)
3.57324 88.93%     85.24%      75.10%

Means
Factor       N   Mean StDev      95% CI
F0.05mg/ml   3 46.45   4.74 (41.40, 51.49)
S0.05mg/ml   3 46.60   2.59 (41.55, 51.64)
SA0.05mg/ml 3 64.06   3.02 (59.02, 69.11)

Pooled StDev = 3.57324
```

Tukey Pairwise Comparisons

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

Factor       N   Mean Grouping
SA0.05mg/ml 3 64.06 A
S0.05mg/ml   3 46.60    B
F0.05mg/ml   3 46.45    B

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

One-way ANOVA: F0.1mg/ml, S0.1mg/ml, SA0.1mg/ml

```
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 F0.1mg/ml, S0.1mg/ml, and SA0.1mg/ml

Analysis of Variance

Source DF Adj SS Adj MS F-Value P-Value
Factor   2 609.88 304.94    24.87    0.001
Error    6   73.56   12.26
Total    8 683.44


Model Summary

S    R-sq R-sq (adj) R-sq(pred)
3.50136 89.24%     85.65%      75.78%


Means

Factor      N   Mean StDev      95% CI
F0.1mg/ml   3 63.81   3.94 (58.87, 68.76)
S0.1mg/ml   3 61.71   2.93 (56.77, 66.66)
SA0.1mg/ml 3 80.13   3.56 (75.18, 85.08)

Pooled StDev = 3.50136
```

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
SA0.1mg/ml	3	80.13	A
F0.1mg/ml	3	63.81	B
S0.1mg/ml	3	61.71	B

Means that do not share a letter are significantly different.

One-way ANOVA: F0.2mg/ml, S0.2mg/ml, SA0.2mg/ml

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	F0.2mg/ml, S0.2mg/ml, and SA0.2mg/ml

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	399.90	199.952	23.99	0.001
Error	6	50.00	8.334		
Total	8	449.91			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2.88689	88.89%	85.18%	74.99%

Means

Factor	N	Mean	StDev	95% CI
F0.2mg/ml	3	76.38	3.33	(72.30, 80.45)
S0.2mg/ml	3	78.33	2.86	(74.25, 82.41)
SA0.2mg/ml	3	91.39	2.39	(87.31, 95.47)

Pooled StDev = 2.88689

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
SA0.2mg/ml	3	91.39	A
S0.2mg/ml	3	78.33	B
F0.2mg/ml	3	76.38	B

Means that do not share a letter are significantly different.

Appendix VII: Statistical analysis of Hydrogen peroxide scavenging activities.

Descriptive Statistics: F0.1mg/ml, S0.1mg/ml, SA0.1mg/ml, F0.2mg/ml, S0.2mg/ml,

Variable	Mean	SE Mean	StDev
F0.1mg/ml	27.72	2.38	4.12
S0.1mg/ml	15.71	1.47	2.55
SA0.1mg/ml	14.22	2.69	4.65
F0.2mg/ml	42.25	2.27	3.93
S0.2mg/ml	31.07	4.15	7.18
SA0.2mg/ml	30.45	2.64	4.57
F0.3mg/ml	60.38	1.77	3.07
S0.3mg/ml	47.24	2.38	4.12
SA0.3mg/ml	48.27	2.50	4.32
F0.4mg/ml	74.24	2.68	4.64
S0.4mg/ml	61.41	2.81	4.87
SA0.4mg/ml	61.57	2.92	5.06
F0.5mg/ml	88.05	2.55	4.42
S0.5mg/ml	75.84	1.66	2.87
SA0.5mg/ml	75.22	2.12	3.68

One-way ANOVA: F0.1mg/ml, S0.1mg/ml, SA0.1mg/ml

Method

Null hypothesis	all means are equal
Alternative hypothesis	At least one mean is different
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor	3	F0.1mg/ml, S0.1mg/ml, and SA0.1mg/ml

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	328.53	164.27	10.92	0.010
Error	6	90.24	15.04		
Total	8	418.77			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
3.87804	78.45%	71.27%	51.52%

Means

Factor	N	Mean	StDev	95% CI
F0.1mg/ml	3	27.72	4.12	(22.24, 33.20)
S0.1mg/ml	3	15.71	2.55	(10.23, 21.19)
SA0.1mg/ml	3	14.22	4.65	(8.74, 19.70)

Pooled StDev = 3.87804

Tukey Pairwise Comparisons

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

Factor      N   Mean Grouping
F0.1mg/ml   3 27.72 A
S0.1mg/ml   3 15.71    B
SA0.1mg/ml 3 14.22    B

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

One-way ANOVA: F0.2mg/ml, S0.2mg/ml, SA0.2mg/ml

