**TITLE PAGE**

# ANTIOXIDANT POTENTIAL OF DIFFERENT TYPES OF TEA

**BY**

**EZE, CHINENYENWA KAYLA**

**(PG/M.Sc/14/76052)**

**A THESIS SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY IN PARTIAL FULFILMENT OF THE REQUIREMENTS OF THE UNIVERSITY OF NIGERIA, NSUKKA FOR THE AWARD OF MASTER OF SCIENCE (M.Sc.) DEGREE IN NUTRITIONAL BIOCHEMISTRY**

**SUPERVISORS: PROF B.C. NWANGUMA& DR. S.O.O. EZE**

**JULY, 2016**

## CERTIFICATION

EZE, Chinenyenwa Kayla, a postgraduate student of the Department of Biochemistry,University of Nigeria Nsukka, with Registration Number, PG/M.Sc/14/76052, hassatisfactorily completed the requirements for the award of the degree of Master of Science(M.Sc.) in NutritionalBiochemistry. The work embodied in this thesis is original and hasnot been submitted in part or full for any other diploma or degree of this or any otherUniversity.

**……** **…….**

**Prof. B.C. Nwanguma** **Dr. S.O.O. Eze**
**Supervisor** **Supervisor**

**……** **..……**

**Prof. O.F.C. Nwodo** **External Examiner**

**Head of Department**

## DEDICATION

This work is dedicated to the Almighty GOD.

## ACKNOWLEDGEMENTS

I must express my gratitude to every person and organizations that have played a part toward the success of this work. May the good GOD bless you.My deepest gratitude goes to my amiable supervisors Prof. B.C. Nwanguma and Dr. S.O.O. Eze, for their unreservedsupportconstructive criticism, commitment, guidance and counsel toward the success of this work; itôs a privilege to tap fromyour streams of knowledge. I must acknowledge the impact of my Head of Department Prof. O.F.C. Nwodoand my lecturers: Prof. I.N.E Onwurah, Prof. L.U.S Ezeanyika, Prof. E.O Alumanah, Prof. H.A Onwubiko, Prof. P.N Uzoegwu, Dr. Parker E. Joshua, Dr.V.N Ogugua, Dr(Mr) C.A Anosike and Mr E. Arinze had on me academically thank all of you.

My sincere appreciation to my friend, Engr. Patuwa Aaron Thank you for being the pillar I rest on when the journey seems tough. I must appreciate Amaka, Munachimso, Cosmos, Innocent, Daniel, Debby, Mrs Nnamani, Lanre, Chiamaka, Tochi, Ochekawo, David, Alpha, Prince, Christian, Joy, Madam Lovinah, Mrs Ogbuabor, Dr (Mrs) E.E Kainde and many others not mention.

I thank members of my family for being there for me, for constantly remembering me in their prayers and for believing in me when I cannot even believe in myself. Words cannot express how grateful I am to sister Onyeoma and husband who took it upon themselves to see that I attain this height.My Lord and all in all JESUS Christ I couldnôt have made it without you, may your name be glorified now and forever.

## TABLE OF CONTENTS

**LIST OF FIGURES**

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF TABLES

## LIST OF ABBREVIATION

BT = Black tea,

UT = Un-caffeinated tea,

GT = Green tea,

HT = Herbal tea

TFC = Total flavonoid content,

TTC = Total tannin content

TPC = Total phenol content

FRAP = Ferric reducing antioxidant power

DPPH = 2,2-Diphenyl-2-picrylhydrazyl

C= Catechin

GC= Gallocatechin

EC = Epicatechin

EGC = Epigallocatechin

ECG= Epicatechin gallate

EGCG = Epigallocatechin gallate

ROS = Reactive oxygen species

MDA = Malondialdehyde

SOD = Superoxide dismutase

CAT = Catalase

GPx = Glutathione peroxidase

GRx = Glutathione reductase

GSH = Reduced glutathione

GSSG = Oxidized glutathione

## ABSTRACT

The present study evaluated the phytochemical constituents and *in vitro* antioxidant potential of different types of tea namely; black tea, un-caffeinated tea, green tea and herbal tea. Radical scavenging capacities of the tea extracts were determined using 2,2-diphenyl-2-picrylhydrazyl (DPPH) assay. Total antioxidant activity was determined using ferric reducing antioxidant power (FRAP) assay. The results showed that the total flavonoid content (TFC) of green tea (215.61±48.83 QE/mg) wassignificantly ($p<0.05$) higher than that of un-caffeinated tea (184.32±33.62 QE/mg) and herbal tea (167.25±31.25 QE/mg). There was no significant ($p > 0.05$) difference between the TFC of un-caffeinated and herbal tea samples. However, the TFC of un-caffeinated and herbal tea samples were found to be significantly ($p<0.05$) higher than that of black tea (142.32±22.73 QE/mg). There was no significant ($p > 0.05$) difference in the total tannin content (TTC) of un-caffeinated tea (411.55±9.21 GAEmg/ml), green tea (406.83±22.71 GAEmg/ml) and herbal tea (402.74±13.2 GAEmg/ml). However, their TTC were found to be significantly ($p < 0.05$) higher than that of black tea (325.14±108 GAEmg/ml). The total phenol content (TPC) of green tea (124.81±79.05 GAEmg/ml) was found to be significantly ($p < 0.05$) higher than that of un-caffeinated tea (63.87±35.76 GAEmg/ml), black tea (51.81±8.90 GAEmg/ml) and herbal tea (15.78±13.02 GAEmg/ml). The antioxidant activity of black tea and herbal tea was found to be significantly ($p < 0.05$) higher than that of un-caffeinated tea. Green tea showed the least radical scavenging activity. A correlation between the antioxidant capacity and the phytochemical constituent of the teas was observed. A positive correlation ($r = 0.060$) was observed between the TTC and FRAP of the tea samples, however, a negative correlation ($r = -0.137$) was observed between the TTC and DPPH radical reducing power of the tea samples. A positive correlation ($r = 0.448$) was observed between the TFC and FRAP as well as between TFC and DPPH ($r = 0.347$) radical scavenging activities of the tea samples. These findings demonstrated that the green tea, black tea, un-caffeinated tea and herbal tea samples are rich in important phytochemicals such as flavonoids and tannins), and possess antioxidant potentials. However, the tea types vary in their content of antioxidants and in their antioxidant potential. Based on the FRAP assay, black tea had the highest antioxidant potential while green tea had the least. Conversely, based on the DPPH assay, black tea, un-caffeinated tea and green tea had equal antioxidant potential while herbal tea had the highest antioxidant potential.

# CHAPTER ONE

## INTRODUCTION

Oxygen is an element indispensable for life. When cells use oxygen to generate energy, free radicals are created as a result of cellular redox process which leads to ATP production by the mitochondria (Kabel, 2014). These products are called reactive oxygen species (ROS). Reactive oxygen species (ROS) is a collective term used for a group of oxygen-centred oxidants, which are either free radicals or molecular species capable of generating free radicals. Free radicals are generated from either endogenous or exogenous sources. Endogenous free radicals are generated from immune cell activation, inflammation, mental stress, excessive exercise, ischemia, infection, cancer and ageing. Exogenous free radicals result from air and water pollution, cigarette smoking, alcohol, heavy metals, certain drugs (cyclosporine, tacrolimus), industrial solvents, cooking and radiation. After penetration into the body, these exogenous compounds are decomposed into free radicals (Valko *et al*., 2007). Under normal physiologic conditions, nearly 2% of the oxygen consumed by the body is converted into reactive oxygen through mitochondrial respiration, phagocytosis, etc. However, free radicals play a dual role as both toxic and beneficial compound (Kunwar and Priyadesh, 2011). At low or moderate level, ROS exert beneficial effects on cellular responses and immune function. At high concentration, they cause oxidative stress, a deleterious process that can damage all cellular structures (Halliwell, 2007). Oxidative stress results from an imbalance between formation and neutralization of free radicals. For example, hydroxyl radical and peroxynitrite in excess can damage cell membranes and lipoproteins by a process called lipid peroxidation. This reaction leads to the formation of malondialdehyde (MDA) and conjugated diene compounds, which are cytotoxic and mutagenic. Lipid peroxidation occurs by a radical chain reaction, i.e once started; it spreads rapidly and affects a great number of lipid molecules (Halliwel, 2007; Valko *et al*., 2007). Oxidative stress plays a major part in the development of chronic and degenerative diseases such as cancer, arthritis, aging, autoimmune disorders, cardiovascular and neurodegenerative diseases. The human body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced *in situ*, or externally supplied through foods and/or supplements. Endogenous and exogenous antioxidants act as free radical scavengers by preventing and repairing damages caused by ROS, and therefore can enhance the immune system

and lower the risk of cancer and degenerative diseases (Valko *et al*., 2007). An Antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Antioxidants do so by terminating chain reactions initiated by free radicals, removing radical intermediates and inhibiting other oxidation reactions by being oxidized themselves. So, antioxidants are often reducing agents such as thiols or polyphenols (Duarte and Lunec, 2005). Hence, plants and animals contain various antioxidants, such as glutathione, vitamin C, and vitamin E; polyphenols as well as enzymes such as catalase, superoxide dismutase and peroxidases. A number of clinical studies reported that the antioxidant in fruits, vegetables, teas and wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases (Li *et al*., 1999; Zaveri 2006). Processed tea, which is one of the most popular beverages, is manufactured from the young tender leaves of the plant *Camellia sinensis* (Cabrera *et al*., 2003). Two types of tea products are most widely consumed; green and black tea. In both cases, it is the chemical composition of the tea shoots and the reactions that occur during processing that determine the nature of the finished product and its quality. Though most of the tea produced in the world can be classified as non-fermented/aerated green tea, semi-fermented (oolong) tea and fermented black tea (Reeves *et al*., 1987), processing and emerging technologies have led to the production of special teas e.g. white tea, flavoured teas, organic teas, decaffeinated teas, herbal teas, scented teas, un-caffeinated teas and various other blends. The tea beverage has continued to be considered medicinal since ancient times because of its polyphenols and there is already growing evidence that tea polyphenols reduce the risk of heart diseases, neurological disease, cancer and obesity in humans (Vanessa and Williamson, 2004). In some studies, tea has been associated with anti-allergic (Yamamoto *et al*., 2004) and antimicrobial properties (Paola *et al*., 2005). Study has demonstrated that the co-administration of drugs with catechins (EC and EGCG) inhibits glucoronidation and sulfonation of orally administered drugs thereby increasing the bioavailability of such drugs (Hang *et al*., 2003). Moreover, some epidemiological studies have associated consumption of tea with a lower risk of several types of cancer including those of the stomach, oral cavity, oesophagus and lungs, therefore, tea appears to be an effective chemo-preventive agent for toxic chemicals and carcinogens (Cabrera *et al.,* 2003; Hakim and Chow, 2004). Research on the effects of tea on human health has been fuelled by the growing need to provide naturally healthy diets that include plant-derived polyphenols. In line with this, there is need to elucidate how known functional components in foods could expand the role of diet in

disease prevention and treatment (Mandel *et al*., 2006). The ability to scavenge free radicals by tea polyphenols is due to possession of a phenolic hydroxyl group attached to the flavan-3-ol structure has been associated with teas therapeutic actions against free radical mediated diseases thereby attracting tremendous research interest (Amie *et al*., 2003). Many plant phenolics have been reported to have antioxidant properties that are even much stronger than vitamins E and C. In addition, currently available synthetic antioxidant like butylated hydroxyl anisole (BHA), butylated hydroxytoluene (BHT) and gallic acid esters have been suspected to cause or prompt negative health effects and hence the need to substitute them with naturally occurring antioxidants (Aqil *et al*., 2006; Pourmorad *et al.,* 2006). There is therefore an increased quest to obtain natural antioxidants with broad-spectrum action. The upsurge of free radical related diseases such as diabetes (type 2), cancer, neurodegeneration e.t.c have provoked the search for food sources with natural antioxidants. Few studies have been carried out using processed tea. Therefore, the present study evaluated the total polyphenols, total flavonoids and *in vitro* antioxidant activities of a set of twenty commercial Nigerian tea samples (black, green, un-caffeinated and herbal tea) using DPPH radical scavenging ability and the ferric reducing antioxidant power.

