HYPOCHOLESTEROLEMIC AND ANTIOXIDANT EFFECTS OF PHENOLIC RICH FRACTION OF *CINNAMOMUM VEROM* (CINNAMON) STEM BARK IN RATS FED WITH HIGH FAT DIET

BY

AISHA SHEHU

SPS/17/MBC/00032

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AUGUST, 2021.

DECLARATION

I hereby declare that this work is the product of my research efforts undertaken under the supervision of Prof. M. K Atiku and has not been presented anywhere for the award of M.Sc in Biochemistry. All Sources have been duly acknowledged.

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Aisha Shehu

SPS/17/MBC/00032

CERTIFICATION

This is to certify that the research work for this dissertation and the subsequent write up of this report by Aisha Shehu (SPS/17/MBC/00032) were carried out under my supervision.

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Prof. M. K. Atiku

Supervisor

APPROVAL

This dissertation has been examined and approved for the award of the degree of MASTER OF SCIENCE in BIOCHEMISTRY (MEDICAL).

EXTERNAL EXAMINER	**DATE**
DR. A.M. GADANYA **INTERNAL EXAMINER**	**DATE**
PROF. M.K ATIKU **SUPERVISOR**	**DATE**
DR. Y.Y MUHAMMAD **HEAD OF DEPARTMENT**	**DATE**

DEDICATION

I dedicate my work to my Late father Alhaji Shehu Muhammad may his gentle soul rest in perfect peace and may Almighty Allaah grant him Al-Jannatul Firdausi “amen”. I will never forget his love and support; he will forever remain in my memory.

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ABSTRACT

Hypercholesterolemia is a metabolic disorder characterized by an increase in the concentration of plasma cholesterol (above 200mg/dL). It is considered the primary risk factor for developing cardiovascular disease (atherosclerosis). Antioxidants are those substances that neutralize free radicals by supplying them with the electron they lack. This study aimed to determine the hypocholesterolemic and antioxidant effects of phenolic rich fraction extracted from stem bark of *Cinnamomum verom*. Phytochemical components, in-vitro antioxidant activity of diethyl ether fraction and LD_{50} of the phenolic rich fraction of the plant extract were analyzed. Lipid profile parameters and in-vivo antioxidant activities of phenolic rich fraction of the plant extract on twenty-eight (28) experimental animals fed with high cholesterol and normal diet were analyzed. The animals were divided into seven (7) groups, Group I was given only normal diet, group II was administered with high fat diet only, group III were administered with high fat diet and 100mg/kg of atorvastatin drug, groups IV and V were administered with high fat diet and 100mg/kg and 200mg/kg of phenolic rich extract respectively and groups VI and VII were administered with normal diet and 100mg/kg and 200mg/kg of extract respectively. The animals were sacrificed after 28 days and blood samples were collected and analyzed for Total Cholesterol (TC), High-Density Lipoprotein-Cholesterol (HDL-CH), Low-Density Lipoprotein-Cholesterol (LDL-CH), Triglyceride (TG), Catalase (CAT), Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx) and Malondyaldehyde (MDA). Gas-Chromatography Mass-Spectroscopy (GC-MS) of phenolic rich fraction(s) of stem bark of cinnamon were carried out to analyze the active compounds of the extract. The result showed the presence of important phytochemicals such as alkaloids, phenols, tannins, flavonoids, terpenoids, glycosides and saponins. It also revealed that the phenol rich fraction is non-toxic to experimental animals even at higher dose of 5000mg/kg body weight. The result shows significant difference ($p<0.05$) in DPPH and reducing power of ascorbic acid, diethyl ether fraction and sodium hydroxide fraction. A significant ($p<0.05$) increase in high –density lipoprotein –cholesterol was observed among the groups (II<VI<I<IV<VII<V<III) and a decrease in total cholesterol, low-density lipoprotein-cholesterol and triglyceride were observed among the groups (II>IV>V>III>>I>VI>VII). The activity of catalase, glutathione peroxidase and superoxide dismutase were significantly ($p<0.05$) increased in group III, IV, V, VI and VII when compared with group II, while the level of malondialdehyde was significantly ($p<0.05$) decreased in groups III, IV, V, VI and VII when compared with II. The result of the GC-MS showed active compounds which may be responsible for the hypocholesterolemic and antioxidant activity of cinnamon bark extract and were found to be mostly phenols such as 1H Indole 2-phenyl, 1,3-benzodioxole, 3-phenyl 2-propyn-1-ol and cinnamaldehyde (E). Thus, the phenolic rich fraction extracted from stem bark of cinnamon showed both hypocholesterolemic and antioxidant effect.

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LIST OF ABBREVIATIONS

Symbol	Interpretation
TC	Total Cholesterol
LDL	Low Density Lipoprotein
HDL	High Density Lipoprotein
TRIG	Triglyceride
SOD	superoxide dismutase
WHO	World Health Organization
MDA	Malondialdehyde
CAT	Catalase
GPx	Glutathione Peroxidase
HCD	High Cholesterol Diet

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CHAPTER ONE

INTRODUCTION

1.1 General Introduction

Medicinal plants also known as medicinal herbs are different types of plants that have medicinal activities used for herbalism and are the back bone of traditional medicines (Singh, 2015). They are frequently used as raw materials for extraction of active ingredients used for the synthesis of many drugs examples in laxatives, antibiotics and antimalarial drugs (Refaz *et al*., 2017). The use of traditional medicine and medicinal plants in most developing countries, as a basis for the maintenance of good health, has been widely observed by UNESCO, (1996). Furthermore, an increasing reliance on their use in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies (UNESCO, 1998).

Phenolics are one of the most sluttish and ubiquitously distributed group of naturally occurring phytoconstituents, all of which have an aromatic ring bearing structure at least one hydroxyl substituent (a phenol). They are commonly found in herbs, fruit, vegetables, grains, tea coffee beans, and red wine. A beneficial biological activity of phenolics in mammals includes antiviral, antibacterial, immune-stimulating, anti-allergic, anti-hypertensive, anti-ischemic, anti-arrhytmic, anti-thrombotic, hypocholesterolaemic, anti lipoperoxidant, hepatoprotective, anti-inflammatory, and anti-carcinogenic actions (Kumar and Pandey, 2013). The medicinal property of phenolics is mostly ascribed to their antioxidant capacity, modulation of gene expression and interaction with the cell signaling pathways. The antioxidant capacity of phenolic compounds is also attributed to their ability to chelate metal ions involved in the production of free radicals (Kumar *et al.*, 2012).

Phenolic structures have capability to strongly interact with proteins, due to their hydrophobic benzenoid rings and hydrogen-bonding virtue of the phenolic hydroxyl groups. These exquisite properties of phenolics gives the ability to act as antioxidants also by virtue of their capacity to inhibit some enzymes involved in radical generation, such as various cytochrome P450 isoforms, lipoxygenases, cyclooxygenase and xanthine oxidase. Phenolics are also capable of acting in redox-sensitive signaling cascades to inhibit DNA damage. Therefore, phenolics may be beneficial to preventing UV-induced oxygen free radical generation and lipid peroxidation. Phenolics can modulate transcriptional factors such as AP-1 (activator protein-1). It can control the expression of various genes implicated in inflammation processes, cell differentiation and proliferation. Phenolics may prevent oxidative stress induced mitochondrial transition pore complex opening by decreasing production of Bax and Bad protein (Mandel and Youdim, 2004). Phenolics are also involved in activation of several protein kinases, phase II antioxidant detoxifying enzymes and modulation of several cell survival/cell-cycle genes.

Cinnamomum belonging to the family *Lauraceae* is a large genus comprising more than 200 species that grow in Asia, Australia and Pacific Islands. The most important constituents of cinnamon are phenols which are cinnamaldehyde and *trans*-cinnamaldehyde (Cin) (Yeh *et al,* 2013). Several *Cinnamomum* species are aromatic and yield essential oils on steam or hydro-distillation. The species that are commercially important are *Cinnamomum zeylanicurz* Blume (syn. *C. verom* JS Presl), *Cinnamomum cassia* Blume, *Cinnamomum tamala* Nees, and *Cinnamomum camphora* Seib. *Cinnamomum* zeylanicurm is a native of Sri-Lanka and tropical Asia. It is grown mostly in Sri Lanka, India, Seychelles and Malagasy Republic. *Cinnamomum cassia* (Chinese cinnamon) is grown mostly in China. *C. cantphora* (camphor tree) is grown in China, India, Taiwan and Japan. The former two species are reputed spices that are used in foods

and pharmaceuticals since ancient times. *Cinnamomum camphora* produces large quantities of natural camphor (Gopal and Rajeswara, 2007).

Cinnamomum verom is a plant that has different uses among different cultures from spicing food to deterring germs from growing (Sonia, *et al.,* 2013). The bark of various cinnamon species is one of the most important and popular spices used worldwide not only for cooking but also in traditional and modern medicines (Sangal, 2011). Overall, approximately 250 species have been identified among the cinnamon genus, with trees being scattered all over the world (Vangalapati *et al.,* 2012). In addition to its culinary uses, cinnamon has been employed as a stomachic and carminative for gastro intestinal complaints as well as other ailments and is still used for these conditions in many countries (Teuscher, 2003).

Cardiovascular disease (CVD) refers to the disease of the heart and blood vessels. Heart disease encompasses a number of diseases that affect the heart example coronary artery disease whereas, vascular disease encompasses a number of diseases that affect the blood vessels. Blood circulatory health problems are a result of cardiac and vascular disease. CVD are the leading cause of stroke and death (Terje, 2011).

Hypercholesterolemia is a condition characterized by very high levels of cholesterol in the blood (Sonia *et al.,* 2013). It usually results from nutritional factors such as obesity and diet high in saturated fats combined with an underlying polygenic predisposition. There is overproduction of LDL (Kane and Havel, 2001) and its genetic component is unlikely to be monogenic, unless it is extreme. Hypercholesterolemia can also have an entirely genetic cause, a common example of this is monogenic familial hypercholesterolemia, an autosomal dominant disorder in which the LDL cholesterol is raised from birth (Goldstein *et al.,* 2001). It is characterized by a dominant

pattern of inheritance of premature coronary disease and/or tendon xanthomata (Stone *et al.,* 2007).

Hypercholesterolemia has been shown to contribute in development and severity of atherosclerosis and cardiovascular diseases (Zahid *et al*., 2016). The level of cholesterol transported by lipoproteins like chylomicrons, the very-low-density lipoproteins (VLDL) and the low density lipoproteins (LDL), is a risk factor for the incidence of cardiovascular disease (Rafieian *et al.,* 2014).

Free radicals are considered as constant threats to the human bodies (Nasri *et al.,* 2013). They are wide groups of highly reactive molecules which may cause damage to cell structures. Reactive oxygen species (ROS) are considered as the most abundant free radicals which are usually formed as natural byproducts of normal metabolism in the body. ROS have crucial role in homeostasis and cell signaling (Rafieian *et al.,* 2013a). Free radicals are present and produced in many chemical configurations, shapes and sizes. They can damage the DNA or oxidize the molecule of circulating low-density lipoprotein (LDL), making it suitable to be trapped in an artery wall (Rafieian *et al.,* 2013b). Free radicals can accelerate atherosclerosis, hence antioxidants should be able to prevent atherosclerosis (Mirhosseini *et al.,* 2014).

Antioxidants are naturally occurring plant substances that protect the body from damage caused by harmful molecules called free radicals. Antioxidants help prevent oxidation, which can cause damage to cells and may contribute to aging. They may improve immune function and perhaps lower the risk for infection, cardiovascular disease and cancer. Antioxidants exist as vitamins, minerals and other compounds in foods. A diet containing plenty of fruits and vegetables, whole grains and nuts can supply all the antioxidants the body needs. Diets rich in antioxidants can be very beneficial (Hamid *et al*., 2010).

1.2 Statement of Problem

Dyslipidemia is generally regarded as an underlying cause of metabolic and cardiovascular disorders such as diabetes mellitus, coronary artery disease /coronary heart diseases (CHD), fatty liver and some cancers. Such disorders are the most common cause of death in both developed as well as developing countries. It is estimated to be the cause of one third of death globally (WHO, 2015). The death is due to Oxidative stress caused by reactive oxygen species. Available means of management may be expensive and are not readily accessible. Therefore, the use of cinnamon with high level of phenolic (antioxidant) in foods may serve in the management of dyslipidemia in the system

1.3 Justification

Most medicinal plants are less expensive, readily accessible and can be used for the treatment of many diseases. *Cinnamomum verom* is one of the plants traditionally used for its antidiabetic, antitumor, antifungal, anti-inflammatory, antiviral and antibacterial properties due to the presence of major cinnamon compound eugenol and cinnamaldehyde (Joerg *et al.,* 2010). Literatures has it that phenols are associated with wide range of biological effect on health, including antioxidative effect and antithrombotic activities (Pyrzynska and Biesaga, 2009). Information on the effect of the isolated phenolic compound is limited, hence the need to know the hypocholesterolaemic and antioxidant effect of phenolic rich fraction extracted from stem bark of *Cinnamomum verom*.

1.4 Aim and Objectives

The aim of this research was to assess the effect of oral administration of phenolic rich fraction extracted from stem bark of *Cinnamomum verom* on serum lipid profile and antioxidant enzymes in hypercholesterolaemic and normolipidaemic albino rats.

The specific objectives include:

1. To extract phenolic rich fraction from stem bark of C*innamomum verom*
2. To determine serum lipid profile (total cholesterol, LDL-Cholesterol, HDL-cholesterol and triglycerides), antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT) and Glutathione peroxidase (GPx)) and Malondyaldehyde (MDA), in hypercholesterolaemic and normolipidaemic rats orally administered with different doses of the phenol rich fraction isolated from stem bark of *Cinnamomum verom* extract.
3. To identify the bioactive compounds of the phenolic rich fraction of *Cinnamomum verom* stem bark using Gas Chromatography Mass Spectroscopy (GC-MS)

CHAPTER TWO

LITERATURE REVIEW

2.1 Medicinal Plants

The term medicinal plants include various types of plants used in herbalism and some of these plants have medicinal activities. Medicinal plants are the “backbone” of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize them on a regular basis (Davidson, 2000). They are considered as rich resources of ingredients which can be used in drug development and synthesis. They play a critical role in the development of human cultures around the whole world. There are about 17.000 species of higher plants, of which approximately 8.000 species are considered medicinal and used mostly by village communities, particularly tribal communities, or in traditional medicinal systems, such as the Ayurveda (Singh, 2015).

The use of traditional medicine and medicinal plants in most developing countries, as a basis for the maintenance of good health, has been widely observed by UNESCO, 1996. Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies (UNESCO, 1998).

During the past decade, traditional systems of medicine have become a topic of global importance. Current estimates suggest that, in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. Although modern medicine may be available in these countries, herbal medicines (phytomedicines) have often maintained popularity for historical and cultural reasons (Singh, 2015).

Medicinal plants are frequently used as raw materials for extraction of active ingredients which are used in the synthesis of different drugs. Like in case of laxatives, blood thinners, antibiotics and anti-malarial medications, contain ingredients from plants. Moreover, the active ingredients of Taxol, vincristine and morphine isolated from foxglove, periwinkle, yew and opium poppy respectively (Singh, 2015).

Medicine in several developing countries using local traditions and beliefs is still the mainstay of health care. As defined by WHO, health is a state of complete physical, mental and social wellbeing and not merely the absence of disease or infirmity. Medicinal plants can make an important contribution to the WHO goal to ensure by the year 2000, that all peoples worldwide will lead a sustainable socioeconomic productive life (Lucy and Edger, 1998).

2.2 Phenolic Compounds as Secondary Metabolites Found in Plants and Foods

Polyphenols are group of secondary metabolites found in all plants, representing the most desirable phytochemicals due to their potential to be used as additives in food industry, cosmetics, medicine and other fields. At present, there is an increased interest to recover them from plant of spontaneous flora, cultivated plants and wastes resulted in agricultural and food industries. Phenolic compounds are considered the most abundant constituents of plants and

processed foods; some phenolic compounds are extremely widespread while others are specific to certain plant families or found only in certain plant organs or at certain development stages (Cheynier, 2012).

Main edible sources of phenolic compounds are fruits and vegetables, seeds, cereals, berries, beverages (wine, tea and juices), olive and aromatic plants. Moure *et al*., 2001; Ignat *et al*., 2011b; and Stevanovic *et al*., 2009 focused a special attention to the presence of these compounds in agricultural and industrial wastes, wood and non-wood forest resources. Spruce and pine wood bark, which represent a waste in the wood industry, have been reported to contain a wide range of phenolic compounds like stilbene glycosides (Balas and Popa, 2007a), gallic acid, catechine, vanillic acid (Hainal *et al*., 2011). Also, cinnamon is said to contain phenols such as cinnamyl acetate, cinnamic aldehyde, eugenols among others (Osuna *et al*., 2005)

2.2.1 Structure and classification of phenols

Phenolic compounds are one of the most numerous and widely distributed group of aromatic compounds in the plant kingdom, with over 8000 phenolic structures currently known, of which more than 6000 are the flavonoids (Garcia *et al*., 2010; Tsao *et al*., 2010; and Vladimir *et al*., 2012). From the chemical point of view, polyphenols are natural compounds with aromatic structures containing one or more aromatic rings with or without the vicinity of a heterocycle and which are grafted with hydroxyl, carboxyl, methoxyl and carbonyl functional groups. According to the biological function, polyphenols can be classified into different classes; however, two main groups of polyphenols can be identified: the flavonoids and the non-flavonoids (Vladimir *et al*., 2012).