```
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 F0.2mg/ml, S0.2mg/ml, and SA0.2mg/ml


Analysis of Variance

Source DF Adj SS Adj MS F-Value P-Value
Factor   2   264.6 132.28     4.51    0.064
Error    6   175.8   29.31
Total    8   440.4


Model Summary
S    R-sq R-sq(adj)  R-sq(pred)
5.41351 60.07%     46.76%      10.17%


Means

Factor      N   Mean StDev      95% CI
F0.2mg/ml   3 42.25   3.93 (34.60, 49.89)
S0.2mg/ml   3 31.07   7.18 (23.42, 38.71)
SA0.2mg/ml 3 30.45   4.57 (22.80, 38.10)

Pooled StDev = 5.41351
```

Tukey Pairwise Comparisons

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

Factor      N   Mean Grouping
F0.2mg/ml   3 42.25 A
S0.2mg/ml   3 31.07 A
SA0.2mg/ml 3 30.45 A
```

Means that do not share a letter are significantly different.

One-way ANOVA: F0.3mg/ml, S0.3mg/ml, SA0.3mg/ml

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 F0.3mg/ml, S0.3mg/ml, and SA0.3mg/ml

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	320.24	160.12	10.66	0.011
Error	6	90.11	15.02		
Total	8	410.35			

Model Summary

S	R-sq.	R-sq(adj)	R-sq(pred)
3.87531	78.04%	70.72%	50.59%

Means

Factor	N	Mean	StDev	95% CI
F0.3mg/ml	3	60.38	3.07	(54.91, 65.86)
S0.3mg/ml	3	47.24	4.12	(41.77, 52.72)
SA0.3mg/ml	3	48.27	4.32	(42.80, 53.75)

Pooled StDev = 3.87531

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
F0.3mg/ml	3	60.38	A
SA0.3mg/ml	3	48.27	B
S0.3mg/ml	3	47.24	B

Means that do not share a letter are significantly different.

One-way ANOVA: F0.4mg/ml, S0.4mg/ml, SA0.4mg/ml

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	F0.4mg/ml, S0.4mg/ml, and SA0.4mg/ml

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	325.2	162.61	6.89	0.028
Error	6	141.7	23.61		
Total	8	466.9			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
4.85901	69.66%	59.54%	31.73%

Means

Factor	N	Mean	StDev	95% CI
F0.4mg/ml	3	74.24	4.64	(67.38, 81.10)
S0.4mg/ml	3	61.41	4.87	(54.55, 68.28)
SA0.4mg/ml	3	61.57	5.06	(54.70, 68.43)

Pooled StDev = 4.85901

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
F0.4mg/ml	3	74.24	A
SA0.4mg/ml	3	61.57	B
S0.4mg/ml	3	61.41	B

Means that do not share a letter are significantly different.

One-way ANOVA: F0.5mg/ml, S0.5mg/ml, SA0.5mg/ml

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	F0.5mg/ml, S0.5mg/ml, and SA0.5mg/ml

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	314.04	157.02	11.41	0.009
Error	6	82.56	13.76		
Total	8	396.60			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
3.70943	79.18%	72.24%	53.16%

Means

Factor	N	Mean	StDev	95% CI
F0.5mg/ml	3	88.05	4.42	(82.81, 93.29)
S0.5mg/ml	3	75.84	2.87	(70.60, 81.08)
SA0.5mg/ml	3	75.22	3.68	(69.98, 80.46)

Pooled StDev = 3.70943

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
F0.5mg/ml	3	88.05	A
S0.5mg/ml	3	75.84	B
SA0.5mg/ml	3	75.22	B

Means that do not share a letter are significantly different.