### 1.1 Antioxidants

An Antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Antioxidants do so by terminating chain reactions initiated by free radicals, removing radical intermediates and inhibiting other oxidation reactions by being oxidized themselves. So, antioxidants are often reducing agents such as thiols or polyphenols (Duarte and Lunec, 2005). Antioxidants are capable of stabilizing, or deactivating free radicals before they attack cells and are absolutely critical for maintaining optimal cellular and systemic health and well-being. Antioxidants are the first line of defence against free radical damage in living organism and are critical for maintaining optimum health and wellbeing. The need for antioxidants becomes even more critical with increased exposure to free radicals. Pollution, cigarette smoke, drugs, illness, stress, and even exercise can increase the chances of exposure to free radicals. Because so many factors can contribute to oxidative stress, individual assessment of susceptibility becomes very important. Many experts believe that the Recommended Dietary Allowance (RDA) for specific antioxidants may be inadequate and in some instances, the need may be several times higher than

the RDA. Antioxidant supplementation is now being recognized as an important means of improving free radical protection, in addition to healthy lifestyle and balanced diet (Mark, 1999).

### 1.1.1 Free Radical Production and Antioxidant Defence Mechanism

The ability to utilize oxygen has provided humans with the benefit of metabolizing fats, proteins, and carbohydrates for energy; however, it does not come without cost. Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called ñfree radicals.ò Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Free radicals have been implicated in the pathogenesis of at least 50 diseases (Langseth, 1993; Halliwell, 1994). Fortunately, free radical formation is controlled naturally by various beneficial compounds known as antioxidants. It is when the availability of antioxidants is limited that this damage can become cumulative and debilitating. However, nature have made provisions for both endogenous and exogenous antioxidant, in such a way that if the ratio of free radicals to endogenous antioxidant is on the rise, exogenous antioxidant can boost up the antioxidant level of biological system.

### 1.1.2 Endogenous and Exogenous Antioxidants

Endogenous antioxidant compounds in cells can be classified as enzymatic antioxidants and non-enzymatic antioxidants. The major antioxidant enzymes directly involved in the neutralization of ROS and RNS are: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GRx) (Genestra, 2007; Pham-Huy *et al*., 2008). SOD, the first line of defence against free radicals, catalyses the dismutation of superoxide anion radical ($O_2 \cdot^-$) into hydrogen peroxide ($H_2O_2$) by reduction. The oxidant formed is transformed into water and oxygen by catalase (CAT) or glutathione peroxidase (GPx). The GPx, a selenoprotein, removes $H_2O_2$ by using it to oxidize reduced glutathione (GSH) into oxidized glutathione (GSSG). Glutathione reductase, a flavoprotein, regenerates GSH from GSSG, with NADPH as a source of reducing power. Besides hydrogen peroxide, GPx also reduces lipid or non-lipid hydroperoxides while oxidizing glutathione (GSH) (Young and Woodside 2001; Bahorun *et al.,* 2006). The non-enzymatic antioxidants are also divided into metabolic antioxidants and nutrient antioxidants. Metabolic antioxidants, belonging to endogenous antioxidants, are produced by metabolism in the body. They include lipoic acid, glutathione, L-arginine, coenzyme Q10, melatonin, uric acid,

bilirubin and metal-chelating proteins such as ferritin, lactoferrin, transferrin e.t.c., While nutrient antioxidants, belonging to exogenous antioxidants, are compounds which cannot be produced in the body and must be provided through foods or supplements. They include vitamins C and E, carotenoids, flavonoids, omega-3 and omega-6 fatty acids, and trace metals such as selenium, manganese, zinc etc. (Droge, 2002; Willcox *et al.,* 2004).

### 1.1.3 Diet, Antioxidant and Health

There has been much interest in the mechanisms of actions of antioxidants which might explain the relationships between dietary quality and health status (Siex, 1997; Aruoma, 1999; Halliwell, 2006). For example, mixtures of fruit and vegetables can increase the antioxidant capacity of blood (Kawashima *et al.,* 2007). Antioxidants from our diet play an important role in helping endogenous antioxidants in the neutralization of oxidative stress. The nutrient antioxidant deficiency is one of the causes of numerous chronic and degenerative pathologies (zinc deficiency hypogonadism, selenium deficiency keshan disease cardiac myoparty). Each of the antioxidant nutrients is unique in terms of its structure and antioxidant function (Wilcox *et al.*, 2004).

#### 1.1.3.1 Flavonoids

Flavonoids are polyphenolic compounds which are present in most plants. Based on chemical structure, over 4000 flavonoids have been identified and classified into flavanols, flavanones, flavones, isoflavones, catechins, anthocyanins and proanthocyanidins. Beneficial effects of flavonoids on human health mainly reside in their potent antioxidant activity (Miller, 1996). They have been reported to prevent or delay a number of chronic and degenerative ailments such as cancer, cardiovascular diseases, arthritis, ageing, cataract, memory loss, stroke, Alzheimerôs disease, inflammation and infection. Every plant contains a unique combination of flavonoids, which is why different herbs rich in these substances have very different effects on the body (Hanneken *et al*., 2006). The main natural sources of flavonoids include green tea, grapes (red wine), apple, cocoa (chocolate), ginkgo biloba, soybean, curcuma, berries, onion, broccoli, etc. For example, green tea is a rich source of flavonoids, especially flavonols (catechins) and quercetin. Catechin levels are 4-6 times greater in green tea than in black tea. Many health

benefits of green tea reside in its antioxidant, anticarcinogenic, anti-hypercholesterolemic, antibacterial (dental caries) and anti-inflammatory activities (Pham-Huy *et al.,* 2008).

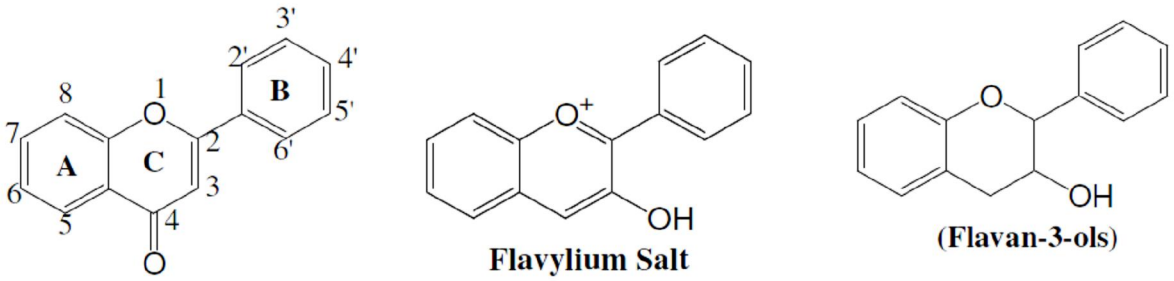


Fig. 1: General structures for various classes of flavonoid

Source: (Andrea *et al.,* 2003)

### 1.1.3.2 Tannins

The term tannin refers to the use of tannins in tanning animal hides into leather; however, the term tannins is widely applied to any large polyphenolic compound containing sufficient hydroxyls and other suitable groups (such as carboxyls) to form strong complexes with proteins and other macromolecules. They have molecular weights ranging from 500 to over 3000 **(**Bate-Smith, 1962**)**. Tannins are astringent, bitter plant polyphenols, the astringency from the tannins is what causes the dry and pucker feeling in the mouth following the consumption of red wine, strong tea, or an unripe fruit; the sensation apparently results from the interaction between tannin constituents and proteins of the saliva and/or the mucous tissue of the mouth (Ashok and Upadhyaya, 2012). Several groups of phenolic compounds, having the general properties of tannins as defined by Bate-Smith, are quite distinct from one another in terms of their chemical structure (Hagerman *et al*., 2005). Phytochemists have classified tannins into three main classes: condensed tannins (*i.e.*, proanthocyanidins) are flavanol-based compounds that release anthocyanidins at high temperatures in alcohol solutions or a strong mineral acid; gallotannins and ellagitannins belong to the family of hydrolysable tannins. Gallotaninns are comprised of galloyl esters of glucose or quinic acid whereas ellagitannins are derivatives of hexahydroxydiphenic acid (HHDP). Phloroglucinols are subunits of phlorotannins, which are present only in marine brown algae (Amarowicz 2007). The tea plant (*Camellia sinensis*) is a good example of a plant that has high tannin content. When any type of tea leaf is steeped in hot

water it brews a "tart" (astringent) flavor that is characteristic of tannins. This is due to the catechins and other flavonoids contained in the tea. Tea "tannins" are chemically distinct from other types of plant tannins such as tannic acid **(**Hamilton-Miller**,** 1995**)** and tea extracts have been reported to contain no tannic acid (Ashok and Upadhyaya, 2012). Black tea and peppermint tea are inhibitors of iron than herb teas like chamomile, vercain, lime flower and pennyroyal (Hurrell *et al*., 1999). Tannins have also been considered a bioactive compound with health promoting potential in plant derived foods and beverages. For instance, tannins have been reported to possess anticarcinogenic and antimutagenic potentials as well as antimicrobial properties. Several studies have reported on the antioxidant and antiradical activities of tannins (Amarowicz, 2007). Tannins do not function solely as primary antioxidants (*i.e.*, they donate hydrogen atom or electrons), they also function as secondary antioxidants. Tannins have the ability to chelate metal ions such as Fe(II) and interfere with one of the reaction steps in the Fenton reaction and thereby retard oxidation (Karamac *et al.,* 2006). The inhibition of lipid peroxidation by tannin constituents can act *via* the inhibition of cyclooxygenase (Zhang *et al.,* 2004).

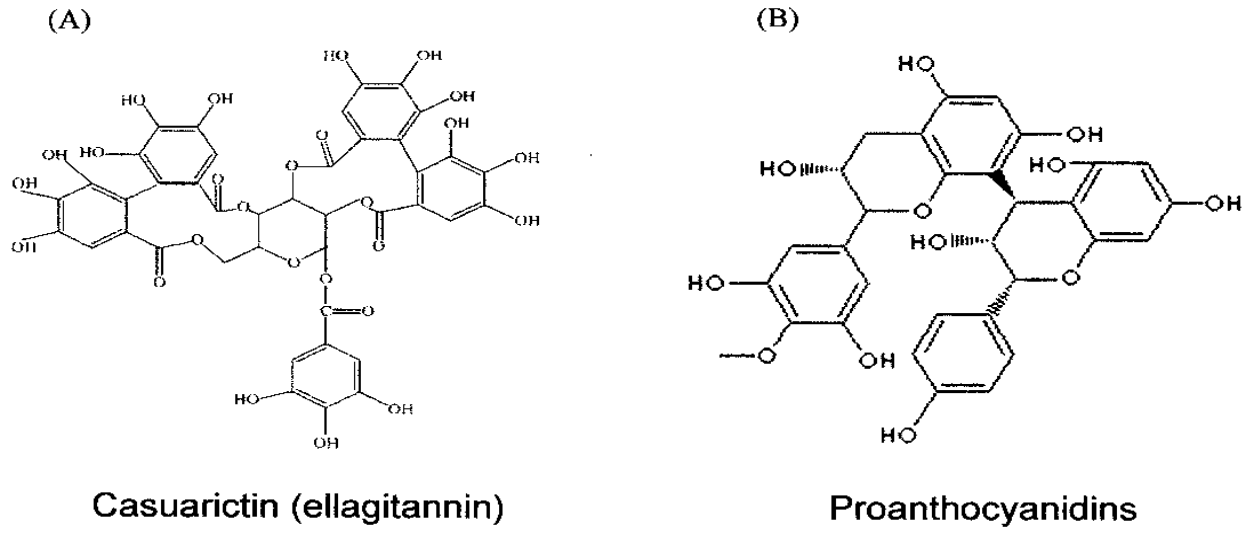


Fig. 2: Chemical Structures of Hydrolysable tannin (A: Ellagitannin) and condensed tannin (B: Proanthocyanidin)

Source: (Karamac *et al*., 2006)

### 1.1.3.3 Ascorbic Acid

Ascorbic acid (vitamin C) is widely known for its antioxidant activity and is therefore used in cosmetics and degenerative disease treatments. Vitamin C has many physiological functions, among them a highly antioxidant power to recycle vitamin E in membrane and lipoprotein/lipid peroxidation. Paradoxically, however, it should also be noted that, *in vitro*, vitamin C is also capable of pro-oxidant activity. It has long been known that the combination of ascorbate and ferrous ions generates hydroxyl radicals, which induces lipid peroxidation (Shekelle, 2003). Vitamin C is a potent antioxidant for hydrophilic radicals, but poor against lipophilic radicals.