2.2.1.1 Non-flavonoid polyphenols

Non-flavonoids can be classified according to their chemical structure into the following groups: phenolic acids with the subclasses derived from hydroxybenzoic acids and from hydroxycinnamic acid, stilbenes, lignans and the polymeric lignins (Han *et al*., 2012; Vladimir *et al*., 2012).

Phenolic acids. From a chemical point of view, phenolic acids containing carboxyl group with one or more hydroxyl groups grafted onto a benzene nucleus. Phenolic acids are the most abundant polyphenols in our diets (30%) and are found in different forms in plants, including aglycones (free phenolic acids), esters, glycosides, and/or bound complexes (Garcia *et al*., 2010; Khoddami *et al*., 2013). Based on position of the hydroxyl group, phenolic acids can be divided into two main types, benzoic acid (C1–C6) and cinnamic acid derivatives (C3–C6) (Tsao, *et al.,* 2010).

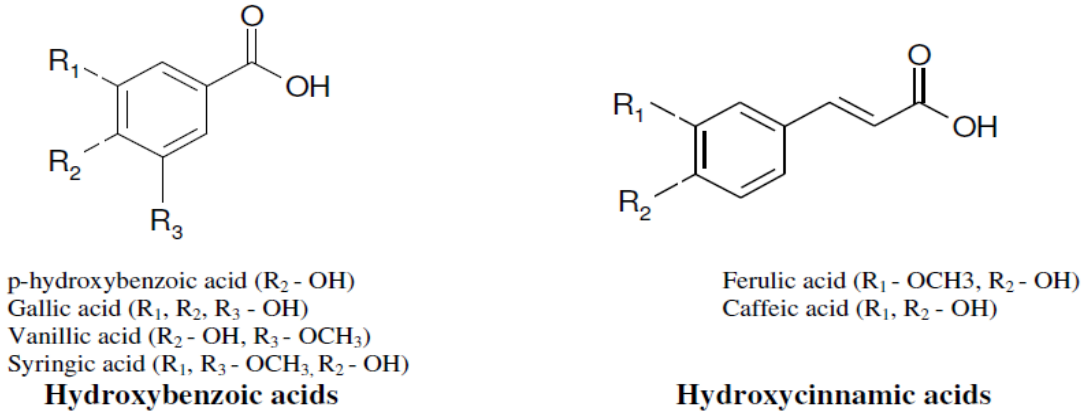

Fig 2.1: structure of hydroxybenzoic acid and hydroxycinnamic acid (Tapas *et al*., 2008).

The most common hydroxybenzoic acids are vanillic, syringic and gallic acids. Among hydroxycinnamic acids, caffeic and ferulic acids are the most abundant compounds in foods. Ferulic acid is mainly found from dietary fiber, sources of which include wheat bran and caffeic

acid occurs mainly as esters (chlorogenic acid) and is largely obtained from coffee, fruits and vegetables (Ndhlala *et al*., 2010).

Stilbenes. Stilbenes are another class of compounds that are part of non-flavonoid polyphenols with 1, 2-diphenylethylene as basic structure.

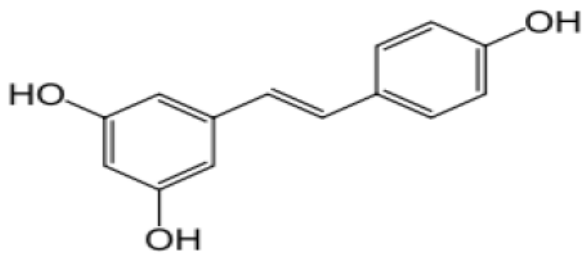

Fig 2.2: Chemical structure of stilbene (resveratrol) (Tapas *et al*., 2008).

Resveratrol is the main representative of this group of phenolic compounds.

This compound exists in two stereo isoforms with configuration *cis*- or *trans*-, the latter being the most widely studied (Giovinazzo *et al*., 2012). Resveratrol is found in small fruits such as grapes and *Vaccinium* berries, peanuts and in *Polygonum* species (Rimando and Suh, 2008). High interest in this compound is linked to its use in the treatment of cardiovascular diseases but also in the fight against motor deficiencies that lead to mobility problems of old people (Kelsey *et al*., 2010).

Lignans: The lignans are a group of natural phenolic compounds with carbon skeletons derived from two phenyl propane units joined together by at least one carbon-carbon bond between the two central β -carbons of the C3 chains (lignans) or by bonds other than the β-β‘-carbon-carbon bond, in which case the resulting compounds are called neolignans (Ferrazzano *et al*., 2011). In nature, lignans are present in the aglycone forms, while their glycosides occur only in small amounts (Ignat *et al.*, 2011b).

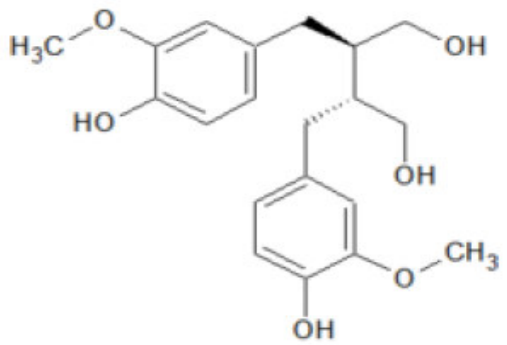

Fig 2.3: Chemical structure of lignans (secoizolariciresinol). (Tapas *et al*., 2008).

The greatest dietary source of lignans is considered to be the flax seeds, but they are also found in appreciable quantities in sesame seed and, to a lesser degree, in a variety of grains, seeds, fruits, and vegetables (Craft *et al*., 2012). In general, the lignan content of foods not exceed 2 mg/100 g with some exceptions: 335 mg/100g in flaxseed and 373 mg/100g in sesame seeds, which have lignan content a hundred times higher than other dietary sources (Peterson *et al*., 2010). Lignans have many biological activities, showing antiviral, anticancer, anti-inflammatory, antimicrobial, antioxidant, immunosuppressive properties and hepatoprotective and osteoporosis prevention (Cunha *et al*., 2012).

Lignins: Lignins are important plant polymers that comprise 16–33% of wood biomass and represent the second largest organic compound after cellulose (Mäki *et al*., 2007). The chemical structure of lignin is the result of polymerization of the *p*coumaryl, coniferyl, and sinapyl hydroxycinnamic alcohols (Yang *et al*., 2013). In plants, lignin strengthens the plant cell walls, aid water transport, protects polysaccharides in the plant cell walls from degrading, help plants to resist on pathogens and other threats, and provide texture in edible plants (Peterson *et al*., 2010).

Due to its so complex structure, lignin valorization is one of the greatest challenges in bio-refining being the only large-volume renewable feedstock that is composed of aromatics

(Ragauskas *et al*., 2014). Lignins are also important to the human health because they possess multiple properties such as antioxidant, UV-absorption antifungal, antibiotic activity, anti-carcinogenic, apoptosis inducing antibiotic, anti-HIV activities and it has been suggested that can be applied for stabilization of food and feed (Dumitru *et al.*, 2013).

2.2.1.2 Flavonoids

Flavonoids are a class of phenolic compounds which together with carotenoids and chlorophyll give color to many species of flowers and fruits. Flavonoids occur only in plants where are present predominantly as glycosides (El Gharras, 2009), in which one or more hydroxyl groups of phenols are combined with reducing sugars. Flavonoids are also associated with a wide range of biological effects on health, including antibacterial, anti-inflammatory, anti-allergic and antithrombotic activities (Pyrzynska and Biesaga, 2009).

The term flavonoid is assigned to the polyphenolic compounds of the general structure C_6-C_3-C_6 in which the two phenolic benzene rings A and C are linked by a pyran ring B s

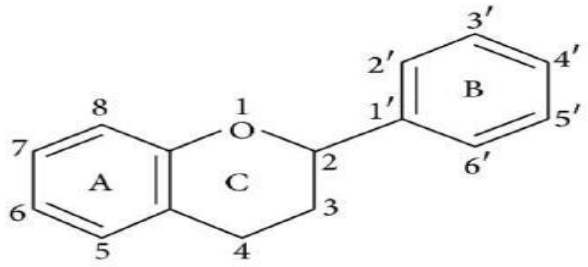

Fig 2.4: Basic structure of flavonoids (2-phenyl-1-benzopyran) (Tapas *et al*., 2008).

According to the oxidation state of the central C ring, flavonoids are divided into six subgroups: flavones, flavonols, flavanols, flavanones, isoflavones, and anthocyanins (Dai and Mumper, 2010).

This subclass is also distinguished by the number, position and nature of the substituent existing in the phenolic ring (free hydroxyl, glycosidic and methylated groups). Regarding the biosynthesis of flavonoids, they are derived from the aromatic amino acids, phenyalanine and tyrosine, and have three-ringed structures (Khoddami *et al*., 2013).

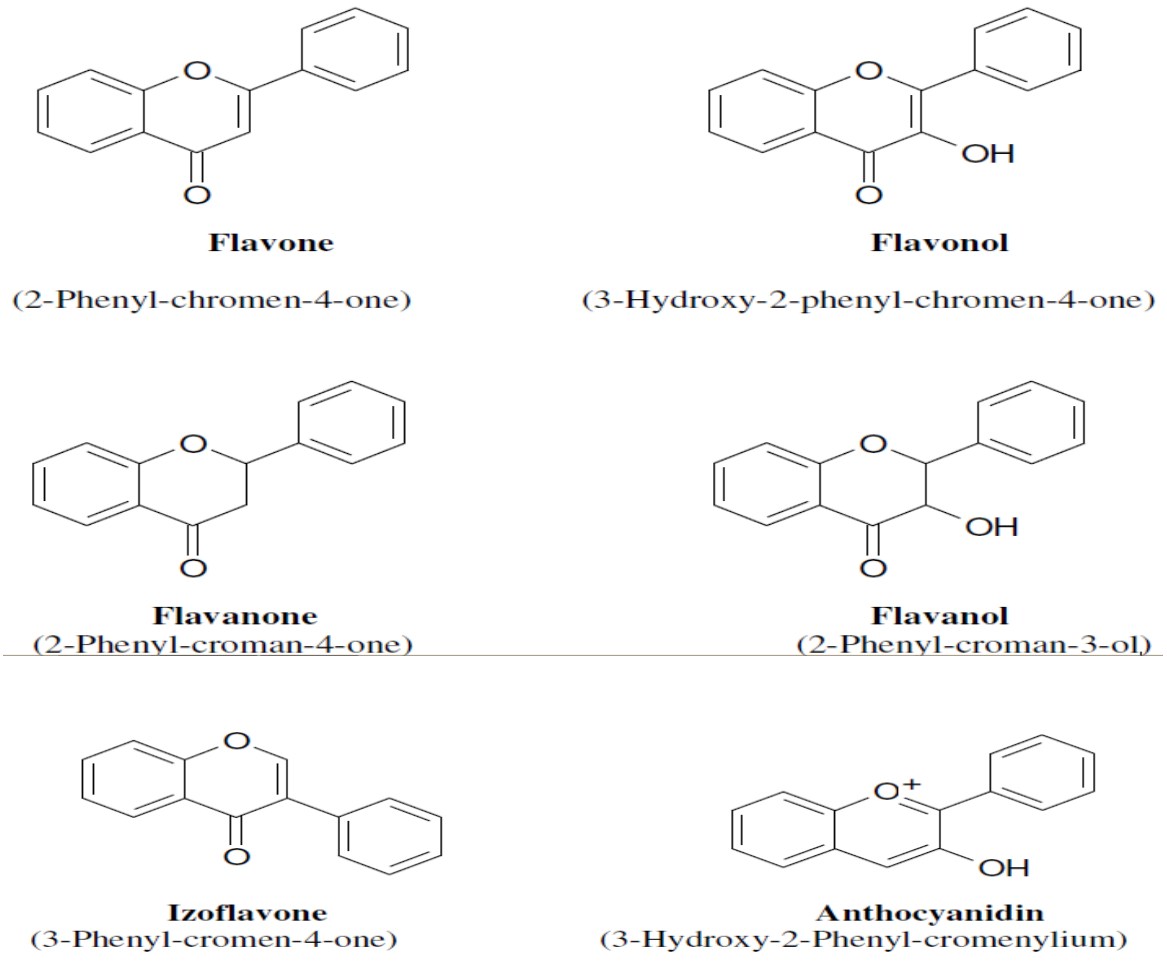

Fig 2.5: Chemical structures of some representative subclasses of flavonoids (Tapas *et al*., 2008).

Flavones and flavonols: Flavones are characterized by the presence of a double bond between C2 and C3 in the heterocycle of the flavan skeleton (Vladimir *et al*., 2012). The most studied flavones are apigenin, luteolin and their derivatives. Flavonols are hydroxylated flavone

derivatives and distinguished by the presence of hydroxyl functional group in C3 position. They are found in many fruits and vegetables, including onions, apples, broccoli, and berries (Patel 2008). Among the flavonols, kaempferol, quercetin and myricetin are most important.

Flavanones and flavanols: Compared with the flavonols and flavones, these two groups are characterized by the absence of the double bond between C2 and C3 and have the precursor 2-phenyl-benzopyrone. The main source of flavanones and flavanols are citrus fruits and juices, flavanols having an important role in generating these fruit taste (Peterson *et al*., 2006). Among flavanones, important ones are: hesperetin, naringin, pinocembrina and eriodictyol. Naringin is present in grapefruit, oranges, grape berries epicarp. This substance has an antibacterial, antiviral, anticancer, depression and antioxidant effect (Sandeep *et al*., 2011). Hesperetin is known for his antibacterial, antiviral, pesticidal, cancer preventive and hepatoprotective action. Flavanols or flavan-3-ols exist as simple monomers such as (+)-catechine and (−)-epicatechine, but also oligomers or polymers are called proanthocyanidins because they release anthocyanidins when are heated in acidic solutions.

Anthocyanins: Anthocyanins are the main class of flavonoids that are responsible for cyanotic colors ranging from pink, red and purple to dark blue of most fruits, flowers and leaves (Andersen and Markham, 2006). Chemically anthocyanins are glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium or flavlium salts, the only differences between individual anthocyanins being the number of hydroxyl groups in the molecule, the degree of methylation of these hydroxyl groups, the nature and number of sugars attached to molecule and the position of the attachment, and the nature and number of aliphatic or aromatic acids attached to the sugars in the molecule (Mazza and Miniati, 1946). The aglycones of anthocyanins, the anthocyanidins, consist of an aromatic ring A bonded to a heterocyclic ring C

that contains oxygen, which is also bonded by a carbon–carbon bond to a third aromatic ring B (Ignat *et al*., 2011b). The diversity of anthocyanins are due to the number and position of hydroxyl and methoxy groups, the identity, number, and positions at which sugars are attached, and the extent of sugar acylation and the identity of the acylating agent, but only six are ubiquitously spread and of great importance in human diet: cyanidin, delphinidin, petunidin, peonidin, pelargonidin, and malvidin. The most commonly types of sugars linked to anthocyanidins are monosaccharides (glucose, galactose, rhamnose and arabinose), and di- or tri-saccharides formed by combination of the four monosaccharides (Ignat et al., 2011c; Ienaşcu *et al*., 2009).

Isoflavones. Found abundantly in vegetable, isoflavones are a group of compounds derived from flavanones. The main factor that differentiates them from other isoflavones is given by the orientation of the C3 position of the benzene ring C (Andersen and Markham, 2006). Isoflavones are also called phytoestrogens because their structure is analogous to the structure of estrogen (Ignat *et al*., 2011c). The most representative compounds of this class are daidzein, genistein, biochanin A and formononetin. Natural sources of isoflavones are soy and products thereof, being found in dry peas, alfalfa seeds and grain / seed of clover, green beans, chickpeas, lima beans and sunflower seeds.

2.3 *Cinnamomum verom*

Cinnamon has been known from remote antiquity. It was imported to Egypt as early as 2000 BC, but those who reported that it had come from China had confused it with *cinnamon cassia*, a related species. Cinnamon was so highly prized among ancient nations that it was regarded as a gift fit for monarchs and even for a deity; a fine inscription records the gift of cinnamon and cassia to the temple of Apollo at Miletus. Its source was kept mysterious in the Mediterranean

world for centuries by those in the spice trade to protect their monopoly as suppliers. *Cinnamomum verom*, which translates as 'true cinnamon', is native to India, Sri Lanka, Bangladesh, and Myanmar. *Cinnamomum cassia* (cassia) is native to China. Related species, all harvested and sold in the modern era as cinnamon, are native to Vietnam, Indonesia and other Southeast Asian countries with warm climates (Gopal and Rajeswara, 2007).

Cinnamon is used as a flavoring agent in some alcoholic beverages, such as cinnamon-flavored whiskey in the United States, and rakomelo, a cinnamon brandy popular in parts of Greece. *Cinnamomum verom*, called true cinnamon tree or Ceylon cinnamon tree is a small ever green tree belonging to the family Lauraceae, native to Sri-Lanka. Among other species, its inner bark is used to make cinnamon. The old botanical synonym for the tree, *Cinnamomum zeylanicum*, is derived from Sri-Lanka's former name; Ceylon. Sri-Lanka still produces 80–90%of the world's supply of *Cinnamomum verom*, which is also cultivated on a commercial scale in the Seychelles and Madagascar. *Cinnamomum verom* trees are 10–15 meters (30–50 feet) tall. The leaves are ovate-oblong in shape and 7–18 cm (3–7inches) long. The flowers, which are arranged in panicles, have a greenish color and a distinct odor. The fruit is a purple 1-cm drupe containing a single seed (Sangal, 2011 and Vangalapati *et al,* 2012).