Appendix VIII. Statistical analysis of Ferric reducing power

Descriptive Statistics: F0.2mg/ml, S0.2mg/ml, SA0.2mg/ml, F0.4mg/ml, S0.4mg/ml,

Variable	Mean	SE Mean	StDev
F0.2mg/ml	0.1503	0.0222	0.0384
S0.2mg/ml	0.3537	0.0156	0.0270
SA0.2mg/ml	0.5677	0.0147	0.0255
F0.4mg/ml	0.2490	0.0232	0.0401
S0.4mg/ml	0.4520	0.0220	0.0382
SA0.4mg/ml	0.6457	0.0180	0.0313
F0.6mg/ml	0.3490	0.0202	0.0350
S0.6mg/ml	0.5607	0.0165	0.0286
SA0.6mg/ml	0.7380	0.0162	0.0281
F0.8mg/ml	0.4383	0.0126	0.0218
S0.8mg/ml	0.6430	0.0231	0.0400
SA0.8mg/ml	0.8643	0.0171	0.0297
F1.0mg/ml	0.5620	0.0172	0.0298
S1.0mg/ml	0.7170	0.0176	0.0304
SA1.0mg/ml	1.0483	0.0136	0.0236

One-way ANOVA: F0.2mg/ml, S0.2mg/ml, SA0.2mg/ml

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 F0.2mg/ml, S0.2mg/ml, SA0.2mg/ml

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0.06620	0.033100	10.86	0.010
Error	6	0.01828	0.003047		
Total	8	0.08448			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0552027	78.36%	71.14%	51.31%

Means

Factor	N	Mean	StDev	95% CI
F0.2mg/ml	3	0.2170	0.0620	(0.1390, 0.2950)
S0.2mg/ml	3	0.3170	0.0621	(0.2390, 0.3950)
SA0.2mg/ml	3	0.4270	0.0380	(0.3490, 0.5050)

Pooled StDev = 0.0552027

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
SA0.2mg/ml	3	0.4270	A
S0.2mg/ml	3	0.3170	A B
F0.2mg/ml	3	0.2170	B

Means that do not share a letter are significantly different.

One-way ANOVA: F0.4mg/ml, S0.4mg/ml, SA0.4mg/ml

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 F0.4mg/ml, S0.4mg/ml, and SA0.4mg/ml

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0.13230	0.066152	32.66	0.001
Error	6	0.01215	0.002025		
Total	8	0.14446			

```
Model Summary
S    R-sq R-sq(adj)  R-sq(pred)
0.0450025 91.59%     88.78%      81.07%

Means

Factor      N    Mean   StDev       95% CI
F0.4mg/ml   3  0.3157  0.0604 (0.2521, 0.3792)
S0.4mg/ml   3  0.4520  0.0382 (0.3884, 0.5156)
SA0.4mg/ml  3  0.6123  0.0313 (0.5488, 0.6759)

Pooled StDev = 0.0450025
```

Tukey Pairwise Comparisons

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

Factor      N    Mean Grouping
SA0.4mg/ml  3  0.6123  A
S0.4mg/ml   3  0.4520    B
F0.4mg/ml   3  0.3157      C

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

One-way ANOVA: F0.6mg/ml, S0.6mg/ml, SA0.6mg/ml

```
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 F0.6mg/ml, S0.6mg/ml, and SA0.6mg/ml


Analysis of Variance

Source DF   Adj SS    Adj MS F-Value P-Value
Factor   2   0.08207 0.041035    18.78    0.003
Error    6   0.01311 0.002185
Total    8   0.09518

Model Summary
S    R-sq R-sq(adj)  R-sq(pred)
0.0467392 86.23%     81.64%      69.01%


Means

Factor      N    Mean   StDev       95% CI
F0.6mg/ml   3 0.4857 0.0392 (0.4196, 0.5517)
S0.6mg/ml   3 0.5240 0.0331 (0.4580, 0.5900)
SA0.6mg/ml 3 0.7047 0.0626 (0.6386, 0.7707)

Pooled StDev = 0.0467392.
```

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
SA0.6mg/ml	3	0.7047	A
S0.6mg/ml	3	0.5240	B
F0.6mg/ml	3	0.4857	B

Means that do not share a letter are significantly different.