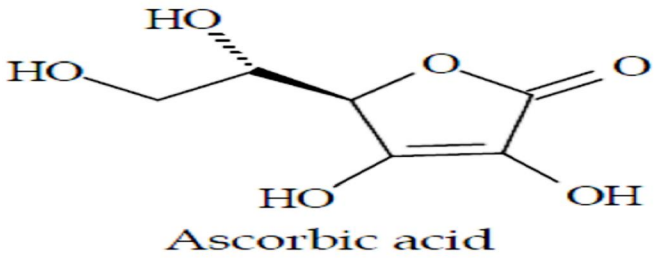


Fig 3: Structure of Ascorbic Acid

Source: (Shekelle, 2003)

### 1.1.3.4 Carotenoids

Carotenoids are group of tetraterpenoids. The basic carotenoid structural backbone consists of isoprenoid units formed either by head-to-tail or by tail-to-tail biosynthesis. There are primarily 2 classes of carotenoids: carotenes and xanthophylls. Carotenes are hydrocarbon carotenoids and xanthophylls contain oxygen in the form of hydroxyl, methoxyl, carboxyl, keto, or epoxy groups. Lycopene and ɓï carotenes are typical carotenes whereas lutein in green leaves and zeaxanthin in corn are typical xanthophylls. The structures of carotenoids are acyclic, monocyclic, or bicyclic. For example, lycopene is acyclic, ï carotene is monocyclic, and Ŭ and ɓï carotenes are bicyclic carotenoids (Rumsey *et al*., 1999). Double bonds in carotenoids are conjugated and trans forms of carotenoids are found in plant tissues. Epidemiological studies have revealed that an increased consumption of a diet rich in carotenoids is correlated with a lower risk of age-related diseases (Glaser *et al*., 2015; Indaser *et al.,* 2015). Carotenoids (Fig. 3) contain conjugated double bonds and their antioxidant activity arises due to/ their ability to delocalize unpaired electrons (DeMan, 1999). This is also responsible for the ability of carotenoids to physically quench singlet oxygen

without degradation and for the chemical reactivity of carotenoids with free radicals. The efficacy of carotenoids for physical quenching is related to the number of conjugated double bonds present in the molecule.

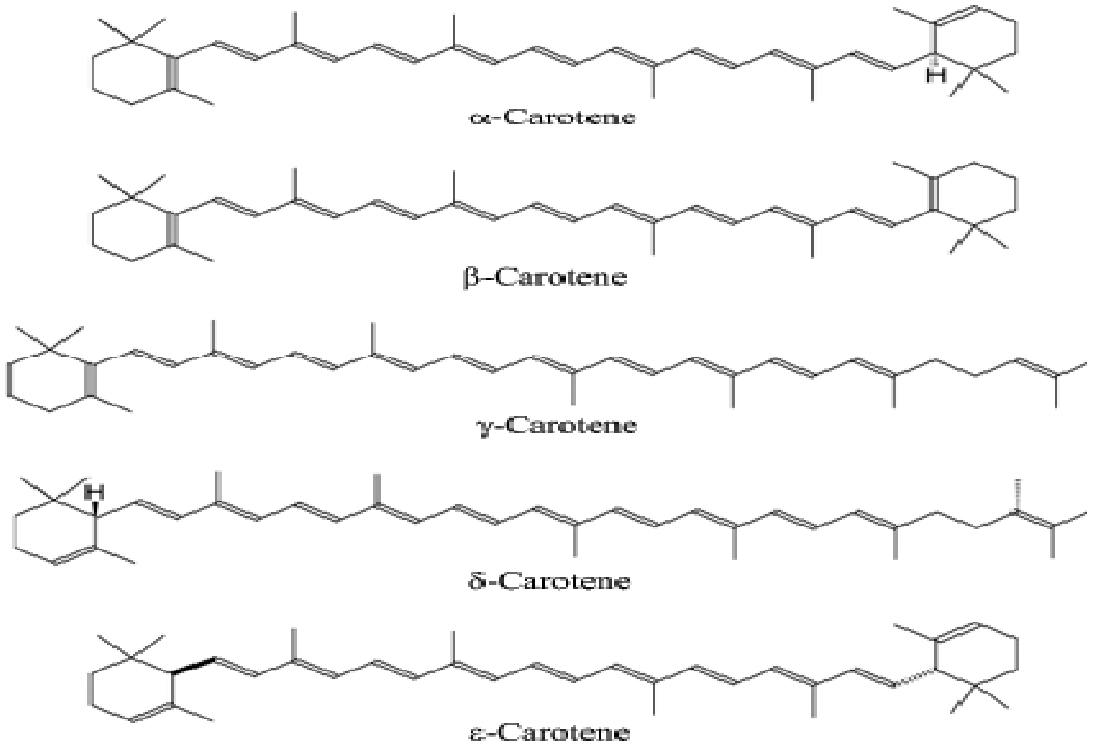


Fig. 4: Structures of Carotene Series
Source: (Jose *et al*., 2008)

**1.1.3.5 Vitamin E**

Vitamin E is a group of eight antioxidant lipophilic molecules, four of which are tocopherols and four others are tocotrienols. It is mostly found in green vegetables, grains, nuts and various vegetable oils, as well as in eggs and milk. Although it is commonly known today for its antioxidant properties, the first biological role attributed to vitamin E was its necessity for fetal survival. Vitamin E is known to possess many biological properties, including antioxidant activity and the ability to modulate protein function and gene expression (Farbstein *et al.,* 2010). All vitamin E compounds are lipophilic. The lipophilicity of the compounds is attributed to their hydrophobic tail, a saturated phytyl chain in the tocopherols and an unsaturated phytyl chain in the tocotrienols. The antioxidant activity is attributed to the chromanol group, whose methylation

differs among members of the vitamin E group. $\alpha$-tocopherol, which is the most abundant vitamin E *in vivo*, is methylated on the 5$^{th}$, 7$^{th}$ and 8$^{th}$ carbon of the chromanol ring (Niki, 2001). Being a lipophilic molecule, vitamin E is most abundant in lipid phase compartments such as the plasma membrane and lipoproteins. It is also found in the membranes of cellular organelles and majorly in the lysosome and the Golgi membrane, where its concentration is more than ten times higher than in other membranes (Zhang *et al*., 1996). Vitamin E is classified as an antioxidant due to its ability to scavenge lipid radicals and terminate oxidative chain reactions. It can terminate radical chain reactions by interacting with the lipid peroxyl radical, preventing it from generating a new radical and perpetuating the chain reaction (oxidizing other lipids). This is due to the rate constant of the reactions between lipids and lipid peroxyl radicals, which are 1,000-fold lower than the rate constant of the reaction between $\alpha$-tocopherol and lipid peroxyl radicals (102 $M^{-1}S^{-1}$ compared to 105‑106 $M^{-1}S^{-1}$). It is unlikely that vitamin E would interfere with the radical chain reaction in other stages. The radical chain reaction is usually initiated by water soluble molecules, where vitamin E is sparse due to its lipophilic nature. Its interaction with lipid radicals is unlikely since the rate constant of the reaction between lipid radicals and oxygen is 100-1,000-fold higher compared to that of lipid radicals and vitamin E. Following its oxidation, vitamin E can be recycled back to its native unoxidized form by various soluble antioxidants such as vitamin C and ubiquinol. This process prevents the accumulation of vitamin E radicals and their subsequent peroxidation of lipids (Brigelius-Flohe, 2009), and is considered by some to be critical for the antioxidant activity of vitamin E (Carr *et al.,* 2000). It has been suggested that all other biological functions of vitamin E are due to its antioxidant activity (Traber and Atkinson, 2007). Antioxidant has been classified as a nutraceutical and tea contain bio-compounds which possess antioxidant activity.

### 1.2 Mechanism of Action of Antioxidant

The role of antioxidants in nutrition and health, as well as their mechanisms of action, have been extensively researched (Serafini, 2006). Although the biological functions of polyphenols and their metabolism in the human body are not completely established, there is a consensus that the antioxidant activity of flavonoids could be a combination of metal chelating properties and free radical scavengers (Bohm *et al*., 1998; Bravo, 1998). Other authors also refer to the inhibition of oxidases, such as lipoxygenase (LO), cyclooxygenase

(CO), myeloperoxidase (MPO), NADPH oxidase and xanthine oxidase (XO) as important mechanisms for avoiding the generation of higher amounts of ROS *in vivo* as well as organic hydroperoxides (Groot and Rauen, 1998). Moreover, they have been also known to inhibit enzymes indirectly involved in the oxidative processes, such as phospholipase A2 (Lindahl and Tagesson, 1997). They stimulate antioxidant enzyme activities such as catalase (CAT) and superoxide dismutase (SOD) (Sudheesh *et al.*, 1999). Therefore, antioxidants interfere with the propagation reactions of free radicals and the radical formation (Van *et al.,* 1996). The chemical structure of polyphenols gives them the ability to act as free radical scavengers. The type of compound, the degree of methoxylation and the number of hydroxyl groups are some of the parameters that determine the antioxidant activity. As for phenolic acids, the oxidation inhibition is related to the chelation of metal ions via the *ortho*-dihydroxy phenolic structure, the scavenging of alkoxyl and peroxyl radicals, and the regeneration of Ŭ-tocopherol through reduction of the tocopheryl radical (Bors *et al*., 1990). The structural features that have been associated with antioxidant activity are: a catechol group on the B-ring, which confers high stability to the radical formed after the capture reaction of the free radical, the 2,3-double bond in conjugation with a 4-oxofunction of a carbonyl group in the C-ring and the presence of hydroxyl groups at the 3 and 5 position as described in (Fig. 4) (Bourne and Rice-Evans, 1998).