2.3.1 Species of Cinnamon

A number of species are often sold as cinnamon:

- *Cinnamomum cassia* (cassia or Chinese *cinnamon*, the most common commercial type)
- *C. burmannii* (Korintje, Padang cassia, or Indonesian cinnamon)
- *C. loureiroi* (Saigon cinnamon*, Vietnamese cassia, or* Vietnamese cinnamon)
- *C. verom* (Sri-Lanka *cinnamon*, Ceylon cinnamon or *Cinnamomum zeylanicum*)
- *C. citriodorum* (Malabar cinnamon)

Cassia induces a strong, spicy flavor and is often used in baking, especially associated with cinnamon rolls, as it handles baking conditions well. Among *cassia*, Chinese cinnamon is generally medium to light reddish brown in color, hard and woody in texture, and thicker (2–3mm (0.079–0.118in) thick), as all of the layers of bark are used. Ceylon cinnamon, using only the thin inner bark, has a lighter brown color, a finer, less dense and crumbly texture. It is considered to be subtle and more aromatic in flavor than cassia and it loses much of its flavor during cooking.

The barks of the species are easily distinguished when whole, both in macroscopic and microscopic characteristics. *Ceylon cinnamon* sticks (quills) have many thin layers and can easily is made into powder using a coffee or spice grinder, whereas cassia sticks are much harder. Indonesian cinnamon is often sold in neat quills made up of one thick layer, capable of damaging a spice or coffee grinder. Saigon cinnamon (*C. loureiroi*) and Chinese cinnamon (*C. cassia*) are always sold as broken pieces of thick bark, as the bark is not supple enough to be rolled into quills (Huang *et al,* 2007).

The powdered bark is harder to distinguish, but if it is treated with tincture of iodine (a test for starch), little effect is visible with pure Ceylon cinnamon, but when Chinese cinnamon is present, a deep-blue tint is produced (Yeh *et al,* 2013).

2.3.2 Cultivation

Cinnamon is an evergreen tree characterized by oval-shaped leaves, thick bark (Figure 2.6) and a berry fruit. When harvesting the spice, the bark and leaves are the primary parts of the plant used. Cinnamon is cultivated by growing the tree for two years, then coppicing it, i.e., cutting the stems. The following year, about a dozen new shoots form from the roots, replacing those that were cut. The stems must be processed immediately after harvesting while the inner bark is still

wet. The cut stems are processed by scraping off the outer bark, then beating the branch evenly with a hammer to loosen the inner bark, which is then pried off in long rolls. Only 0.5mm (0.02in) of the inner bark is used; the outer, woody portion is discarded, leaving meter-long cinnamon strips that curl into rolls ("quills") on drying. The processed bark dries completely in four to six hours, provided it is in a well-ventilated and relatively warm environment. Once dried, the bark is cut into 5 to 10cm (2 to 4in) lengths for sale (Marongiu, *et al,* 2007).

Figure 2.6: Cinnamon bark stick and the powder (Marongiu, *et al,* 2007).

2.3.3 Production

Cinnamon production (tonnes) in 2016 stands as Indonesia (91,300), China (77,055), Vietnam (35,516), Sri-Lanka (16,931), Madagascar (24,640) and production worldwide is 223,575 (FAOSAT,) of the United State. Combined, Indonesia and China produced 75% of the world's cinnamon in 2016 when global production was 223,574 tonnes . Four countries accounted for 99% of the world total: Indonesia, China, Vietnam, and Sri-Lanka (Chou, *et al,* 2013).

2.3.4 Scientific Classification of Cinnamon

Kingdom	Plantae
Clades	Angiosperms
Clades	Magnoliids
Order	Laurales
Family	Lauraceae
Genus	*Cinnamonum*
Species	*C. verom*

2.3.5 Phytochemical Composition of Cinnamon

Cinnamon contains 9.5–10.5% moisture, 3.89–4.65% protein, 59.55–80.59% carbohydrate, 53.1% dietary fiber, 3.55% ash, and vitamins (Charles 2013; Parthasarathy *et-al.,* 2008; Peter 2001). Cinnamon oleoresin is a reddish brown powder which is obtained by solvent extraction such as ethanol (yield 10–12%) and benzene (yield 2.5–4.3%), the latter being a prohibited solvent. Cinnamon contains a wide range of phytochemicals including cinnamaldehyde, cinnamyl acetate, cinnamyl alcohol, eugenol, eugenol acetate, linalool, benzaldehyde, cinnamaldehyde, methyl eugenol, monoterpene, hydrocarbon, benzyl benzoate, caryophyllene, pinene, phellandrene, safrole, cymene, and cineol (Figure 2.7) (Jayaprakasha *et a*l. 2002; Kaefer and Milner 2008; Peter 2001; Vangalapati 2012; Yashin *et al*., 2017). The major constituents of cinnamon bark are essential oil (up to 2.8%), with cinnamaldehyde (60–90%) as a major component. Marongiu *et al.,* (2007) isolated 19 compounds from cinnamon by supercritical CO_2 extraction, as trans-cinnamaldehyde (77.1%), transβ-caryophyllene (6.0%), γ-terpineol (4.4%), as well as eugenol (3.0%). Cinnamon leaves contain 0.24–3.0% volatile oil and the principal component of leaf oil is eugenol, varied from 65 to 97% (Senanayake *et al*. 1978). In addition, the major components of root bark, flower, and fruit oils are camphor, trans-cinnamyl acetate,

and linalool, respectively (Parthasarathy *et al.* 2008). The biological properties of cinnamon are related to its higher polyphenol content and the major phenolics are vanillic acid, caffeic acid, and ferulic acid (Muchuweti *et al.* 2007). Abeysekera *et al.* (2013) reported that the ethanolic extracts of cinnamon leaf and bark had high phenolics and flavonoids contents which were more than those of dichloromethane/methanol extracts. phytochemicals such as glycosides, steroids, alkaloids, saponins, anthraquinones, tannins, terpenoids, and coumarins had also been identified in cinnamon extracts (De Soysa *et al*. 2016; Harsha *et al*., 2013; Sibi *et al*., 2013; Shreya *et al*., 2015; Tacouri *et al*., 2013).

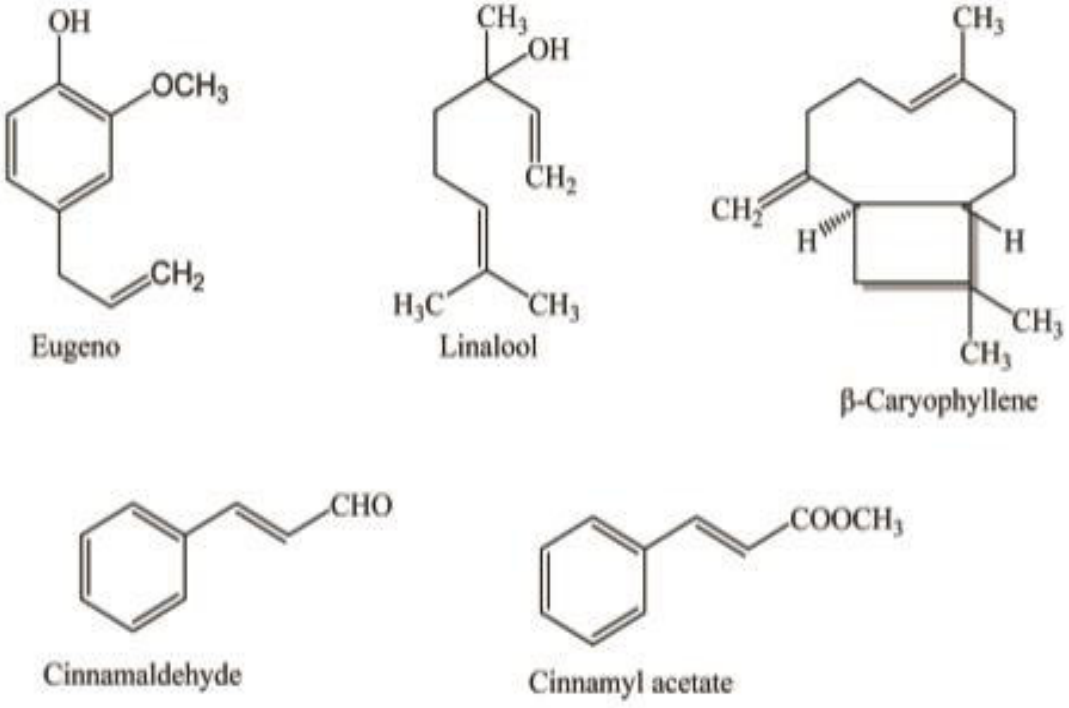

Figure 2.7: Major Phytochemicals of cinnamon (Al-Mashkor, 2015).

2.3.6 Nutrient Composition of Cinnamon

Ground cinnamon is composed of around 11% water, 81% carbohydrates (including 53% dietary fiber), 4% protein, and 1% fat (table 2.1). In a 100 gram reference amount, ground cinnamon is a

rich source of calcium (100% of the Daily Value, DV), iron (64% DV), and vitamin K (30% DV) (Table 2.1).

Table 2.1: Nutritional composition of cinnamon (Li *et al*., 2013).

Nutritional value per 100g (3.5oz.)	
Energy	247kJ (59kcal)
Carbohydrates	80.6 g
Sugars	2.2 g
Dietary fiber	53.1 g
Fat	1.2 g
Protein	4 g
Vitamins	Quantity%DV[†]
Vitamin A equiv.	2%15 µg
Thiamine (B1)	2%0.02 mg
Riboflavin (B2)	3%0.04 mg
Niacin (B3)	9%1.33 mg
Vitamin B6	12%0.16 mg
Folate (B9)	2%6 µg
Vitamin C	5%3.8 mg
Vitamin E	15%2.3 mg
Vitamin K	30%31.2 µg
Minerals	Quantity%DV[†]
Calcium	100%1002 mg
Iron	64%8.3 mg
Magnesium	17%60 mg
Phosphorus	9%64 mg
Potassium	9%431 mg
Sodium	1%10 mg
Zinc	19%1.8 mg
Other constituents	**Quantity**
Water	10.6 g
Unitsµg = micrograms• mg = milligrams, IU = International units	

2.3.7 Food Uses of Cinnamon

Cinnamon bark is used as a spice. It is principally employed in cookery as a condiment and flavoring material. It is used in the preparation of chocolate, especially in Mexico. Cinnamon is often used in savory dishes of chicken and lamb. In the United States, cinnamon and sugar are often used to flavor cereals, bread-based dishes, such as toast, and fruits, especially apples; a cinnamon-sugar mixture is sold separately for such purposes. It is also used in Turkish cuisine for both sweet and savory dishes. Cinnamon can also be used in pickling and Christmas drinks such as eggnog. Cinnamon powder has long been an important spice in enhancing the flavor of Persian cuisine, used in a variety of thick soups, drinks, and sweets.

2.3.8 Properties of *Cinnamomum Verom*

2.3.8.1 Anti-inflammatory Properties.

The inhibitors of prostaglandin biosynthesis and nitric oxide production are potential anti-inflammatory and cancer chemo preventive agents. *Cinnamomum verom* extracts showed potent inhibition of cyclooxygenase-2 (COX-2) activity in lipopolysaccharide (LPS)-induced mouse macrophage RAW264.7 cells (Hong *et al*., 2002). The main constituents of cinnamon, eugenol, and cinnamaldehyde, were found to inhibit COX-2 *in vitro* in a rapid semi-homogeneous COX-2 enzymatic assay (Huss *et al*., 2002).

The redox sensitive, pro-inflammatory nuclear transcription factor NF-kappa B plays a key role in inflammation. Cinnamaldehyde derivatives based on 2_-hydroxycinnamaldehyde isolated from the bark of *C. cassia* significantly inhibited lipopolysaccharide (LPS)-induced nitric oxide (NO) production and NF-kappa B transcriptional activity in a dose-dependent manner (Lee *et al*., 2005). 2_-Hydroxycinnamaldehyde had the strongest inhibitory effect on NO production among

the cinnamaldehyde derivatives through inhibition of NF-kappa B activation, and thus could be used as an anti-inflammatory agent due to its antioxidant properties. Kim *et al*. (2007) recently examined cinnamaldehyde further for its molecular modulation of inflammatory NF-kappa B activation via the redox-related NF-kappa B/I χ B kinases (NIK/IKK) and mitogen-activated protein kinase (MAPK) pathways through the reduction of oxidative stress. Results show that age-related NF-kappa B activation upregulated NF-kappa B targeting genes, inflammatory iNOS, and COX-2, all of which were effectively inhibited by cinnamaldehyde. Cinnamaldehyde furthermore inhibited the activation of NF-kappa B via three signal transduction pathways—NIK/IKK, extracellular signal regulated kinases, and p38 MAPK. It is likely that the anti-oxidative effect of cinnamaldehyde and the restoration of redox balance are responsible for its anti-inflammatory action. The bark of *C. cassia*, probably due to its cinnamaldehyde content, demonstrates clear anti-inflammatory properties *in vitro*.

2.3.8.2 Antibacterial Properties.

Spices have been traditionally used since ancient times for their antiseptic and disinfectant properties. De *et al.* (1999) carried out a preliminary screening for antimicrobial activities of 35 different Indian spices. Cinnamon, among others, has potent antimicrobial activity against the test organisms *Bacillus subtilis* and *Escherichia coli*.

Cinnamon bark oil as well as cinnamaldehyde and eugenol showed potent antibacterial effects against *Bacillus cereus, Campylobacter jejuni, Enterococcus faecalis, Escherichia coli, Listeria monocytogenes, Haemophilus influenzae, Salmonella choleraesuis, S. enterica, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumonia, and S. pyogenes,* as well as *Yersinia enterocolitica* (Friedman *et al*., 2002; Inouye *et al*., 2001; L´opez *et al*., 2005; Smith *et al*., 1998). In general, Gram-positive bacteria were more sensitive to inhibition by the plant

essential oil than Gram-negative bacteria. *Campylobacter jejuni* was the most resistant of the bacteria investigated (Smith *et al*., 1998).

Cinnamaldehyde showed the strongest antibacterial effectiveness of the constituents examined (L´opez *et al*., 2007). Oussalah *et al*. (2006) studied the mechanism of the antimicrobial action of the essential oil of *C. cassia* against cell membranes and walls of bacteria by measurement of intracellular pH and ATP concentration, the release of cell constituents, and electronic microscopy of the cells when the essential oil at its minimum inhibitory concentration was in contact with *E. coli* and *L. monocytogenes*.

2.3.8.3 Antifungal Properties.

The essential oils of several *Cinnamomum* species have been shown to have anti-candidal (*Candida albicans, C. glabrata*) and anti-dermatophytic (*Microsporum canis, Trichophyton mentagrophytes, T. rubrum*) activity *in vitro* (Mastura *et al*., 1999). The essential oil of the leaves of *C. zeylanicum* demonstrated only modest antifungal properties. However, according to Simi *et al*. (2004), the essential oil of *C. zeylanicum* (plant part not specified) showed the strongest antifungal activity compared to *Aniba roaeodora, Laurus nobilis* and *Sassafras albidum* against 17 micromycetes (*Aspergillus niger, A. ochraceus, A. versicolor, A. flavus, A. terreus, Alternaria alternata, Aureobasidium pullulans, Penicillium ochrochloron, P. funiculosum, Cladosporium cladosporioides, C. Fulvium, Trichoderma viride, Fusarium tricinctum, F. sprortrichoides, Phoma macdonaldii, Phomopsis helianthi, Mucor mucedo*) *in vitro*. Trans-cinnamaldehyde was the most active component in the oil of *C. zeylanicum*. Singh *et al*. (1995) identified cinnamic aldehyde as the active fungitoxic constituent of *C. zeylanicum* bark oil. The fungitoxic properties of the vapors of the oil/active constituent were established against fungi involved in respiratory tract infections (mycoses), i.e., *Aspergillus niger, A. fumigatus, A. nidulans A. flavus, Candida*

albicans, C. tropicalis, C. pseudotropicalis, and *Histoplasma capsulatum*. It was concluded that these inhalable vapors appear to approach the ideal chemotherapy for respiratory tract mycoses. Cinnamon oil demonstrated inhibitory activity against *Aspergillus flavus, A. parasiticus, A. ochraceus, Fusarium moniliforme, F. graminearum* and *F. proliferatum* as well as *Saccharomyces cerevisiae* in several further studies (De *et al*., 1999; Soliman and Badeaa, 2002; Ranasinghe *et al*., 2002; Bartine and Tantaoui-Elaraki, 1997; Velluti *et al*., 2003; Velluti *et al*., 2004).

Furthermore, oils obtained from *C. zeylanicum* were found to be most active *in vitro* tested against dermatophyte strains isolated from patients with dermatophytosis inhibiting 80% of the dermatophyte strains tested (Lima *et al*., 1993). Oral candidiasis is a frequent occurrence in patients with HIV infection. Treatment of this condition with an oral azole is generally effective. However, fluconazole-resistant *Candida* species are an emerging problem. *C. zeylanicum* shows *in vitro* activity against fluconazole-resistant and -susceptible *Candida* isolates (Quale *et al*., 1996).