One-way ANOVA: F0.8mg/ml, S0.8mg/ml, SA0.8mg/ml

Method

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

Equal variances were assumed for the analysis.
Factor Information
Factor Levels Values
Factor 3 F0.8mg/ml, S0.8mg/ml, and SA0.8mg/ml

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0.078190	0.039095	39.04	0.000
Error	6	0.006009	0.001001		
Total	8	0.084199			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0316456	92.86%	90.48%	83.94%

Means

Factor	N	Mean	StDev	95% CI
F0.8mg/ml	3	0.6030	0.0274	(0.5583, 0.6477)
S0.8mg/ml	3	0.6430	0.0400	(0.5983, 0.6877)
SA0.8mg/ml	3	0.8177	0.0255	(0.7730, 0.8624)

Pooled StDev = 0.0316456

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
SA0.8mg/ml	3	0.8177	A
S0.8mg/ml	3	0.6430	B
F0.8mg/ml	3	0.6030	B

Means that do not share a letter are significantly different.

One-way ANOVA: F1.0mg/ml, S1.0mg/ml, SA1.0mg/ml

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 F1.0mg/ml, S1.0mg/ml, and SA1.0mg/ml

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0.079953	0.039976	29.72	0.001
Error	6	0.008071	0.001345		
Total	8	0.088024			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0366773	90.83%	87.77%	79.37%

Means

Factor	N	Mean	StDev	95% CI
F1.0mg/ml	3	0.7027	0.0394	(0.6509, 0.7545)
S1.0mg/ml	3	0.7303	0.0325	(0.6785, 0.7821)
SA1.0mg/ml	3	0.9150	0.0377	(0.8632, 0.9668)

Pooled StDev = 0.0366773

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
SA1.0mg/ml	3	0.9150	A
S1.0mg/ml	3	0.7303	B
F1.0mg/ml	3	0.7027	B

Means that do not share a letter are significantly different.

Appendix IX: Statistical analysis of Total flavonoid

Descriptive Statistics: F0.1mg/ml, S0.1mg/ml, SA0.1mg/ml, F0.2mg/ml, S0.2mg/ml,

Variable	Mean	SE Mean	StDev
F0.1mg/ml	27.72	2.38	4.12
S0.1mg/ml	15.71	1.47	2.55
SA0.1mg/ml	14.22	2.69	4.65
F0.2mg/ml	42.25	2.27	3.93
S0.2mg/ml	31.07	4.15	7.18
SA0.2mg/ml	30.45	2.64	4.57
F0.3mg/ml	60.38	1.77	3.07
S0.3mg/ml	47.24	2.38	4.12
SA0.3mg/ml	48.27	2.50	4.32
F0.4mg/ml	74.24	2.68	4.64
S0.4mg/ml	61.41	2.81	4.87
SA0.4mg/ml	61.57	2.92	5.06
F0.5mg/ml	88.05	2.55	4.42
S0.5mg/ml	75.84	1.66	2.87
SA0.5mg/ml	75.22	2.12	3.68

One-way ANOVA: F0.1mg/ml, S0.1mg/ml, SA0.1mg/ml

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 F0.1mg/ml, S0.1mg/ml, and SA0.1mg/ml

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	328.53	164.27	10.92	0.010
Error	6	90.24	15.04		
Total	8	418.77			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
3.87804	78.45%	71.27%	51.52%

Means

Factor	N	Mean	StDev	95% CI
F0.1mg/ml	3	27.72	4.12	(22.24, 33.20)
S0.1mg/ml	3	15.71	2.55	(10.23, 21.19)
SA0.1mg/ml	3	14.22	4.65	(8.74, 19.70)

Pooled StDev = 3.87804

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
F0.1mg/ml	3	27.72	A
S0.1mg/ml	3	15.71	B
SA0.1mg/ml	3	14.22	B

Means that do not share a letter are significantly different.