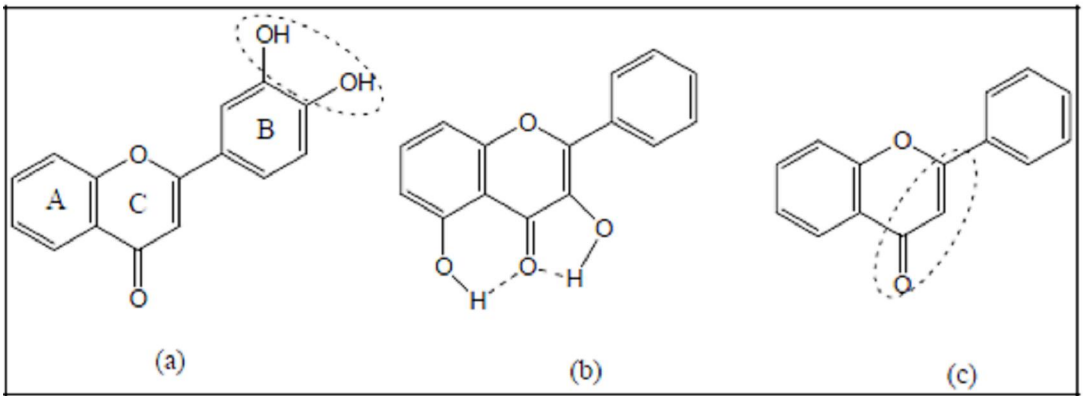


Fig. 5: Antioxidant activity-structure relationship of flavonoids.

(a) a catechol moiety of the B-ring,
(b) presence of hydroxyl groups at the 3 and 5 position,
(c) the 2,3-double bond in conjugation with a 4-oxofunction of a carbonyl group in the C-ring.

Source: (Bourne and Rice-Evans, 1998)

Studies on their metabolism established that flavonoids are located in the membrane at the lipid/water interface, being the first to react with the ROS formed in these areas. As part of this, they can act directly as "scavengers" of free radicals, by hydrogen or electron donation, leading to other more stable compounds, or compounds that can stabilize compounds obtained from free radicals or may have an additive effect on the endogenous antioxidant defence system by increasing or maintaining this antioxidant defence. These mechanisms are also the same for phenolic acids. To evaluate the antioxidant capacity *in vivo* changes in the levels of biomarkers could be used to assess the level of oxidative stress

### 1.2.1 Antioxidant and Oxidative Stress Biomarkers

Biomarkers are essential for assessment of oxidative stress and evaluation of antioxidant capacity *in vivo*. The observed changes in the levels of biomarkers may have additional explanations to antioxidant and its health implication. Products of free radical-mediated damage to lipids, protein and DNA have been identified in biological materials such as plasma, urine and blood cells and are proposed as biomarkers for oxidative damage, these biomarkers can be used to analyse the protective effects of dietary antioxidants *in vivo* (Rietveld and Wiseman, 2003). The biomarker approach has been applied in a number of human intervention trials investigating the biological antioxidant effects of tea and tea flavonoids. Lipids, in particular polyunsaturated fatty acids and their esters are susceptible to oxidation and their oxidation products may serve as appropriate biomarkers (Nikki, 2001). Fatty acids and their esters and cholesterol are important lipid substrates. They are oxidized by three distinct mechanisms: enzymatic oxidation and non-enzymatic, free radical-mediated oxidation and non-enzymatic and non-free radical oxidation. F2-isoprostanes (isoP) and neuroprostanes which consist of series of chemically-stable prostaglandins F2-like compounds formed from arachidonates by a mechanism independent of cyclooxygenase pathway have been widely accepted as one of the most reliable biomarkers of oxidative stress (Roberts et al., 2005). Furthermore, total hydroxyoctadecadienoic acid (HODE) and 7-hydroxycholesterol measured after reduction and saponification of biological fluids may be used as reliable biomarker. Black tea and green tea are powerful *in vitro* antioxidants and effectively protect LDL from oxidation *in vitro*. However, intervention trials to determine the effect of black and green tea on *ex vivo* LDL oxidation in humans have failed to demonstrate a consistent effect of tea (Roberts *et al.,* 2005). Tea is the second most consumed beverages in the

world today however processing method have distinguished the tea beverage into green tea, black tea, white tea, oolong tea, herbal tea, and even differently flavoured tea such as peppermint, ginger spiced e.t.c.

### 1.3 Types of Tea and their Chemical Composition

#### 1.3.1 Green Tea

Green tea is obtained from the tea plant *Camellia sinensis* which belongs to the family Theaceae. Green tea is a 'non-fermented' tea and contains more catechins than black tea or oolong tea. Catechins are *in vitro* and *in vivo* strong antioxidants. In addition, it contains certain minerals and vitamins which increase the antioxidant potential of this type of tea.

#### 1.3.2 Green Tea Composition

The chemical composition of green tea includes complex protein which constitute an important fraction of amino acids such as theanine or 5-*N*-ethylglutamine, glutamic acid, tryptophan, glycine, serine, aspartic acid, tyrosine, valine, leucine, threonine, arginine and lysine. The carbohydrate contents include cellulose, pectins, glucose, fructose and sucrose, while the lipid contents are linoleic and $\alpha$-linolenic acids. It also contains sterols such as stigmasterol. The vitamin contents include vitamins B, C and E. It possesses xanthic bases such as caffeine and theophylline (Huang, 1999). It is rich in pigments such as chlorophyll and carotenoids and also volatile compounds like aldehydes, alcohols, esters, lactones and hydrocarbons. Minerals and trace elements such as Ca, Mg, Cr, Mn, Fe, Cu, Zn, Mo, Se, Na, P, Co, Sr, Ni, K, F and Al are present in green tea (Scholz and Bertram, 1995; Meyer Buchtela, 1999). Polyphenols constitute the most interesting group of green tea leaf components. Tea leaf polyphenols such as catechins, include (+)-catechin (C), (+)-gallocatechin (GC), (ï)-epicatechin (EC), (ï)-epigallocatechin (EGC), (ï)-epicate-chin gallate (ECG), and (ï)-epigallocatechin gallate (EGCG) (Miketova *et al.,* 1998). See appendix II for structures. In consequence, green tea can be considered an important dietary source of polyphenols, particularly flavonoids. Flavonoids are phenol derivatives synthesized in substantial amounts and variety (more than 4000 identified), and widely distributed among plants. Research showed that green tea has flavonols (9ï 10%), other

flavonoids (2ï 4%), xanthine alkaloids (7ï 9%), minerals (6ï 8%), amino acids (4ï 6%), organic acids (4ï 6%), and ascorbic acid (1ï 2%) (Graham, 1992).

### 1.3.3 Black Tea

Black tea is the young leaf of *Camellia sinensis* it accounts for approximately 72% of the worldôs total tea production when fully fermented, and subjected to rapid desiccation with applied heat. It contains no less than 2.5% caffeine (Bruneton, 1999), and must contain no less than 25% water-soluble extractive on a dry basis (Health Canada, 1997). While most of the EGCG antioxidants are oxidized during the fermenting process, black tea retains a high number of the antioxidants polyphenols such as flavonoids. These antioxidants help rid the body of harmful toxins (Health Canada, 1997).

### 1.3.4 Chemical Composition of Black Tea

Black tea leafs contains polyphenols: Polyphenolic compounds including catechins in monomeric, dimeric and oligomeric form such as catechins epicatechin (EC), epigallocatechin (EGC), epigallocatechingallate (EGCG), epicatechingallate (ECG) see appendix II for structures, After fermentation from green tea to black tea, about 15% of the catechins remain unchanged and the rest convert into theaflavines and thearubigins (Bronner and Beecher, 1998). Other flavonoids including (myricetin, quercetin and kaempferol, etc.), amino acids (including l-theanine), methylxanthines such as xanthine alkaloids: caffeine (2.6ï 3.5%), theobromine (0.16ï 0.2%), theophylline (0.02ï 0.04%), carbohydrates, proteins, and minerals. Black tea is also considered as a dietary source of antioxidant nutrients like carotenoids, tocopherols, and minerals such as chromium, manganese, selenium, zinc and a significant amount of aluminium, fluoride, and potassium (Scholz and Bertram, 1995; Meyer-Buchtela, 1999). The components of prepared black tea infusion measured in weight % of extracted solids include catechins (3ï 10%), thearubigins (12ï 18%), theaflavines (3ï 6%), flavonols (6ï 8%), phenolic acids (10ï 12%) and depsides, xanthine alkaloids (8ï 11%), amino acids (13ï 15%),and minerals (10%) (Graham, 1992). These compounds impact the health benefits associated with black tea consumption.

### 1.3.5 White Tea

White tea is an unfermented tea made from young tea leaves or unopened buds covered with tiny, silvery hairs, and the leaves are harvested once a year in the early spring. The leaves are then steamed rapidly and dried, with a minimum amount of processing to prevent oxidation (Hilal and Engelhardt, 2007). Green, oolong and black teas are processed to a greater extent compared to white tea, for this reason white tea retains the greatest levels of antioxidants and the lowest levels of caffeine than any other tea from the *Camellia sinensis* plant (green, black or oolong); though green tea is also an un-fermented.

### 1.3.6 Pu'erh Tea

Pu-erh tea, produced mainly in Yunnan Province of China, is well known for its special flavor and potential health benefits to human beings. Almost all pu-erh tea is produced from the fresh leaves, specifically, the fresh leaves of *Camellia sinensis* (L.) var. *assamica* of artea. Pu-erh tea is a traditional beverage in Hongkong, Taiwan as well as many other areas in Southeast Asia. Sano *et al*. (1986) reported that Pu-erh tea significantly reduced the plasma cholesterol ester and triacylglycerol levels in rat plasma and the similar results have been reported by Miura *et al*. (1995). It was commonly believed that the longer the preservation period, the better the quality and taste of Pu-erh tea. This type of tea comes from a large leaf variety of tea plant and can be picked any time of the year. Its processing is similar to that of black tea. What makes this tea unique is that once it is picked; it is piled and aged for as long as 50ï 100 years.

### 1.3.7 Rooibos (*Aspalathus linearis*)

Rooibos or *Aspalathus linearis* (also *A. contaminata*, *Acorymbosus*, *Borbonia pinifolia* or *Psoralea linaeris*) is a shrub-like leguminous bush native to the Cedarberg Mountains in the Western Cape region of South Africa where it is extensively cultivated for its commercial use as an herbal tea or tisane. After harvesting, the needle-like leaves and stems can be either bruised and fermented prior to drying or dried immediately. The unfermented product remains green in colour and is referred to as green rooibos. During fermentation, the colour changes from green to red with oxidation of the constituent polyphenols, so the final product is often referred to as red tea or red bush tea. Other names include rooibos tea, rooibosch, rooitea or rooitee.

### 1.3.8 Chemical Composition of Rooibos Tea

Brewed rooibos tea (1 tsp/cup) contains 300 mg protein, the amounts of Cu, Fl and Mn present in this single serving provides 7.8%, 5.5ï 7.3% and 1.7ï 2.2% of the U.S. Daily Value (DV), respectively (Erickson, 2003) while Ca, Fe, K, Mg, Na, $P_{O4}$ and Zn (Morton, 1983; Erickson, 2003). Vitamin C is present at Ò15.7 mg/100 mg (Rertyjkk, 1983). According to Habu *et al.* (1985), 99 compounds are present in the volatile oil of rooibos tea. The major components include guaiacol (24.0%), 6-methyl-3,5-heptadien-2-one isomer (5.2%), damascenone (5.0%), geranylacetone (4.2%), ß-phenylethyl alcohol (4.1%) and 6-methyl-5-hepten-2-one (4.0%). Compared with the volatiles present in green and black teas, the concentration of guaiacol in rooibos was much higher, while linalool was moderately lower and geraniol was absent. Kawakami *et al.* (1993) confirmed the presence of these components (except for damascenone) in a later study, although the quantities determined in their analysis varied from those reported by Habu *et al.* (1985).