2.3.8.4 Antiviral Properties

A *Cinnamomum verom* bark extract was highly effective against HIV-1 and HIV-2 replication in terms of inhibition of virus-induced cytopathogenicity in MT-4 cells infected with HIV (Premanathan *et al*., 2000). Cinnamaldehyde derived from cinnamon bark has an inhibitory effect on the growth of influenza A/PR/8 virus in *vitro* (Madin-Darby canine kidney cells) and *in vivo* (mice infected with the lung-adapted PR-8 virus) (Hayashi *et al*., 2007).

The available *in vitro* data demonstrated that C. *cassia* bark oil as well as aqueous and ethanolic extracts have potent antibacterial and highly effective antiviral properties against Gram-positive

and Gram-negative bacteria as well as influenza virus. These properties have not been reported for *C. zeylanicum*, even though the two cinnamon species have similar constituents. The essential oil of *C. zeylanicum*, however, demonstrated potent antifungal activity. Further *in vitro* and *in vivo* research in addition to human data is needed to confirm the antimicrobial properties of cinnamon in free-living individuals.

2.3.8.5 Antioxidant Properties

Spices and vegetables possess antioxidant activity that can reduce lipid peroxidation in biological systems (Shobana and Naidu, 2000). Reactive oxygen species have been implicated in a range of human diseases such as atherosclerosis and certain cancers (Halliwell, 2007). Oxidative processes generally play a key role in inflammatory and immune processes. As oxidative stress has been implicated in the pathogenesis of many human diseases, the use of antioxidants in pharmacology is widely studied (Clark, 2002). Dragland *et al*. (2003) found very high concentrations of antioxidants in the medicinal herb *Cinnamomi cortex*. It was speculated that several of the effects of this herb are mediated by their antioxidant activities.

A water and alcoholic extract (1:1) of cinnamon showed significant inhibition of lipoxygenase-dependent enzymatic lipid peroxidation in an *in vitro* lipid peroxidation assay (Shobana and Naidu, 2000). Etheric, methanolic, and aqueous cinnamon extracts, inhibited *in vitro* oxidation in a beta-carotene/linoleic acid system (Mancini *et al*., 1998).

Ethanol extracts of dry bark of *C. verom* exhibited a greater inhibition of lipid peroxidation of rat liver homogenate *in vitro* than alpha-tocopherol, high superoxide anion scavenging activity, strong anti-superoxide formation activity, and excellent antioxidant activity in enzymatic and non-enzymatic liver tissue oxidative systems (Lin *et al*., 2003). Cinnamon exhibited a higher

percentage of inhibition of oxidation than butylated hydroxyanisole, butylated hydroxytoluene, and propyl gallate as tested by the lipid peroxidation assay (Murcia *et al*., 2004). *Cinnamomi cortex* also has inhibitory effects on lipid peroxidation and protein oxidative modification by copper (Toda, 2003).

Cinnamon oil exhibited superoxide dismutase (SOD)-like activity measured by the inhibition of pyrogallol autoxidation that is catalyzed by the superoxide radical (Kim *et al*., 1995). The volatile extracts of cinnamon showed moderate antioxidant activities in the aldehyde/carboxylic acid assay and in the conjugated diene assay (Lee and Shibamoto, 2002). The essential oil obtained from the bark of *C. zeylanicum* and three of its main components, eugenol, (E)-cinnamaldehyde, and linalool, were tested in two *in vitro* models of peroxy nitrite induced nitration and lipid peroxidation. The essential oil and eugenol showed very powerful activities. (E)-cinnamaldehyde and linalool were completely inactive (Chericoni *et al*., 2005).

However, *C. verom* bark-derived trans-cinnamaldehyde showed potent inhibitory effects on NO production in RAW264.7 cells, determined through the evaluation of NO production and expression of inducible nitric oxide. Little or no activity was observed for cinnamic acid and eugenol (Lee *et al*., 2002).

Several flavonoids obtained from cinnamon that were reported to exhibit antioxidant and free radical scavenging activities were tested for their 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (Okawa *et al*., 2001). Prakash *et al*. (2007) found strong free radical-scavenging activities in the bark of *C. zeylanicum* as indicated by a very low inhibitory concentration value, efficiency concentration value (DPPH), and reducing power value (ascorbic acid equivalents) as well as a reasonably high value of anti-radical power. These findings

confirm earlier results by Shan *et al*. (2005), which indicate very strong activity for *C. zeylanicum* and a relatively high activity for *C. verom*

2.3.8.6 Antitumor Properties

Antitumor and anti-cancer properties of a substance are closely related to its antioxidant and

Immune-modulatory properties. Studies on the antioxidant and immune-modulatory properties of *C. zeylanicum* and *C. cassia* may imply antitumor properties. However, additional studies on cinnamon bark and its main constituent cinnamaldehyde are needed in order to investigate their precise antitumor properties. Ka *et al*. (2003) investigated the effects of cinnamaldehyde on the cytotoxicity, induction of apoptosis and putative pathways of its actions in human promyelocytic leukemia cells. Cinnamaldehyde was a potent inducer of apoptosis in these studies. The authors concluded that the anticancer effects of cinnamaldehyde result from induction of reactive oxygen species (ROS)-mediated mitochondrial permeability transition and resultant cytochrome C release. Nishida *et al*. (2003) found that *C. cassia* induced death of HL-60 cells as demonstrated by the reduction of mitochondrial transmembrane potential and increased caspase-3 activity. According to the authors, the apoptosis induced by *C. cassia* occurred via the mitochondrial route and the apoptosis-conducting mechanism acted through a cascade involving caspase-3.

Kwon *et al*. (1998) synthesized cinnamaldehyde and related compounds from various cinnamic acids based on the 2_-hydroxycinnamaldehyde isolated from the bark of *C. cassia*. Cinnamic acid, cinnamates, and cinnamyl alcohols did not show cytotoxicity against several human solid tumor cells. HCT15 and SK-MEL-2 cells, however, were much more sensitive to these cinnamaldehyde analogues. Cytotoxicity of the saturated aldehydes was much weaker compared to their unsaturated counterparts. Matrix metalloproteinase-9 (MMP-9) degrades type IV

collagen, the major structural component of the basement membrane and the extra cellular membrane (Seo *et al*., 2005). The activity of this enzyme is found to be elevated in tumor tissues. The hexane and chloroform fractions as well as water extracts of *C. cassia* showed a weak inhibitory effect on MMP-9 activity.

However, a strong MMP-9 inhibition was found in the butanol fraction of *C. cassia*. 2_-Hydroxycinnamaldehyde (HCA) and 2_-benzoyloxycinnamaldehyde (BCA) isolated from *C. cassia* strongly inhibited *in vitro* growth of 29 kinds of human cancer cells and *in vivo* growth of SW-620 human tumor xenograft without loss of body weight in nude mice (Lee *et al*., 1999). HCA prevented adherence of SW-620 cells to the culture surface but did not inhibit oncogenic K-Ras processing, implying its antitumor mechanisms are at the cellular level.

Haranaka *et al*. (1985) suggested that one mechanism underlying the antitumor activity of *Cinnamomi cortex* is based on stimulation of the reticulo-endothelial system (RES) and is closely related to tumor necrosis factor (TNF) production. The drug was given to DDY mice in drinking water before and after transplantation of Ehrlich tumors. A good survival rate was found in the group administered *Cinnamomi cortex*. Relatively high levels of TNF activity were noted in the group given cinnamon.

The TNF capacity for production broadly paralleled the survival rate of the mice transplanted to Ehrlich tumors. Abraham *et al*. (1998) assessed anti-genotoxic effects and changes in glutathione S-transferase (GST) activity in mice after oral co-administration of urethane (URE), a carcinogenic substance, with an aqueous extract of cinnamon. The results of the genotoxicity assay (micronucleus test) demonstrated dose-related anti-genotoxic effects after URE was co-administered with the extract. Furthermore, an aqueous extract prepared from cinnamon seemed to interact with phosphorylation/ dephosphorylation signaling activities in three myeloid cell

lines (Jurkat, Wurzburg, and U937), thus reducing cellular proliferation and blocking the G2/M phase of the cell cycle (Schoene *et al*., 2005).

Furthermore, *C. cassia* exerted significant antimutagenic effects against benzo[a]pyrene and cyclophosphamide in mice pretreated with the plant extract as shown by the Ames test, the bone marrow chromosomal aberration assay, and the micronucleus test (Sharma *et al*., 2001).

2.3.8.7 Blood Pressure Lowering Properties

Cinnamomum verom bark affects the blood and cardiovascular system (Cheng *et al.,* 2008). The cardiovascular properties of cinnamon are closely connected to its effects on blood lipids and glucose metabolism. Many agents (nutrients, nutraceuticals, and drugs) that enhance insulin sensitivity and/or reduce circulating insulin concentrations also lower blood pressure (Preuss *et al*., 2006). Cinnamon (8% w/w) in the diet reduced the systolic blood pressure of spontaneously hypertensive rats (SHR) eating sucrose-containing diets to virtually the same levels as SHR consuming diets containing non sucrose. The presence of cinnamon in the diet also decreased the systolic blood pressure of SHR consuming a non-sucrose-containing diet, suggesting that cinnamon reduces more than just sucrose-induced blood pressure elevations.

Cinnamomum verom bark increases the level of atrial natriuretic factor (ANF) in the plasma of mice (Zhou *et al*., 1995). ANF acts to reduce the water, sodium, and adipose loads on the circulatory system, thereby reducing blood pressure.

2.3.8.8 Cholesterol and Lipid Lowering Properties

Several spices, e.g. garlic or ginger, have been shown to have beneficial hypolipidaemic or hypocholesterolaemic properties (Sambaiah and Srinivasan, 1991). In a study undertaken to screen several spices, *C. zeylanicum* did not lower serum or liver cholesterol concentrations of

rats when included in the diet at about 5-fold the normal human intake level. In contrast, cholesterol concentrations were increased by 25% in cinnamon fed animals (Sambaiah and Srinivasan, 1991). However, hypocholesterolaemic effects were reported in a very recent study conducted to isolate and identify the putative antidiabetic compounds of *C. zeylanicum* based on bioassay guided fractionation. Cinnamaldehyde decreased plasma glucose concentration in a dose-dependent manner in Wistar rats (Subash *et al*., 2007). The results of this study indicate that cinnamaldehyde possesses hypolipidaemic effects in streptozotocin-induced diabetic rats. Kim *et al*. (2006b) demonstrated the effect of *C. cassia* extract on blood lipids in an *in vivo* study. HDL-cholesterol concentrations were high in mice fed with cinnamon extract, and the concentrations of triglyceride and intestinal alpha-glycosidase activity were low, after 6 weeks. These results suggest that cinnamon extract has a regulatory role in blood lipids and it may also exert a blood glucose-suppressing effect by improving insulin sensitivity or slowing absorption of carbohydrates in the small intestine.

Several *in vivo* studies strongly suggest that *C. zeylanicum* and *C. verom* have cholesterol-lowering properties. Khan *et al*. (2003) determined whether cinnamon improves blood glucose, triglyceride, total cholesterol, HDL cholesterol, and LDL cholesterol levels in people with type 2 diabetes.

2.3.8.9 Gastro Protective Properties (including H. Pylori Infection)

Helicobacter pylori has been associated with the pathogenesis of antral gastritis, duodenal ulcer, and gastric lymphoma, and the eradication of this organism has shown to reverse or prevent relapse of these diseases. Antimicrobials employed in the eradication of *H. pylori* are not without adverse effects (Chiba *et al*., 1992). Newer treatment modalities, therefore, are required (Nir *et al*., 2000).

Cinnamon extract (from methylene chloride) inhibited *H. pylori* growth at a concentration range typical of common antibiotics (Tabak *et al*., 1999). The efficiency of cinnamon extracts in liquid medium and its resistance to low pH levels may enhance its effect in an environment such as the human stomach.

2.3.8.10 Antidiabetics

Diabetes is a group of metabolic diseases that cause high blood glucose (blood sugar). Diabetes is associated with a number of complications including eye disease (retinopathy), kidney disease (nephropathy), neural damage (neuropathy), cardiovascular diseases, strokes, depression, dementia, and sexual dysfunction (Forbes and Cooper, 2013). Some studies have been conducted to evaluate the ability of cinnamon to reduce the risk of diabetes. C. cassia extract have been reported to have a regulatory role in decreasing blood glucose level in type-2 diabetic animal model. The decrease in glucose level corresponds to the concentration of the extract. In addition, serum insulin became significantly higher during the treatment (Kim *et al.,* 2006). In an advanced study of chronic kidney disease associated with diabetes, Yan *et al*. (2015) found that C. cassia bark extract, which contains sesqui terpenoids, has antidiabetic nephropathy activity.

Cinnamomum burmanii essential oil has been shown to give similar results as C. cassia essential oils against type 2 diabetes mellitus due to its ability to regulate glucose transporter and insulin signaling gene expression when investigated in mouse adipocytes (Cao *et al.,* 2010). In addition, the water extract of *C. burmanii* has been reported to have the ability to enhance liver glycogen synthesis under conditions of reduced insulin sensitivity. Therefore, it has the ability to maintain

the metabolic balance of fat and/or refined carbohydrates in case of fat or carbohydrate overload (excessive dietary intake) (Couturier *et al.,* 2011). The combination of cinnamon essential oil with cumin, fennel, oregano, and myrtle essential oils has a synergetic effect on the reduction of blood glucose levels as well as enhancing insulin sensitivity in type 2 diabetes in a rat model (Talpur *et al.,* 2005). In the study of Cheng *et al*. (2012) defatted soy flour enriched with cinnamon extract had a significant effect on improving glucose metabolism in both in vivo and in vitro experimentation.

It has been reported that the ability of cinnamon to reduce the risks of diabetes stems from its ability to increase the release of insulin, reduce the absorption of intestinal glucose, enhance the synthesis of glycogen, as well as activate the PPAR-γ (Mueller and Jungbauer, 2009). Another possible mechanism is that the polyphenols in cinnamon regulate glucose metabolism and repair pancreatic beta cells (Li *et al.,* 2013).

Cinnamaldehyde may function as a hypoglycemic agent. It could reduce the workload of the pancreas by improving the islet function (Li *et al.,* 2013 and Babu *et al.,* 2007). TAPP may also take part in the antidiabetic activity, (Anderson *et al.,* 2004) through their ability to inhibit the development of toxic hIAPP oligomers and reduce cytotoxicity through alleviating hIAPP-induced membrane damage (Ariaee-Nasab *et al.,* 2014). Likewise, Type-B procyanidin oligomers present in C. cassia water extract have been reported to act as hypoglycemic Agents (Akilen *et al.,* 2013).

Although the above mentioned studies showed that cinnamon extract consumption reduced fasting blood sugar levels on prediabetic patients with metabolic syndrome, the ability of cinnamon to help people who currently have type 2 diabetes mellitus has not been demonstrated (Ziegenfuss *et al.,* 2006 and Akilen *et al.,* 2012). Akilen *et al*. (2013) concluded that

consumption of cinnamon was effective in decreasing systolic blood pressure or diastolic blood pressure for patients with pre-diabetic conditions and with type 2 diabetic mellitus.

2.3.8.11 Anti-hypertriglyceridemia

Hypertriglyceridemia and low HDL cholesterol are two of many consequences of metabolic syndrome (Eckel *et al.,* 2010). Hypertriglyceridemia is a condition in which the plasma triglyceride (TG) levels are higher than a reference value (Brahm and Hegele, 2013). Cinnamon has been found to be beneficial for improving blood lipid profile (Hamidpour *et al.,* 2015). Kim *et al*. (2006b) found that the concentration of TG and total cholesterol becomes lower after consuming cinnamon extract for 6 weeks. At the same time, the HDL cholesterol significantly increased. Cinnamaldehyde has significant hypolipidaemic effects. It can significantly reduce lipid concentrations and increase HDL cholesterol in serum. One possible mechanism is that the cinnamaldehyde could increase the activity of lecithin cholesterol acyl transferase (Babu *et al.,* 2007). Polyphenols present in cinnamon may influence lipid metabolism (Li *et al.,* 2013). Polyphenols effectively inhibit hepatic lipid peroxidation (Lee *et al.,* 2003). This activity is beneficial for human health since lipid peroxidation produces some products that exert cytotoxic and genotoxic effects (Esterbauer, 1993).

2.3.8.12 Digestive system protection

Concerning the digestive system, cinnamon has been reported to reduce the risk of gastric ulcers as well as protect gastrointestinal tract from free radical injury (Hamidpour *et al.,* 2015, Lee *et al.,* 2003 and Helal *et al.,* 2014). Investigated in normal rats, the aqueous extract of cinnamon was proven to regulate multiple metabolic pathways involved in the intestinal lipoprotein

metabolism of small intestinal primary enterocytes. Cinnamon consumption might also improve intestinally derived lipid metabolism in the healthy rats (Qin *et al.,* 2012).

2.4 Basic Classification of Lipoproteins

The lipids can be classified as Total Cholesterol (TC) and its derivatives such as; Triglycerides (TAG), Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL) and Very Low Density Lipoprotein (VLDL) cholesterol. The cholesterol along with some other types of fats cannot dissolve in the blood. In order to be transported to and from cells, they have to be specially carried by certain molecules called lipoproteins, which consist of an outer layer of protein with an inner core of cholesterol and triglycerides (Onwe *et al.,* 2015). In addition, the lipoproteins have been found essential for cholesterol to move around the body.