One-way ANOVA: F0.2mg/ml, S0.2mg/ml, SA0.2mg/ml

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 F0.2mg/ml, S0.2mg/ml, and SA0.2mg/ml

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	264.6	132.28	4.51	0.064
Error	6	175.8	29.31		
Total	8	440.4			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
5.41351	60.07%	46.76%	10.17%

Means

Factor	N	Mean	StDev	95% CI
F0.2mg/ml	3	42.25	3.93	(34.60, 49.89)
S0.2mg/ml	3	31.07	7.18	(23.42, 38.71)
SA0.2mg/ml	3	30.45	4.57	(22.80, 38.10)

Pooled StDev = 5.41351

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
F0.2mg/ml	3	42.25	A
S0.2mg/ml	3	31.07	A
SA0.2mg/ml	3	30.45	A

Means that do not share a letter are significantly different.

One-way ANOVA: F0.3mg/ml, S0.3mg/ml, SA0.3mg/ml

Method

Null hypothesis all means are equal
Alternative hypothesis At least one mean is different
Significance level $\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor Levels Values
Factor 3 F0.3mg/ml, S0.3mg/ml, and SA0.3mg/ml

Analysis of Variance

Source	DF	Ad SS	Adj MS	F-Value	P-Value
Factor	2	320.24	160.12	10.66	0.011
Error	6	90.11	15.02		
Total	8	410.35			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
3.87531	78.04%	70.72%	50.59%

Means

Factor	N	Mean	StDev	95% CI
F0.3mg/ml	3	60.38	3.07	(54.91, 65.86)
S0.3mg/ml	3	47.24	4.12	(41.77, 52.72)
SA0.3mg/ml	3	48.27	4.32	(42.80, 53.75)

Pooled StDev = 3.87531

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
F0.3mg/ml	3	60.38	A
SA0.3mg/ml	3	48.27	B
S0.3mg/ml	3	47.24	B

Means that do not share a letter are significantly different.

One-way ANOVA: F0.4mg/ml, S0.4mg/ml, SA0.4mg/ml

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	F0.4mg/ml, S0.4mg/ml, and SA0.4mg/ml

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	325.2	162.61	6.89	0.028
Error	6	141.7	23.61		
Total	8	466.9			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
4.85901	69.66%	59.54%	31.73%

Means

Factor	N	Mean	StDev	95% CI

```
F0.4mg/ml   3 74.24   4.64 (67.38, 81.10)
S0.4mg/ml   3 61.41   4.87 (54.55, 68.28)
SA0.4mg/ml  3 61.57   5.06  (54.70, 68.43)

Pooled StDev = 4.85901
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence
Factor      N   Mean Grouping
F0.4mg/ml   3 74.24 A
SA0.4mg/ml 3 61.57    B
S0.4mg/ml   3 61.41    B

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

One-way ANOVA: F0.5mg/ml, S0.5mg/ml, SA0.5mg/ml

```
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 F0.5mg/ml, S0.5mg/ml, and SA0.5mg/ml


Analysis of Variance
Source DF Adj SS Adj MS F-Value P-Value
Factor   2 314.04 157.02    11.41    0.009
Error    6   82.56   13.76
Total    8 396.60


Model Summary
      S    R-sq R-sq(adj)  R-sq(pred)
3.70943 79.18%     72.24%      53.16%

Means
Factor      N   Mean  StDev      95% CI
F0.5mg/ml   3  88.05   4.42  (82.81, 93.29)
S0.5mg/ml   3  75.84   2.87  (70.60, 81.08)
SA0.5mg/ml  3  75.22   3.68  (69.98, 80.46)