### 1.3.9 Chamomile Tea

Chamomile belongs to daisy family *Asteraceae* or *Compositae*. Two varieties are widely known; *Matricaria chamomilla* known as wild chamomile and Roman chamomile considered as common species. Both types of chamomiles are used in traditional medicine and herbalism (Hansen and Christensen, 2009). *Matricaria chamomilla* is the most popular source of chamomile. Herbal teas are admired all around the globe and about a million cups of chamomile tea are used on a daily basis in which chamomile flower powder is either pure or blended with medicinal herbs popular for same use. As a tea in larger doses it can be used as sedative, to treat insomnia and in other nervous conditions (Saira *et al*., 2000).

## 1.4 Tea as a Nutraceuticals

Nutraceutical is any substance that is a food or a part of food and provides medical or health benefits, including the prevention and treatment of disease. Such products may range from isolated nutrients, dietary supplements and specific diets to genetically engineered foods and herbal products (De Felice, 1995; Rishida *et al*., 2009). The food products used as nutraceutical are categorized as dietary fibre, probiotics, prebiotics, polyunsaturated fatty acids, spices, polyphenols and antioxidant compounds (Kokate *et al*., 2002). Tea is one of the popular natural

health drinks that contain variety of bioactive compounds, such as tea polyphenols, caffeine, tea pigment, tea polysaccharides, tea saponin, theanine and other functional components (Narotzki *et al*., 2012). Some reports showed that green tea non-nutrient bioactive compounds have antioxidant, anticancer, anti-obesity and other pharmacological functions, making it an excellent candidate for nutraceutical applications (Basu *et al*., 2013; Liang *et al*., 2014). The major hypothesis of the beneficial health effects of tea is associated with its antioxidant properties (Su *et al*., 2007). However, it was determined that theaflavins in black tea and catechins in green tea are equally effective as an antioxidant (Leung *et al*., 2001; Stewart *et al*., 2005). Green tea is rich in phytochemical such as polyphenols (catechins and gallic acid). It also contains carotenoids, tocopherols, ascorbic acid (vitamin C) and minerals such as chromium, manganese, selenium and zinc. They may also function indirectly as antioxidants through: inhibition of the redox-sensitive transcription factors, inhibition of ópro-oxidantô enzymes such as inducible nitric oxide synthase, lipoxygenases, cyclooxygenases and xanthine oxidases. They may also act through induction of antioxidant enzymes such as glutathione-transferases and superoxide dismutases. Using the oxygen radical absorbance capacity (ORAC) assay it was found that green tea has a much higher antioxidant activity against peroxyl radicals than vegetables such as garlic, kale, spinach and Brussels sprouts. Nevertheless, a substantial number of human intervention studies with green tea demonstrate a significant increase in plasma antioxidant capacity in humans after consumption of moderate amounts. There are also initial indications which shows that the enhanced blood antioxidant potential leads to a reduced oxidative damage in macromolecules such as DNA and lipids (Xu *et al*., 2004; Thasleema, 2013). Antioxidant neutralise free radicals by a specific mechanism.

### 1.5 Tea Production in Nigeria

Tea *Camellia sinensis* (L.) was introduced to Nigeria from Kenya in 1972 by Nigerian Beverage Production Company (NBPC). Ten years later tea breeding started with acquisition of 33 clones by Cocoa Research Institute of Nigeria from NBPC. Currently, tea is only grown successfully in Mambilla plateau on an average of 950 ha. Efforts have so far been made to adapt tea to lowland areas of Nigeria such as Ibadan (Oyo State), Ikom (Cross River Estate), Ikorodu (Lagos State) and Mayo-Selbe in (Taraba State) where clones 143, 318, 236 and 357 are promising. Generation of tea planting materials *in vitro* through tissue culture was seriously explored. Information regarding the hybridization of tea is limited. However, vegetative means of propagating tea and

selection pressure for high yielding clones have posed serious problem for genetic erosion which may lead to loss of valuable genetic traits. There is no information whatsoever, on the improvement of tea at DNA level in Nigerian tea germplasm. Therefore, there is urgent need for germplasm collection both from within and outside Nigeria of leading tea clones, hybridization and tea improvement at molecular level in Nigeria. Tea is important in the national economies of some African countries such as Cameroon, Kenya, Malawi and Tanzania (Omolaja and Esan, 2005). Conversely, in Nigeria the production has not been sufficient to satisfy the demand from indigenous processing companies and so cannot contribute to International market. However there are potentials for Nigeria to improve on her current production level. Tea production can contribute immensely to food security and poverty alleviation in Nigeria if adequate attention is given to the improvement of tea production.

**Fig. 6: Mambilla plateau, Taraba state, Nigeria**

Source: Olaniyi *et al*. (2014)

Tea is only grown commercially on the top of Mambilla plateau covering less than 1% of Nigerian land mass. Fig. 6 above shows the area were tea is currently produced in Nigeria.

## 1.6 Bioactivities of Tea

### 1.6.1 Tea and Metabolism, Obesity and Body Fat

Tea catechins can provide modest shifts in metabolism, which may improve weight loss and maintenance (Murase *et al*., 2006; Venables *et al*., 2008; Hursel 2011). Several studies suggest that drinking calorie-free tea may help in weight management. Based on the understanding that it helps elevate metabolic rate, increase fat oxidation and improve insulin activity (Dulloo *et al*., 1999; Murase *et al*., 2005; Shimotoyodome *et al*., 2005; Venables *et al*., 2008; Hursel *et al*., 2011). Furthermore, Vermerelli and Lambert (2012) re-examined the National Health and Nutrition Examination surveys of 2003ï 2006 and discovered that a correlation exist between hot tea consumption and lower mean waist circumference and lower BMI among adult tea drinkers versus non-tea drinkers. In addition, hot tea drinkers had higher HDL, lower C-reactive protein and women had lower level of triacylglycerol.

Green tea extract was found to significantly increase 24-hour energy expenditure and fat oxidation in healthy men (Dulloo *et al*., 1999). In a meta-analysis including six published tea research studies, 24- hour energy expenditure increased by 4.7% or 102 calories over 24 hours with a catechin-caffeine mixture and fat oxidation increased during the same period, revealing that tea may aid weight loss (Hursel *et al*., 2011). In another meta-analysis of 11 published clinical studies, catechins or epigallocatechin gallate (EGCG)-caffeine mixture have a modest positive effect on weight loss and weight maintenance (Hursel *et al*., 2009). Nagao *et al*. (2007) found that in a 12-week, double-blind and placebo-controlled study, green tea catechins led to a reduction in body fat, blood pressure and LDL compared to the control group. The authors also suggested that green tea catechins may help prevent obesity and reduce risk for cardiovascular disease. A follow-up study of the effects of tea catechins on body fat reduction in humans was conducted by examining the effect of drinking Oolong Tea with added Green Tea extract in healthy, moderately overweight men.

A double-blind study was performed in which the test subjects ingested one bottle of Oolong tea containing 690 mg of catechins and control subjects ingested one bottle of Oolong tea containing 22 mg of catechins for 12 weeks. Result that body weight, body mass index (BMI), waist circumference, body fat mass and subcutaneous fat were all significantly lower in the high

catechin-ingesting group as compared to the control group. It was concluded that daily consumption of 690 mg of catechins, the equivalent of five cups of strong green tea, might be useful in preventing and improving obesity (Nagao *et al.,* 2005). Green tea catechins were found to decrease fat storage and help keep adiponectin levels normal on a high-fat diet (Tian *et al.,* 2013). The discovery that supplementation with tea catechins resulted in a significant reduction of high-fat diet-induced body weight gain and visceral and liver fat accumulation (Murase *et al.*, 2002).

Researchers compared the body weight and fat mass of mice that were fed a low-fat or high-fat diet, with swimming or not, and with or without tea catechins. They found that, when fed a high-fat diet, tea catechins helped reduce fat accumulation by 18 percent and exercise alone reduced accumulation by 14 percent. However, mice that exercised and had catechins reduced fat accumulation by 33 percent. This evidence suggests that tea catechins may increase fat metabolism, enabling the body to burn more fat as fuel and store less in the body (Murase *et al.*, 2002). In another study, animals fed a high-fat, high-calorie diet to promote excessive weight gain and obesity were given green tea extract or placebo and their energy expenditure and fat oxidation were measured. They found that green tea extract alone, as well as when combined with exercise, increased energy expenditure and stimulated fat catabolism.

It was concluded that Green Tea extract combined with regular exercise stimulates fat metabolism and may attenuate obesity caused by a high-fat diet more effectively than green tea extract or exercise alone (Shimotoyodome *et al*., 2005). Also, animals fed a diet high in catechin-rich Tea extract were found to increase running times to exhaustion by up to 30 percent compared to a control animal. In addition, green tea extract appeared to shift metabolism so that the animals burned body fat and spared muscle glycogen, thereby increasing endurance time to exhaustion (Murase *et al*., 2006).

### 1.6.2 Role of Tea in Weight Loss

Recent studies suggest that catechins in promotes weight loss (Kao *et al*., 2000). In an animal study by Sayama *et al*. (2000), the antiobesity effect of green tea was evaluated by feeding different levels of green tea (from 1% to 4% of their diets) to female mice for 4 months. The results showed that the mice fed green tea in their diets had a significant suppression of food

intake, body weight gain, and fat tissue accumulation. In addition, levels of cholesterol and triacylglycerol were lower. Perhaps the most interesting finding from the study was that serum leptin levels showed a decrease, indicating that green tea may have a direct beneficial effect leading to weight loss. In some studies, green tea was associated with a mild increase in thermogenesis (increased caloric expenditure), which is generally attributed to its caffeine content (Dulloo *et al*., 2000 and Raymond *et al*., 2005). A study showed that green tea extract stimulates thermogenesis to an extent that is much greater than can be attributed to its caffeine content, meaning that the thermogenic properties of green tea may be due to an interaction between its high content of catechin polyphenols with caffeine. A probable theory for the thermogenic effect of green tea is an increase in levels of norepinephrine, because catechin polyphenols are known to inhibit catechol-O-methyltransferase, the enzyme that degrades norepinephrine. A randomized, placebo-controlled study of 10 individuals was conducted to investigate whether a green tea extract could increase energy expenditure and fat oxidation in humans over 24 hours (Dulloo *et al.,* 1999). Compared to placebo, the green tea extract resulted in a significant (4%) increase in energy expenditure and a significant decrease in respiratory quotient with no change in urinary nitrogen. Twenty-four-hour urinary excretion of norepinephrine was higher during treatment with the green tea extract than with placebo (Cooper *et al.,* 2005)

### 1.6.3 Effect of Tea on Absorption of Metal Ion

Tea catechins can affect iron absorption, particularly in groups at risk of iron deficiency (Samman *et al*., 2001; Nelson and Pauller, 2004). But their effects on other ions are poorly understood. Green tea ingestion over a long period does not affect the apparent absorption of copper, whereas it decreases that of zinc and increases that of manganese (Deng *et al*., 1998). However, catechin intake does not affect the plasma concentration of these ions (Record *et al.,* 1996). Green tea catechins have the potential of affecting absorption and metabolism of ions, because catechins (flavonoids) interact with a variety of metal ions (Mira *et al.,* 2002).

### 1.6.4 Tea, Diabetes and Blood Sugar Control

Type 2 diabetes is considered a global epidemic (American Diabetes Association 2012). Catechins in tea have been shown to help reduce blood sugar and provide insulin-boosting

activity, which may be beneficial for people with both type 1 and type 2 diabetes, although further research is needed (Anderson and Polansky 2002; Stote and Baer, 2013; Zheng *et al.,* 2013). Some studies suggest a link between drinking tea and a reduced risk of Type 2 diabetes (Stote and Baer, 2013). Zheng *et al*. (2013) in a meta-analysis of randomized controlled trials which include 22 studies and 1,584 subjects found that green tea catechins (with or without caffeine) provided a reduction in fasting blood glucose.