2.4.1 Total Cholesterol (TC)

According to guidelines of National Cholesterol Education Program USA (NCEP), TC concentrations below 200 mg/dL have been regarded as desirable, whereas, concentrations greater than 240 mg/dL are referred to as hyperlipidemia. However, epidemiological evidence suggests that the risk of cardiac events decreases as TC levels fall approximately to 150 mg/dL. Moreover, TC should be less than 180 mg/dL for children (Ginsberg and Goldberg, 2001 and Fryar *et al.*, 2010).

2.4.2 Triglyceride

Triglycerides are another type of fat that is carried in the blood by lipoproteins. The excess calories, alcohol or sugar in the body get converted into triglycerides and stored in fat cells throughout the body (Smelt, 2010). The triglyceride concentration less than 150 mg/dL is regarded as normal, whereas, concentrations of 200-499 mg/dL are considered as high.

Moreover, concentrations of 500 mg/dL or higher are considered dangerous for the development and progression of various Cardio-vascular diseases (CVDs) (Ginsberg and Goldberg, 2001).

2.4.3 LDL Cholesterol (LDL-C)

LDL-C is commonly known as the bad cholesterol, which is produced by the liver and transported to different areas of the body like muscles, tissues, organs and heart. High levels of LDL indicate much more cholesterol in the blood stream than necessary and hence, increase the risk of heart disease (Costet, 2010). According to NCEP guidelines, LDL cholesterol concentrations below 100mg/dL are considered optimal, whereas concentrations in the range of 160-189mg/dL are considered to be on the high side. However, increasing evidence supports that normal human LDL cholesterol concentration can be as low as 50 to 70 mg/dL (Ginsberg and Goldberg, 2001). It has been generally accepted that the risk of CVDs decreases as LDL cholesterol concentration decreases.

2.4.4 HDL Cholesterol (HDL-C)

HDL-C is commonly referred to as the good cholesterol, which is produced by the liver to carry cholesterol and other lipids from tissues back to the liver for degradation (Ridker *et al*., 2010). High levels of HDL cholesterol have been considered as a good indicator of a healthy heart. The concentrations of 60 mg/dL or higher have been considered as optimal, whereas, HDL concentrations below 40 mg/dL are considered as major risk factor for CVDs. However, HDL is often interpreted in the context of TC and LDL concentrations, and hence may be regarded as less significant when LDL is low (Ginsberg and Goldberg, 2001 and Ridker *et al*., 2010).

2.4.5 VLDL cholesterol (VLDL-C)

VLDL-C is similar to LDL-C in the sense that it contains mostly fat and not much protein. VLDL cholesterol is the lipoproteins that carry cholesterol from the liver to organs and tissues in the body (Sundaram and Yao, 2010). They are formed by a combination of cholesterol and triglycerides. Moreover, VLDLs are heavier than LDL, and are also associated with atherosclerosis and coronary heart disease (Sundaram and Yao, 2010). Physiology of cholesterol and its derivatives Cholesterol is a lipid (fat) which is produced by the liver and vital for normal body function. Every cell in our body has cholesterol in its outer layer. Cholesterol is a waxy steroid and is transported in the blood plasma of all animals. It is the main sterol synthesized by animals; small amounts are also synthesized in plants and fungi (Hanukoglu, 2012). Since cholesterol is essential for all animal life, each cell synthesizes it from simpler molecules, a complex 37 - step process which starts with the intracellular protein enzyme HMG - CoA reductase. However, normal and especially high levels of fats (including cholesterol) in the blood circulation depending on how it is transported within lipoproteins are strongly associated with progression of atherosclerosis (Onwe *et al.,* 2015).

Most ingested cholesterol is esterified, which is poorly absorbed, however, the body compensates for any absorption of additional cholesterol by reducing cholesterol synthesis (Lecerf and de lorgeril, 2011). For this reasons, cholesterol intake in food has little, if any, effect on total body cholesterol content or concentrations of cholesterol in the blood. Some plants make cholesterol in very small amounts in the form of phytosterols (substances chemically similar to cholesterol), which can compete with cholesterol for re-absorption in the intestinal tract, thus potentially reducing cholesterol re-absorption (Lecerf and de lorgeril, 2011). When intestinal epithelial cells absorb phytosterol in place of cholesterol, they usually secrete it back into the GIT, an important protective mechanism.

2.5 Blood Lipids and Lipid Transport

Lipids are insoluble in water but are soluble in alcohol and other solvents. Hence, they are transported around the body as lipoproteins. A lipoprotein is any complex or compound containing both lipid (fat) and protein (Onwe *et al*., 2015). Lipids originate from two sources: endogenous lipids, synthesized in the liver, and exogenous lipids, which are ingested and absorbed in the intestine. Approximately 7% of body's cholesterol circulates in plasma in the form of low density lipoproteins (LDL). The level of plasma cholesterol is influenced by its synthesis and catabolism in which liver plays a crucial role (Robbins and Cotran, 2004). When dietary fats are digested and absorbed into the small intestines, they eventually reform into triglycerides, which are then packaged into lipoproteins (Bitcher, 2000). For this reason, there are several types of lipoprotein in blood, mentioned in order of increasing density, chylomicrons, very - low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), LDL, and HDL. Of interest in this research are cholesterol, LDL, HDL, and triglycerides. The more lipids and less protein a lipoprotein has, the less dense it is. Dietary fat, are absorbed from the small intestines and transported into the liver by lipoproteins called chylomicrons. Chylomicrons are large droplets of lipids with a thin shell of phospholipids, cholesterol, and protein. Once chylomicrons enter the bloodstream, an enzyme called lipoprotein lipase breaks down the triglycerides into fatty acid and glycerol. After a 12-14 hours fast, chylomicrons are absent from the bloodstream. Thus individuals having a lipid profile test done should fast overnight to ensure that chylomicrons have been cleared (Wardlaw *et al*., 2004). The liver removes the chylomicrons fragments, and the cholesterol is repackaged for transport in the blood in VLDLs which eventually turn into LDL. LDL cholesterol (LDL-C) the "bad cholesterol" consists mainly of cholesterol. Most LDL particles are absorbed from the bloodstream by receptor cells in the liver.

If too much is carried than can be used by cells, there can be a harmful building of LDL. This lipoprotein can increase the risk of arterial disease (atherosclerosis) if levels rise too high (Tymoczko *et al*., 2002). Diets high in saturated fats and cholesterol decrease the uptake of LDL particles by the liver. LDL particles are also removed from the bloodstream by scavenger cells, preventing cholesterol from reentering the bloodstream, but they deposit the cholesterol in the inner walls of blood vessels, eventually leading to the development of plaque (Wardlaw *et al*., 2004). High density lipoproteins are a separate group of lipoproteins that contain more protein and less cholesterol. HDL cholesterol (HDL-c) is also called "good cholesterol". HDL is produced primarily in the liver and intestine, and it travels in the bloodstream, picks up cholesterol, and gives the cholesterol to other lipoproteins for transport back to the liver, so HDL particles are thought to transport cholesterol back to the liver for excretion or to other tissues that use cholesterol to synthesize hormones in a process known as reverse cholesterol transport (RCT) (Lewis and Rader, 2005). Having large numbers of large HDL particles correlates with better health outcomes (Onwe *et al.,* 2015). In contrast, having small numbers of large HDL particles is independently associated with atheromatic disease progression in the arteries (Lewis and Rader, 2005).

2.6 Lipid profile

A lipid profile or lipid panel is a panel of blood tests that serves as an initial broad medical screening tool for abnormalities in lipids, such as cholesterol and triglyceride. The results of this test can identify certain genetic diseases and can determine approximate risks for cardiovascular disease, certain forms of pancreatitis and other diseases (Onwe *et al.,* 2015). A lipid profile measures TC, HDL-C, LDL-C and TAG. Lipid panels are commonly ordered as part of a physical examination, along with other panels such as the complete blood count (CBC) and basic

metabolic panel (BMP). The results of the lipid profile are considered along with other known risk factors of heart disease to develop a plan of treatment and follow up. Depending on the result and other risk factors, treatment options may involve life-style changes such as diet and exercise or lipid - lowering medications such as statin (Bitcher, 2000).

The NCEP (2002) recommends that individuals age twenty and over have a fasting lipoprotein profile every five years. A lipid profile should be done after nine to twelve hours fast without food, liquids or medication. If fasting is not possible the values for total cholesterol and HDL - C may still be useful (Sidhu and Naugler, 2012). If total cholesterol is 200 mg/dl or higher or HDL -C is less than 40 mg/dl, the individual will need to have a follow - up lipoprotein profile done to determine LDL-c and triglyceride levels. Depending on the physician's request, the lipid profile may include the ratio of cholesterol to HDL. This ratio is sometimes used in place of total blood cholesterol.

2.7 Lipid Related Diseases or Conditions

2.7.1 Hyperlipidemia

Hyperlipidemia is a medical condition characterized by an elevation of any or all lipid profile and/or lipoproteins in the blood. It is also called hypercholesterolemia/ hyperlipoproteinemia (Amit *et al*., 2011). Although elevated low density lipoprotein cholesterol (LDL) is thought to be the best indicator of atherosclerosis risk, (Amit *et al*., 2011) dyslipidemia (abnormal amount of lipids in the blood) can also describe elevated total cholesterol (TC) or triglycerides (TG), or low levels of high density lipoprotein cholesterol (HDL). (Onwe *et al.,* 2015). Lipids are fats in the blood stream, commonly divided into cholesterol and triglycerides. Cholesterol circulates in the bloodstream and is involved in the structure and function of cells. Triglycerides (TG) are best

viewed as energy that is either used immediately or stored in fat cells. TG is manufactured in the liver from the foods or by being absorbed from the intestine (Ankur *et al*., 2012). Virchow in 19th century who identified cholesterol crystals in atherosclerotic lesion and stated that endothelial cell injury initiates atherogenesis (Onwe *et al.,* 2015).

In a modification of this hypothesis it was proposed that the endothelium normally influences the behavior of arterial smooth muscle cells by providing a barrier to the passage of plasma proteins, and that the major effect of haemodynamic or other factors that injure endothelium is to reduce the effectiveness of the barrier (Onwe *et al.,* 2015). Arteries are normally smooth and unobstructed on the inside, but in case of increased lipid level, a sticky substance called plaque is formed inside the walls of arteries. This leads to reduced blood flow, leading to stiffening and narrowing of the arteries. It has been proved that elevated plasma levels of cholesterol and of LDL are responsible for atherosclerosis in man, and epidemiological data suggests that elevated plasma levels of HDL have a protective effect (Onwe *et al.,* 2015). This medical condition or problem is divided into two subtypes: primary hyperlipidemia and secondary hyperlipidemia (Onwe *et al*., 2015). Primary hyperlipidemia: This usually take place as a result of genetic problems i.e., mutation within receptor protein, which may be due to single (monogonic) gene defect or multiple (polygenic) gene defect. This type may occur as a result of change in dietary and lack of proper physical activities.

2.7.2 Secondary Hyperlipidemia

This arises as a result of other underlining diseases like diabetes, myxoedema, nephritic syndrome, chronic alcoholism, with use of drugs like corticosteroids, oral contraceptives, Beta blockers (Joseph, 2005).

2.7.3 Causes of Hyperlipidemia

The main cause of hyperlipidemia includes changes in lifestyle habits (Kelly, 2010), diabetes, kidney disease, pregnancy, under active thyroid gland, hereditary factor (Onwe *et al.,* 2015) and female hormones like estrogen. In addition, drugs like diuretics, beta-blockers and medicines used to treat depression have also been reported to raise cholesterol levels (Lipman *et al*., 2000).

2.7.4 Possible Treatment

The National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) recommends that a fasting lipoprotein profile and risk factor assessment be used in the initial classification of adults. There are three categories of risk that modify the goals and modalities of LDL-lowering therapy. The highest risk category is having known CHD or CHD risk equivalents; the risk for major coronary events is equal to or greater than that for established CHD (i.e., more than 20% per 10 years, or 2% per year). The intermediate category includes two or more risk factors, in which the 10-year risk for CHD is 20% or less. The lowest risk category is persons with zero to one risk factor, which is usually associated with a 10-year risk of CHD of less than 10% (Amit *et al*., 2011).

2.8 Hypercholesterolemia

Hypercholesterolemia can be defined as the presence of high plasma cholesterol levels, with normal plasma triglycerides, as a consequence of the rise of cholesterol and apolipoprotein B (apoB)-rich lipoproteins, called low-density lipoprotein (LDL). According to the WHO (1970), hypercholesterolemia would be included in IIa phenotype (Ramasamy, 2016).

Hypercholesterolemia is a condition characterized by very high levels of cholesterol in the blood. Cholesterol is a waxy, fat-like substance that is produced in the body and obtained from foods

that come from animals (particularly egg yolks, meat, poultry, fish, and dairy products). The body needs this substance to build cell membranes, make certain hormones, and produce compounds that aid in fat digestion (Sonia *et al*., 2013).

Hyperlipidemia is major risk factor for the atherosclerosis. Other complications are coronary heart disease, ischemic cerebro vascular disease, hypertension, obesity and diabetes mellitus (Type -II). Although many efficacious lipid-lowering synthetic drugs exist, none is effective for all lipoprotein disorders and all such agents are associated with some adverse effects (Mohd *et al.,* 2016).

Hypercholesterolemia has been associated with enhanced oxidative stress related to increased lipid peroxidation. Increased generation of oxidized LDL is a major factor in the vascular damage associated with high cholesterol levels (Sonia *et al.,* 2013).

2.9 ANTIOXIDANTS

Antioxidants are the molecules that prevent cellular damage caused by oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from one molecule to an oxidizing agent. They may improve immune function and perhaps lower the risk for infection, cardiovascular disease, and cancer. Antioxidants can be classified into natural and enzymatic antioxidants (Hamid *et al*., 2010).

Enzymatic antioxidants that are uniquely produced in the human body are superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) as described below.

2.9.1 Superoxide Dismutase (SOD)

SOD enzyme is found in both the dermis and the epidermis. It removes the superoxide radical (O2.−) and repairs the body cells damaged by free radical. SOD catalyzes the reduction of superoxide anions to hydrogen peroxide. SOD is also known to compete with nitric oxide (NO) for superoxide anion, which inactivates NO to form peroxy-nitrite. Therefore, by scavenging superoxide anions, it promotes the activity of NO (Chakraborty *et al*., 2009).

$$2O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2$$

2.9.2 Catalase enzyme (CAT)

CAT is found in the blood and most of the living cells and decomposes H_2O_2 into water and oxygen. Catalase along with glucose peroxidase is also used commercially for the preservation of the fruit juices, cream consisting of egg yolk, and salad by removing the oxygen (Chakraborty *et al*., 2009).

$$2H_2O_2 \xrightarrow{CAT} 2H_2O + O_2$$

2.9.3 Glutathione Peroxidase (GPx)

GPx is a group of selenium dependent enzymes, and it consists of cytosolic, plasma, phospholipid hydroperoxide, and gastrointestinal glutathione peroxidase (Chakraborty *et al*., 2009). GPx (cellular and plasma) catalyzes the reaction of H2O2 by reduced glutathione (GSH); as a result, oxidized glutathione (GSSG) is produced and it is again recycled to its reduced form by glutathione reductase (GR) and reduced nicotinamide adenine dinucleotide phosphate (NADPH).

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

2.9.4 Malondyaldehyde (MDA)

MDA has been widely used for many years as a convenient biomarker for lipid peroxidation of omega-3 and omega-6 fatty acids because of its facile reaction with thiobarbituric acid (TBA) (Antonio *et al*., 2014). The TBA test is predicated upon the reactivity of TBA toward MDA to yield an intensely colored chromogen fluorescent red adduct; this test was first used by food chemists to evaluate autoxidative degradation of fats and oils (Antonio *et al*., 2014). However, the thiobarbituric acid reacting substances test (TBARS) is notoriously nonspecific which has led to substantial controversy over its use for quantification of MDA from *in vivo* samples. Several technologies for the determination of free and total MDA, such gas chromatography mass spectrometry (GC-MS/MS), liquid chromatography mass spectrometry (LC-MS/MS), and several derivatization based strategies, have been developed during the last decade (Giera *et al*., 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials used in the study

Materials used for this study include equipments and apparatus (appendix I), chemicals and reagents (appendix II).

3.2 Sample Collection and Identification

Dry *Cinnamomum verom* bark (brown in colour) was purchased from Kurmi market, Dala Local Government Area of Kano state, in September 2019. The sample was taken to the Herbarium Unit of the Department of Biological Sciences, Bayero University Kano for identification and authentication on 15th November, 2019. Identification number (BUKHAN:119) was given to the sample (appendix III). The dried sample was crushed using mortar and pestle and to fine powder using mechanical grander.

3.3 Phytochemical Screening (Syed *et al.,* 2015).

Chemical tests were carried out on ethanolic extracts of *Cinnamomum verom* bark using standard procedures to identify the phytochemicals as described by Syed *et al.* (2015).

3.3.1 Alkaloids

Extract (0.2g) was warmed with 2% H_2S0_4 for two minutes on a boiling water bath. The mixture was then cooled, filtered and treated with the Dragendroff's reagent. The sample was observed for presence of orange red precipitation which indicates the presence of alkaloids.

3.3.2 Tannins

Exactly 2g of the extract was mixed with water (1ml) and heated on water bath. The mixture was filtered and 1-3 drops of ferric chloride solution was added to the filtrate and the sample was observed for a dark green coloration, which shows the presence of tannins.

3.3.3 Glycosides

The extract was hydrolyzed by adding 5ml HCl solution and neutralized by adding 5/ml NaOH solution. After this a few drops of Fehling's solutions A and B were added to the mixture and the sample was observed for red precipitates which indicates presence of glycosides.