Pooled StDev = 3.70943
```

Tukey Pairwise Comparisons

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

Factor      N   Mean  Grouping
F0.5mg/ml   3  88.05  A
S0.5mg/ml   3  75.84    B
SA0.5mg/ml  3  75.22    B

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

Appendix X: Statistical analysis of Total phenolic

Descriptive Statistics: F0.1mg/ml, S0.1mg/ml, F0.2mg/ml, S0.2mg/ml, F0.3mg/ml, S0.3mg/ml,

Variable	Mean	SE Mean	StDev
F0.1mg/ml	0.02333	0.00882	0.01528
S0.1mg/ml	0.04333	0.00882	0.01528
F0.2mg/ml	0.05000	0.00577	0.01000
S0.2mg/ml	0.10667	0.00882	0.01528
F0.3mg/ml	0.09667	0.00667	0.01155
S0.3mg/ml	0.16667	0.00882	0.01528
F0.4mg/ml	0.1600	0.0100	0.0173
S0.4mg/ml	0.2200	0.0115	0.0200
F0.5mg/ml	0.2300	0.0153	0.0265
S0.5mg/ml	0.2900	0.0115	0.0200

Two-Sample T-Test and CI: F0.1mg/ml, S0.1mg/ml

Two-sample T for F0.1mg/ml vs S0.1mg/ml

	N	Mean	StDev	SE Mean
F0.1mg/ml	3	0.0233	0.0153	0.0088
S0.1mg/ml	3	0.0433	0.0153	0.0088

Difference = μ (F0.1mg/ml) - μ (S0.1mg/ml)
Estimate for difference: -0.0200
95% CI for difference: (-0.0546, 0.0146)
T-Test of difference = 0 (vs ≠): T-Value = -1.60 P-Value = 0.184 DF = 4

Two-Sample T-Test and CI: S0.2mg/ml, F0.2mg/ml

Two-sample T for S0.2mg/ml vs F0.2mg/ml

	N	Mean	StDev	SE Mean
S0.2mg/ml	3	0.1067	0.0153	0.0088
F0.2mg/ml	3	0.0500	0.0100	0.0058

Difference = μ (S0.2mg/ml) - μ (F0.2mg/ml)
Estimate for difference: 0.0567
95% CI for difference: (0.0231, 0.0902)
T-Test of difference = 0 (vs ≠): T-Value = 5.38 P-Value = 0.013 DF = 3

Two-Sample T-Test and CI: S0.3mg/ml, F0.3mg/ml

Two-sample T for S0.3mg/ml vs F0.3mg/ml

	N	Mean	StDev	SE Mean
S0.3mg/ml	3	0.1667	0.0153	0.0088
F0.3mg/ml	3	0.0967	0.0115	0.0067

Difference = μ (S0.3mg/ml) - μ (F0.3mg/ml)
Estimate for difference: 0.0700
95% CI for difference: (0.0348, 0.1052)
T-Test of difference = 0 (vs ≠): T-Value = 6.33 P-Value = 0.008 DF = 3

Two-Sample T-Test and CI: S0.4mg/ml, F0.4mg/ml

```
Two-sample T for S0.4mg/ml vs F0.4mg/ml
           N    Mean   StDev  SE Mean
S0.4mg/ml  3  0.2200  0.0200    0.012
F0.4mg/ml  3  0.1600  0.0173    0.010


Difference = μ (S0.4mg/ml) - μ (F0.4mg/ml)
Estimate for difference:  0.0600
95% CI for difference:  (0.0114, 0.1086)
T-Test of difference = 0 (vs ≠): T-Value = 3.93  P-Value = 0.029  DF = 3
```

Two-Sample T-Test and CI: F0.5mg/ml, S0.5mg/ml

```
Two-sample T for F0.5mg/ml vs S0.5mg/ml
           N    Mean   StDev  SE Mean
F0.5mg/ml  3  0.2300  0.0265    0.015
S0.5mg/ml  3  0.2900  0.0200    0.012