Some human clinical studies show that tea and its components improved glucose metabolism and endothelial function (Stote and Baer, 2013). Researchers at the United States Department of Agriculture (USDA) conducted a study to examine the insulin-enhancing properties of tea and its components; an *in vitro* test using a fat cell assay found that tea, as normally consumed, increased insulin activity by15-fold. Green, black and Oolong tea all demonstrated insulin-increasing activity. They separated the components of the tea using a high-performance liquid chromatography and discovered that several known compounds found in tea were shown to enhance insulin activity by helping cells to recognize and respond to the hormone. The greatest activity was elicited by EGCG, followed by epicatechin gallate, tannins and theaflavins (Anderson and Polansky, 2002).

### 1.6.5 Effect of Tea on Drug Metabolising Enzymes

Long-term ingestion of green tea increases UDP-glucuronosyl transferase activity in rats (Nelson and Paulter, 2004). And after being absorbed, catechins are metabolized by drug metabolizing enzymes in various organs. Thus, the increased glucuronidation through UDP-glucuronosyl transferase induction is postulated to contribute to the anticarcinogenic effect of green tea by facilitating the metabolism of chemical carcinogens into inactive products that are readily excreted (Okushio *et al*., 2001; Donovan *et al*., 2001). The interaction between 2-amino-3-methylimidazol (4,5-f) quinoline (IQ) and green tea catechin metabolism was examined (Embola *et al.,* 2001). IQ is a pre-carcinogen that was originally detected in an extract of fried meat. The major route of IQ biotransformation in rats is cytochrome P450; this is the first step of IQ biotransformation, followed by conjugation to a sulfate and a glucuronide conjugate, which are subsequently excreted in the urine.

Another effect of green tea catechins through the inhibition of cytochrome P450 metabolism is observed in its protective action against cancers induced by polycyclic aromatic hydrocarbons, but the effect of green tea on cytochrome P450 enzymes depends on the particular form. The long-term consumption of green tea increases cytochrome P450 1A1 and 1A2 activities, but not 2B1 and 2E1 activities, in normal rats. However, it is difficult to draw conclusions about a beneficial effect of green tea against carcinogens involving only modulation of this metabolic pathway (Chacko *et al.,* 2010).

### 1.7.1 Role of Tea in Immune Functions

Researchers from Brigham Women Hospital and Harvard University published novel data indicating that tea contains a component that can help the body ward off infection and disease and that drinking tea may strengthen the immune system (Kamath *et al*., 2003). The researchers identified a substance in tea called L-theanine. This bioactive compound primes the immune system in fighting infections caused by bacteria, viruses and fungi. A subsequent human clinical trial showed that certain immune cells of participants who drank five cups of black tea a day for two to four weeks secreted up to four times more interferon; an important part of the bodyôs immune defence, than consumption of the same amount of coffee for the same duration, for which no effect was observed on interferon levels. According to the authors, this study suggests that drinking black tea provides the bodyôs immune system with natural resistance to microbial infection (Kamath *et al*., 2003).

### 1.7.2 Role of Tea in Neurological Health

Age-related declines in memory and cognition occur naturally, but research suggests that modifiable factors such as diet and exercise may help slow the progression of age-related neurodegeneration (Scarmeas *et al*., 2009). Tea helps in improving biomarkers that play a role in brain health. The bioactive compounds found in tea may promote neurological health through various actions (Mendel *et al*., 2008). In addition, L-theanine in tea has been shown to directly affect areas of the brain that control attention and ability to solve complex problems (Kelly *et al.,* 2008; De Bruin *et al*., 2011; Pack *et al*., 2011). In an animal model study, Japanese researchers also found that theanine, the amino acid present almost exclusively in tea, may help prevent memory declines as we age by decreasing neuronal cell death. In their study, animals that were

given theanine and were then subjected to repeated memory impairment, had less memory damage to their brains compared to animals who did not receive theanine (Egashira and Ishigami, 2007).

Research reports indicate that tea polyphenols, particularly (-)-epigallocatechin-3-gallate, are bioavailable to the brain and can act via antioxidant, iron-chelation, signal transduction modulation, and other mechanisms to effect neuroprotective and neuro-rescue action, with potential implications for age-related dementia, Alzheimerôs and Parkinsonôs diseases (Mandel *et al*., 2008). EGCG was capable of reducing biomarkers associated with Alzheimerôs disease. Using strains of mice transgenically bred to be prone to developing Alzheimerôs disease, the researchers found that exposure to EGCG resulted in reduced production of amyloid protein, a marker for the development of plaques associated with Alzheimerôs disease (Rezai-Zadeh *et al*., 2005). A prospective cohort study of nearly 30,000 Finnish adults aged 25 ï 74 years old, who were followed for 13 years, found that tea drinking was associated with a reduced risk of Parkinsonôs disease. Among tea drinkers, those who reported drinking three or more cups of tea per day were 69% less likely to develop Parkinsonôs disease compared to those who reported not drinking tea (Hu and Bidel, 2007).

### 1.7.3 Cognitive Health

A recent human study examined the effect of the unique tea amino acid L-theanine (-glutamylethylamide) on attention-related task performance. Task performance was measured by electroencephalographic (EEG), or the measurement of electrical activity produced by the brain as recorded from electrodes placed on the scalp. The results suggest L-theanine plays a role in attentional processing in synergy with caffeine (Kelly, 2008). A published randomized human clinical trial also found that subjects given a daily supplement with green tea extract and L-theanine extracted from tea experienced improvements in mild cognitive impairments (MCI). In a test of attention and self-reported measure of alertness, subjects consumed two cups of tea (100 mg caffeine and 46 mg L-theanine) versus a placebo beverage. Results indicated that accuracy on the Attention Switching task was improved after tea as compared to the placebo, as well as performance on two of the four subtasks from the Intersensory Attention task (De Bruin *et al*., 2011). Caffeine and L-theanine in tea may offer cognitive benefits and improve mental clarity

and work performance. A cross-sectional study showed that participants who consumed more tea felt less tired and reported higher levels of subjective work performance (Bryan *et al*., 2012).

### 1.7.4 Cardiovascular Disease Risk Factors

Tea consumption improves endothelial function by increasing nitric oxide bioavailability and enhancing vasorelaxation. Tea catechins, epigallocatechin-3-gallate and epicatechin, provided at concentrations achievable in human tissues, relaxed blood vessel tone of isolated arterial walls in an animal model (Aggio *et al*., 2012). In a randomized, double-blind, placebo-controlled study of 19 males, daily black tea consumption increased flow mediated dilation (FMD) from an average of 7.8% to up to 10.3%, depending on flavonoid dosages. The flavonoids in as little as one cup of tea (about 100 mg flavonoids) were found to improve FMD. Black Tea decreased systolic blood pressure by 2.6 mm/Hg and diastolic by 2.2 mm/Hg (Grassi *et al*., 2009). Moreover, Dutch researchers found that study participants who drank one to two cups of black tea daily had a 46% lower risk of severe aortic atherosclerosis, which is a strong indicator of cardiovascular disease. Those who drank more than four cups of tea per day had a 69% lower risk (Geleijnse *et al.,* 1999).

### 1.7.5 Cholesterol Reduction

Researchers from the United States Department of Agriculture (USDA) studied the effect of tea on 15 mildly hypercholesterolemic adult participants following a ñStep Iò type diet moderately low in fat and cholesterol, as described by the American Heart Association and the National Cholesterol Education Program. After three weeks, researchers found that five servings of black tea per day reduced LDL (ñbadò) cholesterol by 11.1% and total cholesterol (TC) by 6.5% compared to placebo beverages. Recent clinical trials have not confirmed these results. However, additional work is being done in this area. The mechanism behind the blood cholesterol lowering effects of tea may be rooted in the effect of theaflavins, through their interference with the formation of dietary mixed micelles, which could result in reduced intestinal cholesterol absorption. Theaflavin treated micelles/particles were analyzed and theaflavins were shown to have a dose-dependent inhibitory effect on the incorporation of cholesterol into micelles. The primary theaflavin identified to display this effect was theaflavin-3-gallate (Vermeer *et al*., 2008).

### 1.7.6 Role of Tea Theaflavin in Cardiac Health

Green and black teas are sources of bioactive flavonoids which possess antioxidant activity (Leung *et al.*, 2001). The fermentation process used to prepare black tea converts many of the simpler catechin flavonoids in green tea leaves to more complex phenolic constituents such as theaflavins (Wang and Halliwell, 2001). Human population studies have revealed that people who regularly consume three or more cups of black tea per day have a reduced risk of heart disease and stroke (Larsson and Walk 2005). Clinical studies suggest that the reduced risk of heart disease associated with tea consumption may be due to improvement in some risk factors for cardiovascular disease, including blood vessel function, platelet function and a reduction in oxidative damage (Hakim *et al.*, 2003).

### 1.7.7 Role of Theanine in Stress Management

One of the reasons green tea has been used in the Orient for centuries, is its calming and curative properties due to the presence of L-theanine, an amino acid found primarily in green and black teas, that produces tranquilizing effects in the brain (Huber, 2003). Through the natural production of polyphenols, the tea plant converts theanine into catechins. This means that tea leaves harvested during one part of the growing season may be high in catechins (with good antioxidant potentials), while leaves harvested during another time of year may be higher in theanine. Three to four cups of green tea are expected to contain from 60 to 160 mg of theanine. Recently, L-theanine has been linked to the feelings of relaxation reported by those who drink green tea.

Experimental studies have also shown that L-theanine appears to negate some of the effects of caffeine (Talbott, 2002). L-theanine facilitates the generation of alpha waves in the brain which is believed to be associated with a relaxed yet alert mental state. A clinical study on L-theanine using young women subjects showed that L-theanine seemed to have the greatest impact on the production of alpha waves among those women who had been categorized as high-anxiety subjects. Theanine is believed to lower cortisol levels during stress periods (cortisol production in the body increases during physical stress) (Talbott, 2002). Research studies have also revealed that people who produce more alpha brain waves also have less anxiety and highly creative people generate more alpha waves when faced with a problem to solve. Elite athletes tend to

produce a burst of alpha waves on the left side of their brains during their best performances. One of the specific aspects of theanine activity is its ability to increase the brainôs output of alpha waves.

Alpha waves are one of the four basic brainwave patterns (delta, theta, alpha, and beta) that can be monitored using an electroencephalogram (EEG). Each wave pattern is associated with a particular oscillating electrical voltage in the brain, and the different brainwave patterns are associated with different mental states and states of consciousness (theta_drowsiness; alpha _ relaxed/alert; beta _ stress/anxiety). Studies in rats have shown theanine to be an effective antihypertensive agent (Zhang[a] *et al*., 2002). It is interesting to know that theanine was able to bring elevated blood pressure back to normal levels, but had no effect on normal blood pressure levels (Yokogoshi *et al*., 1995; Yokogoshi and Kabayashi, 1998). Because theanine reaches its maximum levels in the blood between 30 minutes and 2 hours after ingestion, it can be used as both a daily anti-stress and mental focus regimen and as needed as a supplement during stressful times. Studies on rodents have shown that the ability to learn and remember may be enhanced with theanine supplementation (Kakuda *et al*., 2000; Yokogoshi and Tareshima, 2000). This natural relaxant works to diminish stress, worry, and anxiety, and may allow the brain to focus and concentrate better (Huber, 2003).