3.3.4 Saponin

Extract (0.2g) was shaken with 5ml of distilled water and then heated to boil. Then the sample was observed for the frothing (appearance of creamy mix of small bubbles).

3.3.5 Flavonoids

Extract (0.2g) was dissolved in 2ml diluted (2%) NaOH and made a solution (a) having yellow color. After this, 2%HCl was added to solution (a) and the sample was observed for discoloration which indicates presence of flavonoids.

3.3.6 Terpenoids (Salkowski test)

Extract (0.2g) of the plant sample was mixed with 2ml of $CHCl_3$ and 3ml concentrated H_2S0_4 was carefully added to form a layer. A reddish brown coloration in the interface was observed indicating the presence of terpenoids.

3.3.7 Phenolic compounds

The extract (5mg) was dissolved in 0.5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A purple, red, brown or dark green, color indicated the presence of phenolic compounds.

3.4 Extraction and Isolation of Phenolics from *Cinnamomum verom*

3.4.1 Hydrochloric Acid Extraction/ Digestion

Cinnamomum verom (200g) was steeped in 200 ml of 2M HCl and was subjected to extraction using sonication in a GT-sonic ultrasonic cleaning instrument (model VGT-1620QTD) for 30 min at 50 °C. The HCl extract (supernatant) was reserved for further analysis, while the *Cinnamomum verom* was washed with hot tap water (Deidre *et al*., 2012).

3.4.2 Sodium Hydroxide Extraction/ Digestion

Phenolic compounds were extracted using a modified method by Lazaro and Favier (2000). *Cinnamomum verom* was steeped in 200 ml of 2M NaOH at 50 °C for 30 min using GT-sonic ultra-sonication extraction method. The NaOH extract (supernatant) was reserved for further analysis and the residue was washed with hot tap water. Alkaline-treated *Cinnamomum verom* was neutralized by vortexing for 5 min in 10 ml HCl. The hot water wash was repeated and the *Cinnamomum verom* left to air dry at ambient conditions. The NaOH extracts was neutralized using 2M HCl to approximately 70% of the original concentration. An aliquot of the neutralized samples was freeze dried. Prior to analysis, the freeze-dried extracts were rehydrated back to the initial volume with Deionized water (Deidre *et al*., 2012).

3.4.3 Isolation of Phenols from *Cinnamomum verom*

A 200ml of the acid extract and alkaline extract each was poured into a separatory funnel and 200ml of diethyl ether added. The mixture was shaken thoroughly for evenly distribution and allowed to separate. The diethyl ether layer was collected and evaporated to dryness (Deidre *et al*., 2012).

3.5 In Vitro Antioxidant Capacity of Different Fractions (NaOH and Diethyl Ether) of *Cinnamomum verom*

The scavenging effects of *Cinnamomum verom* were evaluated against 1,1-diphenyl-2-picrylhydrazyl DPPH and ferric reducing assay.

3.5.1 DPPH Radical Scavenging Assay (Zohreh *et al*., 2018)

Principle

DPPH solution has a deep violet color characterized by absorption band in ethanol solution centered at about 517nm. The principle is based on the reduction of DPPH by antioxidant (able to donate hydrogen atom) leading to change of violet color to light yellow which is measured at 517nm in a spectrophotometer.

Procedure

A stock solution of 1mg/ml concentration of each fraction (phenolics-rich fraction and sodium hydroxide fraction) was prepared with methanol in a plain tube. Ascorbic acid of similar concentration was prepared and used as standard. DPPH solution (95% methanol) was also prepared in a separate tube wrapped with aluminum foil to avoid radiation. Each of the stock solutions (1ml) was serially diluted with methanol to achieve concentrations of 1000, 500, 250,

125, 62.5, and 31.25µg/ml of methanol. The absorbance of the blank solution was measured at 700nm and recorded. To each of the respective concentrations 2ml of DPPH solution was added. The mixture was shaken and incubated in a dark chamber for 30 minutes. The absorbance of the sample solution was then measured at 517nm in a spectrophotometer.

The percentage inhibition of DPPH radical was calculated using the equation

% inhibition of DPPH radical = ([Ac -As]/Ac) x 100

Where Ac is the absorbance of control and As is the absorbance of sample.

The scavenging ability of the natural antioxidants of the stem bark towards the stable free radical DPPH was measured by the method of Nur Alam *et al.,* (2012).

3.5.2 Reducing Power (Hira *et al*., 2011).

Principle

Antioxidant compounds form a colored complex with potassium ferricyanide, Trichloroacetic acid and Ferric chloride which is measured at 700nm.

Potassium ferricyanide + Ferric chloride $\xrightarrow{\text{Antioxidant}}$ Potassium ferrocyanide + Ferrous chloride

Procedure

Various concentrations of the plant fraction/standard (2.5ml) were mixed with 2.5mL of 0.2M sodium phosphate buffer and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min and 2.5 ml of trichloroacetic acid solution (100 mg/l) was added. The mixture was centrifuged at 650 rpm for 10 min, and 5mL of the supernatant was mixed with 5 ml of

distilled water and 1 ml of 0.1% ferric chloride solution. The absorbance was measured at 700nm in a spectrophotometer.

3.6 Experimental Animals

A total of forty (40) healthy albino rats weighing 90-120g were used in this study. Twelve (12) were selected randomly and used for acute toxicity (LD_{50}) study. The rats were purchased from animal house of Biological Science Department of Bayero University Kano. All the animals were allowed free access to diet and distilled water in an animal house with conditions of temperature (25±1°C) and 12/12 hours' light/dark cycle. The animals were allowed to acclimatize for one week before the start of the experiment. This experiment took appropriate measures to reduce pain and discomfort of the laboratory animals in accordance to guides provided by (Olfert *et al.,* 1993). The basal diet of the animal was commercially prepared pelletized grower mash of CHUKUN brand purchased from Kofar Famfo, Kano state.

3.6.1 Determination of Acute Toxicity in Animal Models

Lorke's method (lorke, 1983).

This method has two phases which are phases I and II.

Phase I

Nine (9) rats were used in this phase; the rats were divided into three groups of three animals each. Each group was administered different doses (10, 100 and 1000 mg/kg) of phenolic rich fraction extracted from *Cinnamomum verom* stem bark. The rats were placed under observation for 24 hours (to monitor their behavior as well as mortality).

Phase II

This phase involved three rats, which were distributed into three groups of one animal each. The animals were administered higher doses (1600, 2900 and 5000 mg/kg) of test substance and then observed for 24 hours for behavior as well as mortality (Lorke, 1983).

Then the LD_{50} was calculated by the formula:

$LD_{50} = \sqrt{(D_0 \times D_{100})}$

D_0 = Highest dose that gave no mortality,

D_{100} = Lowest dose that produced mortality.

3.6.2 Preparation of High Fat Diet Feed

Normal animal food pellets of CHUKUN brand was crushed using mortar and pestle into small pieces and then grinded into fine powder in a grinder. Cholesterol 2%, cholic acid 1%, Sucrose 40% and Coconut oil 10% were added in the mixer grinder in an ascending order of their quantity and mixed well (Nishina *et al.,* 1990)

3.6.3 High Fat Diet Induced Model

Twenty (20) rats were selected and divided into five (5) groups each of four (4) animals. Atorvastatin and phenolic cinnamon extract were dissolved in distilled water. The treatment protocol was planned to study the effect of herbal extracts in preventive aspect of high fat diet (HFD) induced hyperlipidemia. The animals of all groups except normal group were given a

lipid (HFD) diet consisting of 2.5% cholesterol, 1% cholic acid and 2ml coconut oil with standard pellet diet for 21 days (Mohd *et al*., 2016).

3.6.4 Basal Diet Induced Model

Eight (8) rats were selected and divided into two (2) groups each containing four rat. Phenolic cinnamon extract was dissolved in distilled water. The treatment protocol was planned to study the effect of herbal extracts in preventive aspect of normal diet induced rats.

3.6.5 Experimental Design

A total number of twenty-eight (28) rats were used for the experiment. The work was conducted in two (2) phases.

Phase I- This phase focused on the effect of phenolic-rich fraction of *C. verom* on albino rats fed on high fat diet for a period of 28 days. The rats in this phase of the study were weighed and grouped randomly into five groups (1-5) of four animals (n=4) each.

Group I: Normal control group that were administered basal diet and distilled water

Group II: Animals in this group were administered high fat diet (HFD)

Group III: Animals in this group were administered HFD and treated with 100mg/kg of Atorvastatin drug orally.

Group IV: Animals in this group were administered HFD and 100mg/kg body weight of *C. verom* phenolic-rich fraction orally.

Group V: Animals in this group were administered HFD and 200mg/kg body weight of *C. verom* phenolic-rich fraction orally

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Phase II- focused on the effect of *C. verom* phenolic rich fraction extract on lipid profile and antioxidant enzymes of normolipidaemic rats fed on basal diet of CHUKUN brand. The animals in this phase were weighed and grouped randomly as groups VI and VII with four animals (n=4) each

Group VI; Animals in this group were administered basal diet and 100mg/kg body weight of *C. verom* phenolic rich fraction orally

Group VII; Animals in this group were administered basal diet and 200mg/kg body weight of *C, verom* phenolic rich fraction orally.

Diet and water were accessed *ad libitum* by the animals in all groups.

3.6.6 Weight of Experimental Animals

The weight of the experimental animals was weighted before and after the experiment using digital weighing balance.

3.6.7 Collection of Blood Samples

After fourth weeks of extract administration, the animals were fasted for 12 hours, and all rats were sacrificed by cervical dislocation under anesthesia. Serum samples were collected by centrifuging blood (4,000 rpm/min, at 4°C for 15 minutes) and used for antioxidant enzymes determination.

3.7 Serum Lipid Profile

The following were assayed: Total cholesterol (TC), Low density lipoproteins-cholesterol (LDL-CH), High density lipoproteins- cholesterol (HDL-CH), and triglycerides (TGs).

3.7.1 Estimation of Serum Lipid Profile

The serum lipid profile was estimated using cardio chek® P.A meter and pts panels® (lipid panel test strips). The strips were used to measure cholesterol, HDL cholesterol and triglycerides in whole blood/serum/plasma with the cardio chek PA analyzer. The pts panel strips are designed for use with fresh capillary whole blood or venous whole blood collected in EDTA or heparin tubes plasma or serum.

3.7.2 Principles of the Test

When blood is applied to a test strip, the blood reacts with the chemical on the strip to produce color that is read by the analyzer using reflectance photometry. The amount of color produced is proportional to the concentration of cholesterol, HDL and triglyceride. The enzymatic reactions that occur are listed below

Cholesterol

Cholesterol ester + H_2O $\xrightarrow{\text{cholesterol esterase}}$ cholesterol + fatty acid

Cholesterol + H_2O + O_2 $\xrightarrow{\text{cholesterol oxidase}}$ cholesterol-4-en-3-0ne + H_2O_2

$2H_2O_2$ +4-AAP + Disubstituted Aniline $\xrightarrow{\text{peroxidase}}$ Quinoneimine dye + $4H_2O$

HDL Cholesterol

VLDL, LDL, HDL plasma $\longrightarrow$ VLDL, LDL, depleted plasma

Cholesterol ester + H_2O $\xrightarrow{\text{cholesterol esterase}}$ cholesterol + fatty acid

Cholesterol + H_2O +O_2 $\xrightarrow{\text{cholesterol oxidase}}$ cholesterol-4-en-3-0ne + H_2O_2

$2H_2O_2$ +4-AAP + Disubstituted Aniline $\xrightarrow{\text{peroxidase}}$ Quinoneimine dye + $4H_2O$

Triglycerides

Triglyceride + $3H_2O$ $\xrightarrow{\text{lipoprotein lipase}}$ glycerol + 3 fatty acids

Glycerol + ATP $\xrightarrow{\text{glycerol kinase /Mg}}$ glycerol-3-PO_4 +ADP

Glycerol-3-PO_4 + O_2 $\xrightarrow{\text{glycerophosphate oxidase}}$ Dihydroxyacetone-PO_4 + H_2O_2

$2H_2O_2$ + 4-AAP +N, N-disubstituted aniline $\xrightarrow{\text{peroxidase}}$ Quinoneimine dye + $4H_2O$

LDL Cholesterol

The LDL was calculated using the formula by Fredwald's (Fredwald's *et al.*, 1972).

[LDL] = [TC] – [HDL] – [Trig/5]

Where;

TC= Total cholesterol

HDL= High Density Lipoprotein Cholesterol

Trig= Triglyceride

LDL= Low Density Lipoprotein Cholesterol

3.7.3 Procedure

The MEMo chip that matches the lot number of the test strip vial was inserted and one of the buttons was pressed to turn the analyzer ON and strip was held at the end marked PTS. The opposite end of the strip was inserted into the analyzer and the strip was pushed in as far as it can go.

When APPLY SAMPLE appeared, a capillary blood collector was used to apply 35-40µl of the serum on the test strip blood application window. In as little as 90 seconds, the results were displayed automatically on the analyzer. As necessary, next was pressed to additional results.

3. 8 In-vivo Antioxidant Assay

In this phase, the antioxidant status of the phenolic *Cinnamomum verom* administered to the rat was assayed.

3.8.1 Serum Catalase (CAT)

Catalase activity was assayed following the instruction on the assay kit Cat number BC 0200 size 50T/48S.

Principle

CAT is the main enzyme for clearing hydrogen peroxide. The UV absorption of hydrogen peroxide can be measured at 240nm, whose absorbance decreases when degraded by the enzyme catalase. From the decrease in absorbance, the enzyme activity can be calculated.

Procedure

The spectrophotometer was preheated for 30 minutes, wavelength adjusted to 240nm and set to zero with distilled water. CAT working solution: 20ml reagent l was added to 100µl reagent II and was mixed thoroughly before use. The reagent was preheated in water bath at 37^{o}C.

CAT working reagent (1ml) was added into 1ml cuvette, then 35µl of sample was also added and mixed for 5 seconds. Absorbance A1 and A2 (after 1 minutes) was measured at 240nm.

- ΔA_T = A1 –A2 was calculated

Calculations

CAT (U/mL) = ΔA_T X 678

3.8.2 Superoxide Dismutase (SOD) Assay

SOD was assayed following the assay kit instruction. Cat number BC0170 size 50T

Principle

Superoxide dismutase (SOD) is an enzyme that alternatively catalyze the dismutation of the superoxide (O^2) radical into either ordinary molecular oxygen (O_2) or hydrogen peroxide (H_2O_2). Superoxide is produced as a by-product of oxygen metabolism.

SOD enzymes deal with superoxide radicals by either adding or removing an electron from the superoxide molecule it encounters, thus changing the O^{2-} into one of the two less damaging species. The reduction reaction between O^{2-} and the reducant nitroblue tetrazolium (NBT) forms blue color, which has absorbance at 560nm; SOD reacts with O^{2-} to suppress the blue reaction. The deeper blue means lower SOD activity.

Procedure

The spectrophotometer was preheated for 30 minutes, wavelength adjusted to 560nm and set to zero with distilled water. Reagents I, II, and V were kept in water bath for 5 minutes at 37^{o}C. Reagents were added into four (4) test tubes labelled test tube (T), control tube (C) and blank tubes (B1 and B2).

Reagent I (240µl) was added to all the four tubes, reagent II (6µl) to tubes T and B1, reagent V (30µl) to all the tubes, sample (90µl) to tubes T and C, distilled water (480µl, 486µl, 570µl and 576µl) to tubes T, C, B1 and B2 respectively and then 180µl of reagent III to all the tubes.

Tubes were mixed thoroughly, placed at room temperature for 30 minutes and added to 1ml cuvette and absorbance value from each tube was taken at 560nm wavelength in a spectrophotometer.

$\Delta A_T = A_T - A_{C,}$ $\Delta A_B = A_{B1} - A_{B2}$ was calculated.

Where ΔA_T= change in test concentration

A_C= absorbance of control

A_T= absorbance of test

A_B= absorbance of blank

Calculation

%inhibition (P) = $(\Delta A_B - \Delta A_T) / \Delta A_B$ X 100

SOD (u/ml) = 11.4 (1-P/P)

3.8.3 Glutathione peroxidase (GPx) assay

This test was carried out following the instruction on the assay kit manual Cat number BC1190 size 50T/24S.

Principle

Glutathione peroxidase (GPx or GSH-PX) is an important peroxidase widely distributed in the body. GPx can catalyze the formation of oxidized glutathione (GSSG) from reduced glutathione (GSH) and reduce toxic hydrogen peroxide to non-toxic hydroxyl compounds.

GPx catalyzes the oxidation of GSH by hydrogen peroxide to produce GSSG. GSH can react with 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) to form compounds with characteristic absorption peaks at 412nm. The decrease of absorbance at 412nm can reflect the activity of GPx.

Procedure

The spectrophotometer was preheated for 30 minutes, wavelength adjusted to 412nm and set to zero with distilled water. Standard solution of 20µmol/ml was diluted to 0.25µmol/ml with the extraction solution. Standard solution of 100µl was mixed with 400µl reagent IV, and the concentration of the standard solution was 0.05µmol/ml.

The sample (150µl) was mixed with reagent I (150µl) and placed at room temperature for 5 minutes. The reagents were added in turn to 1.5 ml centrifugal tubes labelled test tube (T) and control tube (C). Sample mixture (100µl) was added to T, reagent II (100µl) to both tubes and the mixture was preheated for 5 minutes at 37^{o}C. Reagent III (100µl) was added to both tubes and was allowed to react for 5 minute at 37^{o}C. Reagent IV (1µl) was added to both tubes and finally sample mixture (100µl) to C. The tubes were centrifuged at 4000rpm at room temperature for 5 minutes and the supernatant was taken to Eppendorf tube.