Difference = μ (F0.5mg/ml) - μ (S0.5mg/ml)
Estimate for difference:  -0.0600
95% CI for difference:  (-0.1209, 0.0009)
T-Test of difference = 0 (vs ≠): T-Value = -3.13  P-Value = 0.052  DF = 3
```

One-way ANOVA: F0.1mg/ml, S0.1mg/ml, F0.2mg/ml, S0.2mg/ml, F0.3mg/ml, S0.3mg/ml,

```
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      10  F0.1mg/ml, S0.1mg/ml, F0.2mg/ml, S0.2mg/ml, F0.3mg/ml, S0.3mg/ml,
F0.4mg/ml,S0.4mg/ml, F0.5mg/ml, S0.5mg/ml

Analysis of Variance
Source  DF    Adj SS    Adj MS  F-Value  P-Value
Factor   9  0.216413  0.024046    81.05    0.000
Error   20  0.005933  0.000297
Total   29  0.222347

Model Summary
S    R-sq  R-sq(adj)  R-sq(pred)
0.0172240  97.33%     96.13%      94.00%
Means

Factor     N     Mean    StDev        95% CI
F0.1mg/ml  3  0.02333  0.01528  (0.00259, 0.04408)
S0.1mg/ml  3  0.04333  0.01528  (0.02259, 0.06408)
F0.2mg/ml  3  0.05000  0.01000  (0.02926, 0.07074)
S0.2mg/ml  3  0.10667  0.01528  (0.08592, 0.12741)
```

```
F0.3mg/ml  3  0.09667  0.01155  (0.07592, 0.11741)
S0.3mg/ml  3  0.16667  0.01528  (0.14592, 0.18741)
F0.4mg/ml  3   0.1600   0.0173  ( 0.1393,  0.1807)
S0.4mg/ml  3   0.2200   0.0200  ( 0.1993,  0.2407)
F0.5mg/ml  3   0.2300   0.0265  ( 0.2093,  0.2507)
S0.5mg/ml  3   0.2900   0.0200  ( 0.2693,  0.3107)

Pooled StDev = 0.0172240
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence
Factor     N     Mean  Grouping
S0.5mg/ml  3   0.2900  A
F0.5mg/ml  3   0.2300    B
S0.4mg/ml  3   0.2200    B
S0.3mg/ml  3  0.16667      C
F0.4mg/ml  3   0.1600      C
S0.2mg/ml  3  0.10667        D
F0.3mg/ml  3  0.09667        D E
F0.2mg/ml  3  0.05000          E F
S0.1mg/ml  3  0.04333            F
F0.1mg/ml  3  0.02333            F

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

One-way ANOVA: F0.1mg/ml, S0.1mg/ml

```
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       2  F0.1mg/ml, S0.1mg/ml


Analysis of Variance
Source  DF    Adj SS    Adj MS  F-Value  P-Value
Factor   1  0.000600  0.000600     2.57    0.184
Error    4  0.000933  0.000233
Total    5  0.001533

Model Summary
S    R-sq  R-sq(adj)  R-sq(pred)
0.0152753  39.13%     23.91%       0.00%


Means
Factor     N     Mean    StDev         95% CI
F0.1mg/ml  3  0.02333  0.01528  (-0.00115, 0.04782)
S0.1mg/ml  3  0.04333  0.01528  ( 0.01885, 0.06782)

Pooled StDev = 0.0152753
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence
Factor     N     Mean  Grouping
S0.1mg/ml  3  0.04333  A
```

```
F0.1mg/ml  3  0.02333  A

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

One-way ANOVA: F0.2mg/ml, S0.2mg/ml

```
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       2  F0.2mg/ml, S0.2mg/ml

Analysis of Variance
Source  DF    Adj SS    Adj MS  F-Value  P-Value
Factor   1  0.004817  0.004817    28.90    0.006
Error    4  0.000667  0.000167
Total    5  0.005483


Model Summary
S    R-sq  R-sq(adj)  R-sq(pred)
0.0129099  87.84%     84.80%      72.64%