### 1.7.8 Anticarcinogenic Effect of Tea

More than 3,000 published result of studies that evaluated the role of tea whether white, green, oolong or black tea for anticancer potential. Tea compounds such as epigallocatechin gallate (EGCG), may play a chemo preventive role, in cancers of various sites. The result suggest that tea compounds have many mechanisms by which they provide chemo-protection: by reducing free radical and DNA damage; inhibiting uncontrolled cell growth (cell proliferation) by promoting programmed cell death (apoptosis); and boosting the immune system to help fend off the development and promotion of cancer cells (Roy *et al*., 2003; Bhattacharyya *et al*., 2004; Hakim *et al*., 2008). Leading scientists worldwide are actively studying these potential mechanisms, with clinical trials and population studies underway. More evidence is needed before any definitive conclusions can be drawn. Preliminary research suggests that tea may provide protection against various types of cancer including digestive, skin, lung, prostate, breast

and ovarian cancers (Katiyar *et al*., 2001; Siddiqui and Saleem, 2007; Hakim *et al.,* 2008; Zhang *et al*., 2012). Recent findings will include:

### 1.7.8.1 DNA Damage

Oxidative DNA damage is implicated in the development of various forms of cancer. Recent studies have found that smokers who drank four cups of decaffeinated green tea per day demonstrated a 31% decrease in biomarkers of oxidative DNA damage in white blood cells as compared to those who drank four cups of water after four months (Hakim *et al.,* 2003; Hakim *et al.,* 2008). Epigallocatechin gallate (EGCG) may protect normal cells from cancer-causing hazards and eliminate cancer cells through apoptosis. Researchers tested the potential anti-cancer benefits of the green tea polyphenol EGCG in hamster cells and discovered that EGCG suppressed DNA changes and damage from carcinogens, EGCG also protected against further damage from the carcinogens and inhibited growth and multiplication of cancer cells (Roy *et al.,* 2003). An animal study identified beneficial changes in immune functions after black tea ingestion in cancer-bearing animals; black tea altered immune responses that helped protect immune cells against harmful cancerous cells, black tea acted like anti-cancer drugs that help boost the immune system without promoting the proliferation of cancerous cells (Bhattacharyya *et al.,* 2004).

### 1.7.8.2 Breast Cancer

Results from a large epidemiologic study examining the association of regular tea consumption with the risk of breast cancer and found that, women under 50 years with moderate tea consumption (three or more cups per day) had a 37% reduced breast cancer risk (Kumar *et al*., 2009). In a study of 10 women with advanced breast cancer undergoing radiation therapy for treatment, half were given radiation therapy plus 400 mg epigallocatechin-3-gallate (EGCG) three times daily, the results showed that the EGCG helped inhibit cell proliferation, cell invasion and angiogenesis, then the authors concluded that EGCG may be an effective strategy to inhibit the spread of invasive breast cancer cells (Zhang *et al*., 2012).

### 1.7.8.3 Prostate Cancer

Researchers at the University of Wisconsin, Madison reviewed the existing literature about tea as a preventive measure for prostate cancer among men. Based on epidemiological *in vitro* and *in*

*vivo* studies, the researchers suggest that tea especially green tea may be a good public health recommendation that may help prevent prostate cancer (Siddiqui and Saleem, 2007).

### 1.7.8.4 Skin Cancer

According to a study conducted by the University of Arizona, participants who drank iced Black Tea and citrus peel had a 42% reduced risk of skin cancer (Hakim and Harris, 2001). consumption of hot black tea was associated with a significantly lower risk of squamous cell carcinoma (SCC), a form of skin cancer; tea concentration (strength), brewing time and temperature all influenced the potential protective effects of hot black tea on SCC (Hakim *et al*., 2000). Oral consumption of green or black tea decreased the number of tumours in mice following exposure to UV radiation (Lu *et al.,* 2001). Green tea polyphenols may have cancer prevention potential, especially in the case of solar UV-induced cancer (Ahmad and Mukhtar 2001; Katiyar *et al*., 2001). Also topical application of green tea polyphenols on human skin prior to UV exposure inhibited indicators of DNA damage, thus inhibiting photocarcinogenesis, or UV-induced skin cancer (Katiyar *et al*., 2000).

Experiments show that administration of green tea, black tea or specific flavonoids in tea inhibited the growth of established non-malignant and malignant skin tumors in tumor-bearing mice. In addition, oral administration of black tea inhibited DNA synthesis and enhanced cell death (apoptosis) in both non-malignant and malignant tumours in tumour-bearing mice (Conney *et al*., 1999). In a human clinical trial, 16 healthy adults were given 540 mg Green Tea catechins with vitamin C for 12 weeks. The researchers then exposed their skin to UV radiation and they reported a reduction in UV-induced inflammation as a result of Green Tea polyphenols and vitamin C (Rhodes *et al*., 2013).

### 1.7.8.5 Lung Cancer

Studies comparing groups of mice treated with a tobacco-specific carcinogen and receiving either water or water enriched with tea-derived polyphenols found that the tea-fed mice developed 24% fewer lung tumours and the average size of the tumours was 38% smaller as compared to the water-fed mice (Yang[a] *et al*., 1997). Tea catechins were evaluated for their effects on cell proliferation, apoptosis and associated gene expression in highly metastatic human lung cancer cells, a significant reduction in cell proliferation after exposure to tea catechins was

noted. It is suggested that tea compounds can influence genetic alteration to reduce the growth and survival of human lung cancer cells (Ganguly *et al*., 2005). In addition in an *in vitro* study, researchers exposed highly metastatic lung cancer cell lines to tea polyphenols and found that tea polyphenols inhibited the expression of cancer cells to the endothelial cell walls. The mechanism of tea polyphenol prevention of human lung carcinoma metastasis might be through inhibiting adhesion molecule expression to block cancer cell adhesion (Zheng *et al*., 2012).

#### 1.7.8.6 Ovarian Cancer

A case-control study conducted in China, which employed 254 patients with histologically confirmed epithelial ovarian cancer and 652 control subjects, determined tea consumption based on a validated questionnaire and found that, after accounting for demographic, lifestyle and familial factors, ovarian cancer risk declined with increasing frequency and duration of overall tea consumption (Zhang *et al*., 2012). A population-based study involving more than 61,000 Swedish women aged 40 ï 76 showed that drinking black tea was associated with a reduced risk of ovarian cancer, the study found that women who drank the most tea green or black were least likely to develop ovarian cancer over the 15-year study follow-up.

Women who drank two or more cups of tea daily experienced a 46% reduction in risk compared to women who reported not drinking tea. Even small amounts of tea (less than one cup per day) reduced risk by 18%, while one cup per day reduced risk by 24%. Although previous studies evaluating the effects of tea consumption and ovarian cancer found inconsistent results, the researchers noted that the large size of this study and long-term follow-up provides compelling evidence that tea drinking may indeed offer protection against this type of cancer.

### 1.7.9 Role of Tea in Oral Health

Tea may contribute to oral health through various means, which include anti-bacterial properties and fluoride content. These factors may help protect against cavities and gum disease and may strengthen tooth enamel (Kushiyama *et al*., 2009; Nugala *et al*., 2012). Research has shown that tea flavonoids may inhibit the plaque-forming ability of oral bacteria, and the fluoride in tea may support healthy tooth enamel (Yu *et al*., 1995; Sarka *et al.*, 2000). A study conducted at the New York university dental center, examined the effects of black tea extract on dental caries formation in hamsters, compared to those who were fed water with their food, hamsters that were

fed water with black tea extract developed up to 63.7% fewer dental caries (Linke and LeGerose, 2003). Drinking tea is minimally erosive to tooth enamel according to a recent study comparing tea (green and black) to soda and orange juice using *in vitro* tests. Water was used as the non-erosive control, and vinegar was the erosive control. The 20-week study was conducted under controlled conditions, and results were categorized as highly, moderately, or minimally erosive. Soda and orange juice were shown to be moderately erosive and vinegar remained highly erosive (Bassiouny *et al*., 2008).

A research study postulated that drinking green tea was inversely related to periodontal (gum) disease, the study analyzed 940 Japanese men, aged 49ï 59 years old who took part in a comprehensive health examination. The effect of tea on gum disease seems to be dose-dependent; each additional cup of tea was associated with a greater decrease in gum disease factors (Kushiyama *et al*., 2009). Based on a review of the evidence supporting green tea catechins for the prevention of periodontal disease, researchers recommend two to three cups of green tea per day (Nugala *et al*., 2012).

### 1.8 Adverse Effect of Tea

Although green tea has several beneficial effects on health, the effects of green tea and its constituents may be beneficial at a certain dose yet higher doses may cause some unknown adverse effects. Moreover, green tea catechins may not have similar effects in all individuals. EGCG of green tea extract is cytotoxic, and higher consumption of green tea can exert acute cytotoxicity in liver cells, a major metabolic organ in the body (Schmidt *et al.,* 2005). Another study found that higher intake of green tea may be responsible for oxidative DNA damage of hamster pancreas and liver (Takabayashi *et al.*, 2004).

Yun *et al*. (2006) clarified that EGCG acts as a pro-oxidant, rather than an antioxidant, in pancreatic ɓ cells *in vivo*. Therefore, high intake of green tea for hyperglycemia control may be detrimental for the diabetic individuals. At a high dose (5% of diet for 13 weeks), green tea extract induced a thyroid enlargement (goiter) in normal rats (Sakamoto *et al*., 2001; Satoh *et al*., 2002). This high-level treatment modified the plasma concentrations of the thyroid hormones. However, drinking even a very high dietary amount of green tea would be unlikely to cause these adverse effects in humans. Harmful effects of tea overconsumption are due to three main factors:

(1) its caffeine content, (2) the presence of aluminium, and (3) the effects of tea polyphenols on iron bioavailability. Green tea should not be taken by patients suffering from heart conditions or major cardiovascular problems. Pregnant and breastfeeding women should drink no more than one or two cups per day, because caffeine can cause an increase in heart rhythm. It is also important to control the concomitant consumption of green tea and some drugs, due to caffeineôs diuretic effects (Bruneton, 2001).

Some studies revealed the capacity of tea plants to accumulate high levels of aluminium. This aspect is important for patients with renal failure because aluminium can be accumulated by the body, resulting in neurological diseases. It is therefore necessary to control the intake of food with high amounts of this metal (Costa *et al*., 2002). Likewise, green tea catechins may have an affinity for iron and green tea infusions can cause a significant decrease of the iron bioavailability from the diet (Hamdaoui *et al*., 2003).

## 1.9 Aim and Specific Objectives of the Study

### 1.9.1 Aim of the Study

This research was aimed at determining the antioxidant potential of different types of tea.

### 1.9.2 Specific Objectives of the Study

The specific objectives of this study were:

- To determine the phytochemical constituent of different types of tea
- To determine the free radical scavenging activity of different types of tea using the stable radical 2,2-Diphenyl-2-picrylhydrazyl (DPPH)
- To determine the total antioxidant capacity using the ferric reducing antioxidant power of different types of tea.

# CHAPTER TWO

# MATERIALS AND METHODS

## 2.1 Materials

### 2.1.1 Collection of tea samples

Twenty commercially available teas of different types (green, black, un-caffeinated and herbal teas) were purchased at shop-rite in Enugu city, and various other local grocery stores in Nsukka, Enugu State.

### 2.1.2 Equipment and Instruments

Equipment and instruments used for this study were in the laboratory of Biochemistry Department University of Nigeria, Nsukka. They include: centrifuge (model 800D) (New life medical instrument, England), micropipette (Volume Range 0-1000µl; Swastic Science Instrument Private Ltd, Mumbai, India), test spectrophotometer (model SPM721-2000; Biodiagnostic Inc., USA).