Another set of tubes were labelled test tube (T), control tube (C), standard tube (S) and blank tube (B). Supernatant (500µl) was added to T and C, sample mixture (500µl) to S only, reagent IV (500µl) to B only, reagent V and VI each (200µl) to all the tubes, and finally distilled water (100µl) to all the tubes. The tubes were mixed thoroughly after placing them at room temperature for 2 minutes, the absorbance at 412 was measured. The absorbance was recorded as $A_{T,}$ $A_{C,}$ A_S and A_B respectively.

$\Delta A_T = A_C - A_T$, $\Delta A_S = A_S - A_B$.

Where ΔA_T= change in absorbance of test

A_C= absorbance of control

A_T= absorbance of test

ΔA_S=change in absorbance of standard

A_S= absorbance of standard

A_B= absorbance of blank

Calculation

Percentage inhibition= $(A_C - A_T) / (A_C-) \times 100\%$

GPx= $260 \times \Delta A_T / \Delta A_S$.

3.8.4 Malondyaldehyde (MDA) Assay kit

The test was carried out following the instruction on the assay kit manual. Cat number BC0020 size 50T.

Principle

Lipid peroxide is produced by the action of oxygen free radicals on unsaturated fatty acid, then resolves to compounds, including Malondyaldehyde (MDA), the level of lipid peroxidation can be showed by detecting level of MDA.

Under acidic and high temperature conditions, the brown red 3,5,5- three methylsulfamethoxazole-2,4-two ketone was synthesized with MDA and thiobarbituric acid (TBA) and condensation reaction occured, the largest absorption wavelength is 532nm. The content of lipid peroxidation can be estimated after colorimetry. But the soluble sugar will disturb the detection, the production (color reaction of soluble sugar with TBA) have absorption wavelength in 450nm and 532nm. In this kit, the MDA content is calculated by the difference between the absorbance at 532nm, 450nm and 600nm.

Procedure

The spectrophotometer was preheated for more than 30 minutes, and was set to zero with distilled water. Reagents were added to two tubes labelled test tube (T) and blank tube (B). MDA working reagent (600µl) was added to both tubes, sample (200µl) to T, distilled water (200µl) to B and reagent III (200µl) to both tubes.

The mixture was incubated at $100^{O}C$ for 30 minutes, then cooled in an ice bath and centrifuged at 1000rpm for 10 minutes at room temperature to remove insoluble materials. The supernatant was taken into 1mL cuvette. The absorbance was measured at 450nm, 532nm and 600nm.

$\Delta A450 = A450\ (T) - A450\ (B)$, $\Delta A532 = A532\ (T) - A532\ (B)$, $\Delta 600 = A600\ (T) - A600\ (B)$.

Where

A (T) = absorbance of test, A (B) = absorbance of blank

Calculation

MDA (nmol/mL) = 5(12.9 X (ΔA532 - Δ600) - 2.58 X ΔA450) X 5.

3.9 GC-MS Analysis

A gas chromatography from Agilent USA hyphenated to a mass spectrophotometer (7890A GC system, 5675C inter MSD with triple axis detector equipped with an auto injector (10µl string) was used. Helium gas was used as a carrier gas, then chromatographic separation was performed on capillary column having specification: length; 30m, internal diameter 0.2µm, thickness; 250µm, treated with phenyl methyl silox. Other GC/MS conditions were ion sources (EI), 250^0C, interface temperature; 300^0C, pressure; 16.2psia, out time, 1.8mm, 1µl injector in split mode with a split ratio 1:50, the injection temperature of 300^0C the column temperature started at 35^0C for 5mins and changed to 150^0C at the rate of 4^0C/min, the temperature was raised to 250^0C at the rate of 20^0C/min and held for 5mins. The total elution was 47.5 minute. MS Solution software provided by supplier was used to control the system and to acquire the data; identification of the compounds was based on the comparison of their mass spectra and their retention time with standard mass spectra from NIST library (NIST11).

3.10 Data Analysis

All data were collected and statistically analyzed using SPSS20.0. The results were expressed as the mean ± standard deviation. Significance of differences between group means were analyzed with one-way ANOVA followed by Duncan multiple comparison test at $P<0.05$.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Phytochemical Screening of stem bark of *Cinnamomum verom*

Table 4.1.1 shows the results of the phytochemicals composition of Cinnamon bark extract. It revealed the presence of saponins, tannins, flavonoids, alkaloids glycoside and terpenoids in cinnamon bark.

Table 4.1.1: Phytochemical Screening of *Cinnamomum verom*

Phytochemicals test	Inference
Saponins	+
Tannins	+
Glycosides	+
Alkaloids	+
Flavonoid	+

Terpenoids	+
Phenols	+

Key += present

4.1.2 Percentage yield of *Cinnamomum verom* after extraction

In the extraction and isolation phase of phenol rich fraction from stem bark of *Cinnamomum verom* alkaline hydrolysis gave the highest yield of 13.15 and percentage yield of 6.25% (Table 4.1.2).

Table 4.1.2: Phenolic extract yield from *Cinnamomum verom*

Parameters/ methods	Acid hydrolysis/extraction	Alkaline hydrolysis/extraction
Weight of sample (g)	200	200
Weight of Extract yield (g)	1.08	13.15
Percentage yield (%)	0.54	6.58

4.1.3 Acute Toxicity Study

In the initial phase of the determination of acute toxicity, mortality and toxic symptoms were not observed when the phenolic rich fraction extracted from stem bark of *Cinnamomum verom* was administered orally to the experimental rats (Table 4.1.3a). In the second phase, no mortality was observed although some rats exhibited symptoms of weakness (Table 4.1.3b).

Table 4.1.**3a: Phase I LD50 (oral, rat) of the phenolic bark extract of *Cinnamomum verom***

Dose (mg/kg body weight)	Mortality
10	0/3
100	0/3
1000	0/3

Table 4.1.3**b: Phase II LD50 (oral, rat) of the phenolic bark extract of *Cinnamomum verom***

Dose (mg/kg body weight)	Mortality
1600	0/1
2900	0/1
5000	0/1

4.1.4 In-vitro antioxidant activity

Table 4.1.4 shows the result of IC_{50} of DPPH and reducing power with highest value in NaOH fraction (109.202 and 1620.541) µg/ml followed by diethylether fraction (95.6197 and 608.75) µg/ml and lastly ascorbic acid (1.044 and 49.379) µg/ml in both DPPH and reducing power. Ascorbic acid which is the control has the highest antioxidant activity, then diethylether and NaOH

Table 4.1.4 In-vitro antioxidant activity of *Cinnamomum verom*

Parameters	IC_{50} DPPH (µg/ml)	IC_{50} reducing power (µg/ml)
Ascorbic acid	1.044	49.379
NaOH fraction	109.202	1620.541
Diethyl ether fraction	95.6197	608.75

4.1.5 Weight of Experimental Animal

The weight of the experimental animals is presented in table 4.1.5 for the initial weight and final weight used for the study. After four weeks of oral administration, the final body weight of albino rats in the HFD group (139. .00±0.82) was higher compared to animals in the control group (112. 50±0.58, P<0.05). There was no significant difference between control and atorvastatin treated group. Final body weights of all the treated groups were all significantly lower than HFD groups (P<0.05). The final body weights of basal diet rat groups (BD+200) treated with phenols were significantly lower than that of the control (P<0.05).

Table 4.1.5: Body weight of experimental animals before and after administration of *Cinnamomum verom*

Groups	**specification**	**Initial weight (g)**	**Final weight (g)**
I	Control	90.75±1.50	112.25 ± 0.96^{bc}
II	HFD	90.50±2.08	138.50 ± 1.29^{f}
III	HFD + AVS	90.25±0.96	113.25 ± 0.96^{c}
IV	HFD + CVP100	90.50±1.29	130.25 ± 0.95^{d}
V	HFD + CVP200	90.50±0.58	120.25 ± 1.26^{e}
VI	BD + CVP100	90.50±1.73	111.25 ± 0.95^{b}
VII	BD + CVP200	89.50±1.91	108.50 ± 1.29^{a}

Values are expressed as mean ± SD (n=4). Mean values down the column with the different superscript are significantly different; P<0.05 according to Duncan's multiple range test

Key: BD= Basal Diet, HFD= High Fat Diet, CVP100= *C. verom* phenolic 100mg/Kg, CVP200= *C. verom* phenolic 200mg/Kg, AVS= Atorvastatin 100 mg/kg body weight.

4.1.6 Lipid profile of experimental animals

Table 4.1.6 depicts the effect of phenol rich fraction extracted from stem bark of *Cinnamomum verom* on hypercholesterolemic status of rat fed with high fat diet and normal diet. The result showed significant decrease ($P<0.05$) in the mean concentrations of serum TC, LDL-C and Triglycerides on treated groups and significant increase ($P<0.05$) in the mean concentrations of serum HDL-C of the untreated groups. HFD group has the highest concentration of TC (172.00±9.63), LDL-C (93.20±15.45) and Trig (120.25±8.14) and lowest HDL-C level (54.75±6.80) when compared with the control group (BD) and HFD+AVS group. Furthermore, the TC, LDL-C and Trig levels of Basal diet groups treated with phenol-rich fraction (BD+100, BD+200,) was significantly lower than that of the control.

Table 4.1.6: Lipid profile of rats fed with high fat and normal diets.

Groups	Specification	TC (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	TG (mg/dl)
I	Control	117.25±8.92^{a}	69.50±6.61^{b}	32.65±11.34^{ab}	78.50±12.87^{a}
II	HFD	172.00±9.63^{c}	54.75±6.80^{c}	93.20±15.45^{c}	120.25±8.14^{c}
III	HFD + AVS	120.50±10.25^{a}	79.00±7.07^{ab}	24.75±15.94^{ab}	83.75±7.59^{a}
IV	HFD + CVP100	139.00±15.77^{b}	74.75±9.74^{b}	43.35±13.57^{b}	104.50±5.80^{b}
V	HFD + CVP200	127.75±14.86^{ab}	76.25±8.92^{b}	33.85±13.65^{ab}	88.25±4.99^{a}
VI	BD + CVP100	115.25±8.73^{a}	69.00±6.68^{b}	31.40±11.44^{ab}	74.25±12.74^{a}
VII	BD + CVP200	112.50±9.04^{a}	75.25±4.99^{b}	21.95±6.35^{a}	76.50±12.39^{a}

Values are expressed as mean ± SD (n=4). Mean values down the column with the different superscripts are significantly different; $P<0.05$ according to Duncan's multiple range test

Key: TC= Total Cholesterol, HDL-C= High Density Lipoprotein-Cholesterol, LDL-C= Low Density Lipoprotein-Cholesterol, TRIG= Triglyceride, Key: BD= Basal Diet, HFD= High Fat Diet, CVP100= *C. verom* phenolic 100mg/Kg, CVP200= *C. verom* phenolic 200mg/Kg, AVS= Atorvastatin 100 mg/kg body weight.

4.1.7 Antioxidant of experimental animals

Table 4.1.7 depicts the effect of phenol rich fraction extracted from stem bark of *Cinnamomum verom* on antioxidant status of rats fed with high fat and normal diet. The result showed significant decrease ($P<0.05$) in serum glutathione peroxidase (GPx, 26.72±2.47), superoxide dismutase (SOD, 5.92±1.57) and catalase (CAT, 13.12±3.26) of HFD group when compared with the control group (28.55±6.57, 7.87±1.12 and 17.49±0.49) respectively and an increase in the concentration of malondyaldehyde (MDA, 44.16±5.17) when compared with the control group (BD, 28.35±2.88). Furthermore, the level of GPx, SOD and CAT of the groups (BD+100 and BD+200) increases significantly ($P<0.05$) and significant decrease in MDA when compared with the control group.

Table 4.1.7: Antioxidant effect of *Cinnamomum verom* on rats fed with high fat and normal diet

Groups	Specification	GPx (U/ml)	SOD (U/mg/min)	Catalase (U/mg)	MDA (µmol/L/mg)
I	Control	28.55±6.57^{a}	7.87±1.12^{bc}	17.49±0.49^{b}	28.35±2.88^{c}
II	HFD	26.72±2.47^{a}	5.92±1.57^{ab}	13.12±3.26^{a}	44.16±5.17^{e}
III	HFD + AVS	27.51±2.10^{a}	8.33±1.83^{c}	21.59±3.34^{c}	32.04±4.30^{c}
IV	HFD + CVP100	26.99±1.01^{a}	6.73±0.88^{a}	16.55±0.31^{a}	42.18±0.98^{b}
V	HFD + CVP200	27.03±1.97^{a}	7.97±0.55^{bc}	18.29±0.85^{a}	37.49±0.75^{d}
VI	BD + CVP100	29.83±0.94^{a}	8.43±0.85^{bc}	17.94±0.82^{ab}	27.56±3.30^{a}
VII	BD + CVP200	30.99±5.06^{a}	9.85±1.53^{ab}	18.38±0.85^{b}	26.74±3.08^{a}

Values are expressed as mean ± SD (n=4). Mean values in the same column with the different superscript are significantly different; $P<0.05$ according to Duncan's multiple range test

Key: GPx = Glutathione peroxidase, SOD= Superoxide dismutase, MDA= Malondyaldehyde,
Key: BD= Basal Diet, HFD= High Fat Diet, CVP100= *C. verom* phenolic 100mg/Kg, CVP200= *C. verom* phenolic 200mg/Kg, AVS= Atorvastatin 100 mg/kg body weight

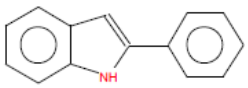
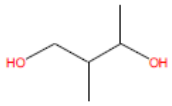
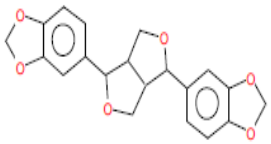
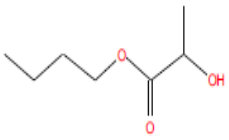
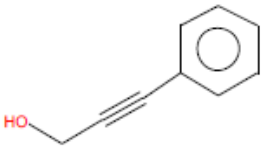
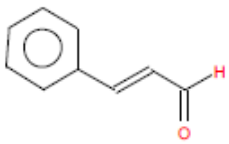
Fig 4.1.1 Present the Preparatory Thin Layer Chromatography (TLC) of the phenol rich extract isolated from stem bark of *Cinnamomum verom* which shows six (6) bands with solvent system of n-Butanol and 2N HCl in the ratio of 1:1.

Fig 4.1.1: Prep Thin Layer Chromatography of diethyl ether fraction of stem bark of *Cinnamomum verom*.

4.1.8 Gas Chromatography-Mass Spectroscopy of Phenolic Rich Fraction Extracted from Stem Bark of *Cinnamomum verom*

Table 4.1.8 Present the bioactive compound identified by Gas Chromatography-Mass Spectroscopy (GC-MS) of phenol rich fraction isolated from stem bark of *Cinnamomum verom*.

Table 4.1.8: Bioactive component identified by Gas Chromatography-Mass Spectroscopy (GC-MS) of diethyl ether fraction isolated from stem bark of *Cinnamomum verom*.

RT	%AREA	COMPOUND NAME/ IUPAC NAME	MOLECULAR FORMULA	MOLECULAR WEIGHT	MOLECULAR STRUCTURE
2.898	1167856.56	1H-Indole, 2-phenyl-	$C_{14}H_{11}N$	193	
8.729	1209475.94	1,3-Butanediol, 2-methyl-	$C_5H_{12}O_2$	104	
10.182	879702.8	1,3-Benzodioxole, 5,5'-(tetrahydro-1H,3H-furo[3,4-c]furan-1,4-diyl)bis-, [1S-(1α,3aα,4β,6aα)]-	$C_{20}H_{18}O_6$	354	
10.97	879702.8	Propanoic acid, 2-hydroxy-, butyl ester	$C_7H_{14}O_3$	146	
12.826	236626.56	3-Phenyl-2-propyn-1-ol	C_9H_8O	132	
13.089	262357	Cinnamaldehyde, (E)-	C_9H_8O	132	

KEY: RT= Retention Time

4.2 DISCUSSION

From the results of the phytochemical screening of *Cinnamomum verom* stem bark (table 4.1.1), it was found to contain saponins, tannins, alkaloids, flavonoids, glycosides, terpenoids and phenols. This corroborated with the work of Rajesh. (2016), Mazimba (2015), Dhanalaxmi and Jyoti (2014), (Li *et al.,* 2013) and Paliwal *et al.,* (2018) who reported the presence of these same phytochemicals and attributed the antioxidant and hypercholesterolaemic activity of the *Cinnamonum verom* to the presence of these compounds. The presence of these constituents gives an indication of their medicinal value. Flavonoids, tannins, phenolic compounds have been found to have antioxidant hypercholesterolaemic and antimicrobial properties (Shreya *et al*., 2015 and Qian and Nihorimbere, 2004). Phytochemicals in plants and plants parts have been extensively reported to possess antioxidant and antimicrobial activities both *in vitro* and *in vivo*. Alkaloids, saponins, tannins, glycosides, terpenoids and phenolic compounds etc. have been found to have bactericidal effect by inhibiting vital metabolic processes such as DNA and cell wall synthesis inhibition among others (Ahuja *et al*., 2015, Haslam, 1996; Rahman and Choudhary, 1995; Tsuchiya *et al*., 1996).