Means

Factor     N     Mean    StDev        95% CI
F0.2mg/ml  3  0.05000  0.01000  (0.02931, 0.07069)
S0.2mg/ml  3  0.10667  0.01528  (0.08597, 0.12736)

Pooled StDev = 0.0129099
```

Tukey Pairwise Comparisons

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

Factor     N     Mean  Grouping
S0.2mg/ml  3  0.10667  A
F0.2mg/ml  3  0.05000    B

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

One-way ANOVA: F0.3mg/ml, S0.3mg/ml

```
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	2	F0.3mg/ml, S0.3mg/ml

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	1	0.007350	0.007350	40.09	0.003
Error	4	0.000733	0.000183		
Total	5	0.008083			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0135401	90.93%	88.66%	79.59%

Means

Factor	N	Mean	StDev	95% CI
F0.3mg/ml	3	0.09667	0.01155	(0.07496, 0.11837)
S0.3mg/ml	3	0.16667	0.01528	(0.14496, 0.18837)

Pooled StDev = 0.0135401

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
S0.3mg/ml	3	0.16667	A
F0.3mg/ml	3	0.09667	B

Means that do not share a letter are significantly different.

One-way ANOVA: F0.4mg/ml, S0.4mg/ml

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	2	F0.4mg/ml, S0.4mg/ml

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value

```
Factor   1  0.005400  0.005400    15.43    0.017
Error    4  0.001400  0.000350
Total    5  0.006800


Model Summary

        S    R-sq  R-sq(adj)  R-sq(pred)
0.0187083  79.41%     74.26%      53.68%


Means

Factor     N    Mean   StDev       95% CI
F0.4mg/ml  3  0.1600  0.0173  (0.1300, 0.1900)
S0.4mg/ml  3  0.2200  0.0200  (0.1900, 0.2500)

Pooled StDev = 0.0187083
```

Tukey Pairwise Comparisons

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

Factor     N    Mean  Grouping
S0.4mg/ml  3  0.2200  A
F0.4mg/ml  3  0.1600    B

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

One-way ANOVA: F0.5mg/ml, S0.5mg/ml

```
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       2  F0.5mg/ml, S0.5mg/ml


Analysis of Variance

Source  DF    Adj SS    Adj MS  F-Value  P-Value
Factor   1  0.005400  0.005400     9.82    0.035
Error    4  0.002200  0.000550
Total    5  0.007600


Model Summary

        S    R-sq  R-sq(adj)  R-sq(pred)
0.0234521  71.05%     63.82%      34.87%


Means
```

```
Factor      N    Mean   StDev        95% CI
F0.5mg/ml   3  0.2300  0.0265  (0.1924, 0.2676)
S0.5mg/ml   3  0.2900  0.0200  (0.2524, 0.3276)

Pooled StDev = 0.0234521
```

Tukey Pairwise Comparisons

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

Factor      N    Mean  Grouping
S0.5mg/ml   3  0.2900  A
F0.5mg/ml   3  0.2300    B

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

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NAIROBI, KENYA
Tel. 8710901 Ext. 57530

Our Ref: I56/CE/24744/11

DATE: 2nd August 2015

Director General,
National Commission for Science, Technology
& Innovation
P.O Box 36023-00100
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION FOR KENEDY W. WAFULA– REG. NO. I56/CE/24744/11.

I write to introduce Mr. Kenedy W. Wafula who is a Postgraduate Student of this University. She is registered for M.Sc degree programme in the **Department of Biochemistry & Biotechnology.**

Mr. Wafula intends to conduct research for a M.Sc. Proposal entitled, **"Antioxidant Activities of Dichloromethane Extracts of *Strychnos henningsii* and *Ficus sycomorous* in Pancreas od Alloxan Induced Diabetic Mice".**

Any assistance given will be highly appreciated.

Yours faithfully,

MRS. LUCY N. MBAABU
FOR: DEAN, GRADUATE SCHOOL

KM/nn