The solubility of phenolic compounds in solvents with different polarity depends on the number of hydroxyl groups in the molecules as well as the molecular size and extent of hydrogen bonding. From the result obtained the percentage yield (table 4.1.2) alkaline hydrolysis is higher than that of the acid hydrolysis which is in accordance with the result obtained by Eva *et al.* (2015) and Rommel and Wrolstad (1993). Phenolic compounds usually occur in conjugated forms and many phenolic compounds occur as glycosides or esters. Thus, sample preparation included alkaline and acid hydrolysis to free bound phenolic compounds. Hydrolysis of phenolic glycosides to corresponding aglycones offers a practical method for quantification of phenolic

compounds in natural matrices (Eva *et al.,* 2015). Phenolic compounds obtained as hydrolysates were substantially more abundant than free derivate. Rommel and Wrolstad (1993) used alkaline hydrolysis (2 M NaOH) to study hydroxycinnamic and hydroxybenzoic acids in red raspberry juices and reported that the rate of acid/base hydrolysis of glycosides depended on the acid/base strength, the nature of the sugar moiety, and the position of the moiety in the phenolic nucleus. Glucuronides resist acid hydrolysis better than glucosides, which are rapidly cleaved (Eva *et al.,* 2015).

The acute toxicity test result of this study (table 4.1.3) showed that the LD_{50} of the diethyl ether fraction of bark extract of *Cinnamomum verom* was greater than 5000mg/kg body weight. The fact that the experimental rats survived the acute toxicity testing up to a dose of 5000mg/kg showed that the aqueous bark extract of *Cinnamomum verom* is practically non-toxic (Hodge and Staner, 2005). This is in accordance with a study by Shah *et al*., (1998) who demonstrated that the ethanolic extract of *Cinnamomum zeynalicum bark* was non-toxic after acute toxicity tests. Rabiatu *et al*., (2015) also reported that *Cinnamomum verom* possess low moderate toxicity, evidenced by high LD50 values with no lethality, thus the low toxicity of *Cinnamomum verom* offers a wide margin of safety for beneficial doses.

The stem bark methanol extract shows good activity in reducing Fe^{3+} to Fe^{2+}. Reductones in an extract exhibit their antioxidant activities through the action of breaking the free radical chain by donating a hydrogen atom to convert radicals into stable and non-harmful products (Ofentse *et al.,* 2015). Studies have shown that fruit and vegetables possess a large spectrum of biological activities that are principally due to their antioxidant property. These control reactive oxygen species from exogenous factors and thus prevent free radical damage and lipid peroxidation (Liu *et al.,* 2007 and Pan *et al.,* 2008). From the result of the study (table 4.1.4) showed that the total

phenolic content has good positive correlation with DPPH radical scavenging and reducing power. This is in accordance with literature report which reported high correlation between antioxidant activity and total phenolic content (Rakshit and Ramalingam2011, Mazimba *et al.,* 2011, and Rezaeizadeh *et al.,* 2011).

Antioxidants have been considered the most important drivers in the progress and existence of humans, as they respond to free radicals and damage in metabolic diseases and age-related syndromes of humans and other animals, (Halliwell, 2006, Halliwell, 2011). Mancini-Filho *et al* (1998) reported various extracts of cinnamon, such as ether, aqueous, and methanolic extracts that have shown considerable antioxidant activities. A research group reported that cinnamon oil potentially exhibits superoxide-dismutase- (SOD-) like activity as indicated by the inhibition of the inhibiting capacity of pyrogallol autoxidation (Kim, *et al.,* 1995). Cinnamon exhibited higher antioxidant activities compared to that of other dessert spices (Murcia, *et al.,* 2004). MDA, the product of lipid peroxidation, is an index of the level of oxygen free radicals. A decrease in lipid peroxidation leads to a reduction of atherosclerosis caused by hypercholesterolemia (Ines *et al*., 2005). The content of MDA in rats fed a cholesterol-rich diet was increased compared to rats fed standard laboratory diet, suggesting that hypercholesterolemia could enhance the process of lipid peroxidation. The oral administration of phenol rich extract of cinnamon prevented a cholesterol-rich diet induced elevation of MDA and resulted in a significantly decreased content of MDA in liver, heart and kidney. The data obtained suggested that cinnamon phenolics might be capable of lowering or slowing down oxidative- stress-related lipid peroxidation. A cholesterol-rich diet brings about remarkable modifications in antioxidant defense mechanisms. Studies have shown that hypercholesterolemia diminishes the antioxidant defense system and decreases the activities of SOD and CAT, elevating the lipid peroxide content. (Ines *et al*., 2005). In the present study,

the activities of SOD and CAT in the HFD group were significantly decreased compared with those of control rats. Administration of drug (100mg/kg) and phenol rich extract of cinnamon (100mg/kg and 200mg/kg) to the rats fed cholesterol-rich diet significantly elevated the activities of SOD. These results have suggested that phenolic compounds extracted from cinnamon could improve the efficiency of superoxide anion to hydrogen peroxide due to increased SOD activity, which catalyzes dismutation of superoxide anion into hydrogen peroxide. Phenolic extracts of cinnamon also increased the activity of CAT, which, in turn, detoxifies hydrogen peroxide and converts lipid hydroperoxides to nontoxic substances (Ines *et al*., 2005).

Cinnamon extracts have also been shown to be useful in decreasing fasting plasma glucose, cholesterol, and triglycerides in diabetic patients. Similarly, application of cinnamon extract reduced liver MDA levels in carbon tetrachloride-poisoned rats and improved SOD, CAT, and GPx activities. Cinnamon has been shown to prevent hyperlipidemia and improved glucose tolerance in rats fed fructose/high fat diet (Zeynep *et al.,* 2017)

Hypertriglyceridemia and low HDL cholesterol are two of many consequences of metabolic syndrome (Eckel *et al.,* 2010). Hypertriglyceridemia is a condition in which the plasma triglyceride (TG) levels are higher than a reference value (Brahm and Hegele, 2013). Cinnamon has been found to be beneficial for improving blood lipid profile (Hamidpour *et al.,* 2015).

Many studies revealed that diet plays an important role in the development of hyperlipidemia and atherosclerosis. Several studies on animals and humans have demonstrated that saturated fatty acids cause hypercholesterolemia by elevating total cholesterol and changing lipoprotein pattern and the mechanisms involved remain under study. Feeding of cholesterol has been often carried out to increase the cholesterol levels to induce hypercholesterolemia and related metabolic changes in various animal models (Zahid *et al.*, 2016).

Result of the study (table 4.1.7) indicated that supplementation of high fat diet was sufficient to induce hyperlipidemia. Similar findings were reported by Zahid *et al.* (2016), Mohd *et al.* (2016). Dhulasavant *et al.* (2010) and Iqbal *et al.* (2015) that rats and rabbits fed with a high cholesterol diet elevated their serum lipid profile parameters. Administration of phenol rich extract of cinnamon (100 and 200mg/kg, body weight) for 28 days in fat diet and normal diet model, successfully prevented the elevation of serum Total Cholesterol, Triglycerides, Low Density Lipoproteins Cholesterol (LDL-C), and increased serum High Density Lipoprotein Cholesterol (HDL-C) in Fat diet and normal diet model rats respectively (Table.4.1.5). The findings of this study correlated with the results of Dhulasavant *et al.* (2010) who reported that ethanolic and aqueous extracts of *Cinnamomum tamala* Nees leaves significantly reduced the levels of total cholesterol, triglyceride, LDL, VLDL and atherogenic index whereas the extracts significantly increased the HDL level. Zahid *et al.* (2016), and Mohd *et al.* (2016) also reported similar findings which correlate with the finding in the current study.

The result of the GC-MS analysis of the diethyl ether from stem bark of *Cinnamomum verom* (table 4.1.8) shows that it is mostly dominated by phenols, and other compounds. The phenols are said to have antioxidant and anti-hypercholesterolaemic effect.1, 3-Butanediol is found in pepper (C. annuum). 1, 3-Butanediol is a solvent for flavoring agents 1, 3-Butanediol is an organic chemical, an alcohol. It is commonly used as a solvent for food flavoring agents and is a co-monomer used in certain polyurethane and polyester resins. It is one of four stable isomers of butanediol. In biology, 1, 3-butanediol is used as a hypoglycemic agent. The compound Propanoic acid is said to have antioxidant activity (Bhama *et al.,* 2018). Sesamin is a lignan that consists of tetrahydro-1H,3H-furo[3,4-c]furan substituted by 1,3-benzodioxole groups at positions 1 and 4 (the 1S,3aR,4S,6aR stereoisomer). Isolated from Cinnamomum camphora, it

exhibits cytotoxic activity. It has a role as an antineoplastic agent, a neuroprotective agent and a plant metabolite. It is a lignan, a member of benzodioxoles and a furofuran. They have anticholesteroleimic and antioxidant properties (Ali *et al*., 2007). Cinnamaldehyde is said to have hypercholesterolemic and shows significant decrease in total cholesterol LDL and triglyceride level and significant increase in HDL level (Kritika and Uma, 2018, and Asghari *et al.,* 2017).

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATION

5.1 Summary

Phytochemical analysis of *Cinnamomum verom* was analyzed and the phenolic was isolated. Diethyl ether fraction isolated from stem bark of *Cinnamomum verom* containing phenols was administered to rats fed high fat diet and normal diet. The phenolic rich fraction has been shown to be non-toxic to the experimental animals even at higher dose (5000mg/kg) and also showed In-vitro antioxidant activity. Blood sample collected from the rats were analyzed for hypocholesterolemic and antioxidant effects of the phenolic rich fraction of *Cinnamomum verom*. The total cholesterol, LDL and Triglycerides were significantly reduced in rats fed high fat diet and normal diet compared with the control group while there is significant increase in HDL level.

Moreover, the level of MDA significantly reduced and the activity of SOD, CAT and GPx increased between the high fat diet, normal diet and the control groups. GC-MS analysis showed the active compound of cinnamon which may be responsible for hypocholesterolemic and antioxidant effect such as propanoic acid, 1,3-benzodioxole and cinnamaldehyde.

5.2 Conclusion

1. This study showed the presence of phytochemical constituents in *Cinnamomum verom*
2. Based on this study, phenol rich fraction isolated from stem bark of *Cinnamomum verom* was not toxic even at high dose

3. The study showed that the diethyl ether fraction contain phenols which shows low In-vitro antioxidant activity compared to that of ascorbic acid.

4. Based on the study, the diethyl ether fraction containing phenol showed significant decrease in Total cholesterol, LDL and TG level of high fat diet and normal diet rat and increase in HDL level.

5. The study showed decrease in MDA level and increase in CAT, SOD and GPx activity of the high fat diet and normal groups compared with the control group.

6. The GCMS result shows some active compounds which are phenols found in cinnamon and may be responsible for hypocholesterolemic and antioxidant effect.

5.3 Recommendation

A further study on the hypocholesterolemic and antioxidant effects should be carried out on the purified phenolic isolated from cinnamon and other phytochemical compounds found in cinnamon.

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APPENDIX I

List of equipment/apparatus

Spectrophotometer, water bath, glass wares (beakers, test tubes etc), Utrasonicator (GT sonic), weighing balance, PTS lipid panel test strips (Manufactured by Polymer technology system, inc., Indianapolis USA), MEMo Chip, CardioCheck professional analyser, Gas chromatography (Agilent USA), Column (Agilent 19091-433HP-5Ms 5% methyl silox). Mass spectrohotometre (5975C), heating mantles, Rotary evaporator, freeze dryer,

APPENDIX II

List of reagents and Reagent Preparation

HCL

NaOH

diethylether.

2N hydrochloric acid (HCL) was prepared adding 165ml concentrated HCL with specific gravity 1.18 to distilled water and making it to 1000ml in the volumetric flask

2M sodium hydroxide was prepared by adding 80g of NaOH pallet in distilled water and making it to 1000ml in the volumetric flask.

APPENDIX III

Plant identification

BAYERO UNIVERSITY, KANO

DEPARTMENT OF PLANT BIOLOGY

Herbarium Plant Identification Form

Name of inquirer: Aisha Shehu SPS/17/MBC/00032

Kingdom: Plantae

Division:

Order: Laurales

Family: Lauraceae

Genus: *Cinnamomum*

Species: *Cinnamomum verom*

Common name: Cinnamon

Local name: Kirfa

Certification

Bayero University, Kano Herbarium Accession Number BUKHAN 119

Identified by Dr Yusuf Nuhu

Sign &Date 15th November, 2019

APPENDIX IV
ELEMENTAL RESULT

Weight of animals

Initial weight

N	Basal diet	High Cholesterol diet	High Cholesterol diet + drug	High Cholesterol diet + 100mg/kg extract	High Cholesterol diet + 200mg/kg extract	Basal diet + 100mg/kg extract	Basal diet + 200mg/kg extract
1	90	91	91	92	90	88	88
2	92	90	92	91	91	91	88
3	89	89	90	89	91	91	90
4	92	93	89	90	90	92	92

Final weight

N	Basal diet	High Cholesterol diet	High Cholesterol diet + drug	High Cholesterol diet + 100mg/kg extract	High Cholesterol diet + 200mg/kg extract	Basal diet + 100mg/kg extract	Basal diet + 200mg/kg extract
1	113	138	113	129	120	112	110
2	112	139	114	130	119	111	109
3	112	139	114	131	120	110	108
4	113	140	112	131	122	112	107

Lipid profile

Total cholesterol

N	Basal diet	High Cholesterol diet	High Cholesterol diet + drug	High Cholesterol diet + 100mg/kg extract	High Cholesterol diet + 200mg/kg extract	Basal diet + 100mg/kg extract	Basal diet + 200mg/kg extract
1	128	167	107	157	110	117	124
2	114	162	119	146	123	105	111
3	107	175	125	132	133	113	102
4	120	184	131	121	145	126	113

Low-density lipoprotein-cholesterol

N	Basal diet	High Cholesterol diet	High Cholesterol diet + drug	High Cholesterol diet + 100mg/kg extract	High Cholesterol diet + 200mg/kg extract	Basal diet + 100mg/kg extract	Basal diet + 200mg/kg extract
1	48	87.4	4.2	56.8	19.6	35	26.4
2	23	74.6	24.4	43.2	26.4	21.6	28.4
3	25	101	27.4	48.6	50.4	23	16.2
4	32.2	109.8	43	24.8	39	46	16.8

High-density lipoprotein-cholesterol

N	Basal diet	High Cholesterol diet	High Cholesterol diet + drug	High Cholesterol diet + 100mg/kg extract	High Cholesterol diet + 200mg/kg extract	Basal diet + 100mg/kg extract	Basal diet + 200mg/kg extract
1	62	55	86	80	73	65	80
2	78	64	77	83	79	70	70
3	68	48	83	61	66	78	72
4	70	52	70	75	87	63	79

Triglyceride

N	Basal diet	High Cholesterol diet	High Cholesterol diet + drug	High Cholesterol diet + 100mg/kg extract	High Cholesterol diet + 200mg/kg extract	Basal diet + 100mg/kg extract	Basal diet + 200mg/kg extract
1	90	123	84	101	87	85	88
2	65	117	88	99	88	67	63
3	70	130	73	112	83	60	69
4	89	111	90	106	95	85	86

Antioxidants

Catalase

N	Basal diet	High Cholesterol diet	High Cholesterol diet + drug	High Cholesterol diet + 100mg/kg extract	High Cholesterol diet + 200mg/kg extract	Basal diet + 100mg/kg extract	Basal diet + 200mg/kg extract
1	16.95	17.7	19.89	12.65	11.36	15.5	17.03
2	17.2	12.2	26.44	12.71	12.39	15.77	17.46
3	17.8	12.59	21.02	12.76	13.39	14.07	16.5
4	18	10	18.99	12.09	12.02	14.41	18.51

Superoxide dismutase

N	Basal diet	High fat diet	High fat diet + drug	High fat diet + 100mg/kg extract	High fat diet + 200mg/kg extract	Basal diet + 100mg/kg extract	Basal diet + 200mg/kg extract
1	6.89	5.36	5.7	6.67	7.43	5.21	9.71
2	7.99	7.6	8.96	6.17	7.67	6.87	6.03
3	7.21	4.01	9.95	4.66	8.67	6.6	8.16
4	9.4	6.7	8.71	5.4	8.12	7.03	7.5

Glutathione peroxidase

N	Basal diet	High Cholesterol diet	High Cholesterol diet + drug	High Cholesterol diet + 100mg/kg extract	High Cholesterol diet + 200mg/kg extract	Basal diet + 100mg/kg extract	Basal diet + 200mg/kg extract
1	25.65	29.36	25.12	28.09	26.88	24.82	27.24
2	21.76	28.3	27.24	27.51	25.35	26.88	25.2
3	37.14	24.76	30.24	26.52	29.84	25.29	21.76
4	29.65	24.47	27.45	25.82	26.05	26.34	33.78

Malondyaldehyde

N	Basal diet	High Cholesterol diet	High Cholesterol diet + drug	High Cholesterol diet + 100mg/kg extract	High Cholesterol diet + 200mg/kg extract	Basal diet + 100mg/kg extract	Basal diet + 200mg/kg extract
1	28.67	51.00	37.40	42.76	38.00	26.51	26.51
2	25.23	38.7	33.3	41.45	36.67	32.08	24.05
3	32.10	42.34	30.01	43.25	37.05	24.23	31.11
4	27.40	44.60	27.45	41.25	38.25	27.40	25.30