### 2.1.3 Chemicals and Reagents

All chemicals used in this study were of analytical grade and products of BDH, England; Karmel, India; Sigma Aldrich, Germany; Qualikems, JHD, Guanghua Guangdong, China; Cambrian chemicals, and Cultivalae chemical Ltd., others were purchased from standard chemical and reagent stores within Enugu State.

| Chemicals | Producer |
|---|---|
| Trichloro acetic acid | Qualikams |
| Ethyl Acetate | JHD Guangdom |
| Sodium hydrogen phosphate dihydrate | Guangdom Guanghua |
| Disodium hydrogen phosphate dodecahydrate | Sci-Tech Co Ltd |
| Gallic Acid | Karmel India |

| | |
|---|---|
| Ferric Chloride | BDH Poole England |
| Thiobarbituric Acid | Cambrian chemicals |
| Ammonia solution 25% | Guanghua Sci Tech Co Ltd |
| Sodium Carbonate | Karmel India |
| Potassium fericyanide | BDH pool Eng |
| Ascorbic acid | Cartivalae Chemicals Ltd |
| Folin Ciocalteam | Sigma Aldrich Germany |
| DPPH | Sigma Aldrich Germany |

## 2.2 Methods

### 2.2.1 Preparation of Extract

Commercially available tea (10 g each) was extracted in 100 ml of distilled water at $100^0$C and kept below 80°C to brew for 10 minutes. The mixture was decanted and filtered using Whatman No.1 filter paper. The resulting filtrate was used for the phytochemical and antioxidant assay.

### 2.2.2 Experimental Design

A total of 20 different brands of tea were divided into four groups as follows:

Group 1: Five different brands of black tea denoted by the letter B and numbers 1-5 (BT1=Ahmad Tea, BT2 = LOYD lemon tea, BT3 = Lipton tea, BT4 = Dilmah and BT5 = Shenghai Bozehng).

Group 2: Five different brands of un-caffeinated tea denoted by the letter U and numbers 1-5. (UT1 = Tetley tea, UT2 = Chamommile tea, UT3 = Lemon and Ginger tea, UT4 = Pepermint tea and UT5 = Red bush tea).

Group 3: Five different brands of green tea denoted by the letter G and numbers 1-5. (GT1 = Hillway tea, GT2 = Moringa tea, GT3 = Green life CRT tea, GT4 = Legend tea and GT5 = Tianshi tea.

Group 4: Five different brands of herbal teas denoted by the letter H numbers 1-5. (HT1 = Slim Dieters tea, HT2 = Ginseng tea, HT3 = Typhoid tea, HT4 = Fat Burner tea and HT5 = Anti-Diabetes tea).

### 2.2.3 Phytochemical Studies

#### 2.2.3.1 Test for Tannins

Commercially available tea (10 g each) was extracted in 100 ml of distilled water at $100^0$C and kept below 80°C to brew for 10 minutes. The mixture was decanted and filtered using Whatman No. 1 filter paper two milliliter of the brewed tea was made up to 10ml with distilled water. Few drops of 1% ferric chloride solution was added to 2 ml of the diluted aqueous extract. Usually of a blue-black, green or blue-green precipitate indicates the presence of tannins (Evans, 2002). Folin-Ciocalteau method was used for the determination of the total tannins content (TTC) of the tea extract using gallic acid as an internal standard with slight modification as previously reported Mythili *et al*. (2014). A known volume, 0.1 ml of the extract (1 mg/ml) was mixed with 7.5 ml of distilled water in a 10 ml volumetric flask. Aliquot (0.5 mL) of a 10 fold dilute Folin-Ciocalteau phenol reagent (FCPR, 1:10) was added. One milliliter of 35% $Na_2CO_3$ solution was added to the mixture and made up to the mark with distilled water.

The mixture was incubated in the dark for 30 mins at room temperature. A set of standard solutions of gallic acid (100, 80, 60, 40 and 20 µg/ml) were prepared in the same manner as described for the extract. The absorbance of the extract and standard solutions were read against the reagent blank at 725 nm with a UV/Visible spectrophotometer (UV-1800, Shimadzu, Japan). The total tannins content was determined from the calibration curve and expressed as milligram of gallic acid equivalent (GAE) per gram of the extracts (Singh *et al*., 2012). The determination of the total tannins content in the extract was carried out in triplicate.

#### 2.2.3.2 Test for Flavonoids

The presence of flavonoids in tea sample was detected using ferric chloride test. Commercially available tea (10 g each) was extracted in 100 ml of distilled water at $100^0$C and kept below 80°C to brew for 10 minute. The mixture was decanted and filtered using Whatman No. 1 filter paper. To 2 ml of the filtrate, few drops of 10% ferric chloride were then added. A green-blue or violet

colouration indicated the presence of a phenolic hydroxyl groups (Evans, 2002). The total flavonoid content of the aqueous extracts was determined spectrophotometrically using the aluminium chloride ($AlCl_3$) assay, as reported by Ghasemzadeh *et al*. (2011).

A known volume, 1 ml aliquot of each extract (1 mg/ml) or standard solution of quercetin (31.5, 62.5,125, 250, 500 and 1 mg/LS) was added to a volumetric flask (10 ml) and diluted with 4 ml double distilled water at time 0. After which, 0.3 mL of 5 % (w/v) sodium nitrite ($NaNO_2$) was added and after 5 min, 0.6 ml $AlCl_3$ (10 %) was added. At 6 min, 2 ml of sodium hydroxide (1 M NaOH) was added to the mixture, and the final total volume was made up to 10 ml with double-distilled water. The solution was mixed completely, and the absorbance was measured against a prepared reagent blank in triplicate at 430 nm. The total flavonoid content was expressed as quercetin equivalents in mg/100 g of dry extract weight.

#### 2.2.3.3 Test for Total Phenolic Contents

Total phenolic contents of the extract were determined using the Folin-Ciocalteu reagent as previously reported by Gholivand and Piryaei (2014), using gallic acid as standard, with some modifications. Aliquot 0.1 ml solution containing 1000 ε g of the extract was mixed with 46 ml of distilled water in a volumetric flask and 1 ml Folin-Ciocalteu reagent was added, and the content of the flask was thoroughly mixed. The mixture was allowed to react for 3 min and 3 ml aqueous solution of 2% $Na_2CO_3$ was added. After 2 hours at room temperature, absorbance of each mixture was measured at 760 nm. The same procedure was also applied to the standard solutions of gallic acid, and a standard curve was obtained. Total phenol contents were expressed as ε g gallic acid equivalents per mg of the extract. All tests were carried out in triplicates.

### 2.2.4 Determination of Antioxidant Activity Using DPPH Radical Scavenging Model

The antioxidant activities of the extracts were measured by determining the hydrogen donating or radical scavenging ability, using the stable radical, DPPH as reported previously reported Mensor *et al*., (2001). An aliquot (120 ε l) of 0.25 mM DPPH solution in methanol and 30 ε l of each extract at increasing concentrations (31.3, 62.5, 125, 250, 500 and 1000 ε g/ml) were mixed and shaken together and left at room temperature in the dark. The absorbance was measured at a wavelength of 518 nm after 30 min against different concentrations of the extracts in distilled water as blanks and DPPH in methanol without extract as the control. The standard synthetic

antioxidant, butylhydroxytoluene was used as the positive control. The percentage antiradical activity (AA%) of the extracts was calculated using the following formula (Ghasemzadeh *et al*., 2011).

$$AA\% = \left(100 - \left(\frac{Abs_{sample} - Abs_{blank}}{Abs_{control}}\right) \times 100\right)$$

Where, Abs sample, Abs blank, and Abs control are the absorbance value of the extract, blank, and control samples, respectively.

### 2.2.5 Ferric Reducing Antioxidant Power Assay

The ferric reducing antioxidant power (FRAP) of the extracts were assayed based on the blue coloration that developed due to the reduction of ferric iron to the ferrous form as described by Loizzo *et al*. (2012). Extract solutions were prepared by infusing 10 g of extracts in $100^0$C distilled water. An aliquot (0.2 ml) of each extract solution was added to a test tube containing 1.8 ml of freshly prepared FRAP reagent that consisted of 2.5 ml of 10 mM TPTZ solution in 40 mM of HCl and 2.5 mL of 20 mM $FeCl_3.6H_2O$ in 25 ml of 0.3 M acetate buffer (pH 3.6). The mixture was incubated at 37 ℃ for 5 min. The photometric absorbance was recorded at a sswavelength of 593 nm. The reducing power was ascertained by comparing the spectrophotometric absorbance of each sample against a standard curve obtained with $Fe_2SO_4$.

### 2.2.6 Statistical Analysis

The data obtained were analysed using IBM Statistical Product and Service Solutions (SPSS), version 18.0 and graph pad prism, version 6. The results were expressed as mean ± standard deviation (SD). Significant differences were established by one-way analysis of variance (ANOVA). Considered significant at ($p<0.05$).

# CHAPTER THREE

# RESULTS

## 3.1 Results of Qualitative Phytochemical Analysis of Different types of Tea

The results of the phytochemical screening of the tea samples are presented in Table 1. The two phytochemical screened for, namely flavonoids and tannins were present in the tea samples.

**Table 1: Qualitative Phytochemical Screening of Different Types of Tea**

| Teas | Flavonoid | Tannin |
|---|---|---|
| $BT_1$ | +++ | ++ |
| $BT_2$ | +++ | ++ |
| $BT_3$ | ++ | ++ |
| $BT_4$ | ++ | ++ |
| $BT_5$ | + | + |
| $UT_1$ | ++ | ++ |
| $UT_2$ | + | + |
| $UT_3$ | ++ | + |
| $UT_4$ | + | + |
| $UT_5$ | + | + |
| $GT_1$ | ++ | ++ |
| $GT_2$ | ++ | + |
| $GT_3$ | ++ | ++ |
| $GT_4$ | +++ | + |
| $GT_5$ | ++ | ++ |
| $HT_1$ | ++ | ++ |
| $HT_2$ | + | + |
| $HT_3$ | + | ++ |
| $HT_4$ | ++ | + |
| $HT_5$ | + | + |

+ = detected in low quantity; ++ = detected in moderate quantity,
+++ = detected in high quantity; - = not detected

BT1=Ahmad black tea, BT2 = LOYD lemon black tea, BT3 = Lipton black tea, BT4 = Dilmah black tea and BT5 = Shenghai Bozehng black tea

UT1 = Tetley un-caffeinated tea, UT2 = Chamommile un-caffeinated tea, UT3 = Lemon and Ginger un-caffeinated tea, UT4 = Pepermint un-caffeinated tea and UT5 = Red bush un-caffeinated tea).

GT1 = Hillway green tea, GT2 = Moringa green tea, GT3 = Green life CRT green tea, GT4 = Legend green tea and GT5 = Tianshi green tea.

HT1 = Slim Dieters herbal tea, HT2 = Ginseng herbal tea, HT3 = Typhoid herbal tea, HT4 = Fat Burner herbal tea and HT5 = Anti-Diabetes herbal tea.

### 3.2 Result of Quantitative Phytochemical Analysis of Different types of Tea

The results of the quantitative analysis of total flavonoid, total tannin and total phenol contents of the tea samples are shown in Figs. 7,8 and 9. The highest concentration of flavonoid (269.65 QE/mg) was observed in a green tea sample (GT3), while the least concentration (115.06 QE/mg) was recorded in a black tea sample (BT5) as shown in Fig.7. The total tannin content of the tea samples is shown in Fig. 8. The highest concentration of tannin (446.36 GAE/mg) was observed in a green tea sample (GT1), while the least concentration (233.42 GAE/mg) was observed in two black tea samples (BT2 and BT3). The results of the total phenol analysis of the tea samples are shown in Fig. 9. The highest concentration of phenol (229.74 GAE/mg) was observed in a green tea sample (GT1), while the least concentration (14.11 mg/GAE) was recorded in an herbal tea sample (HT2).

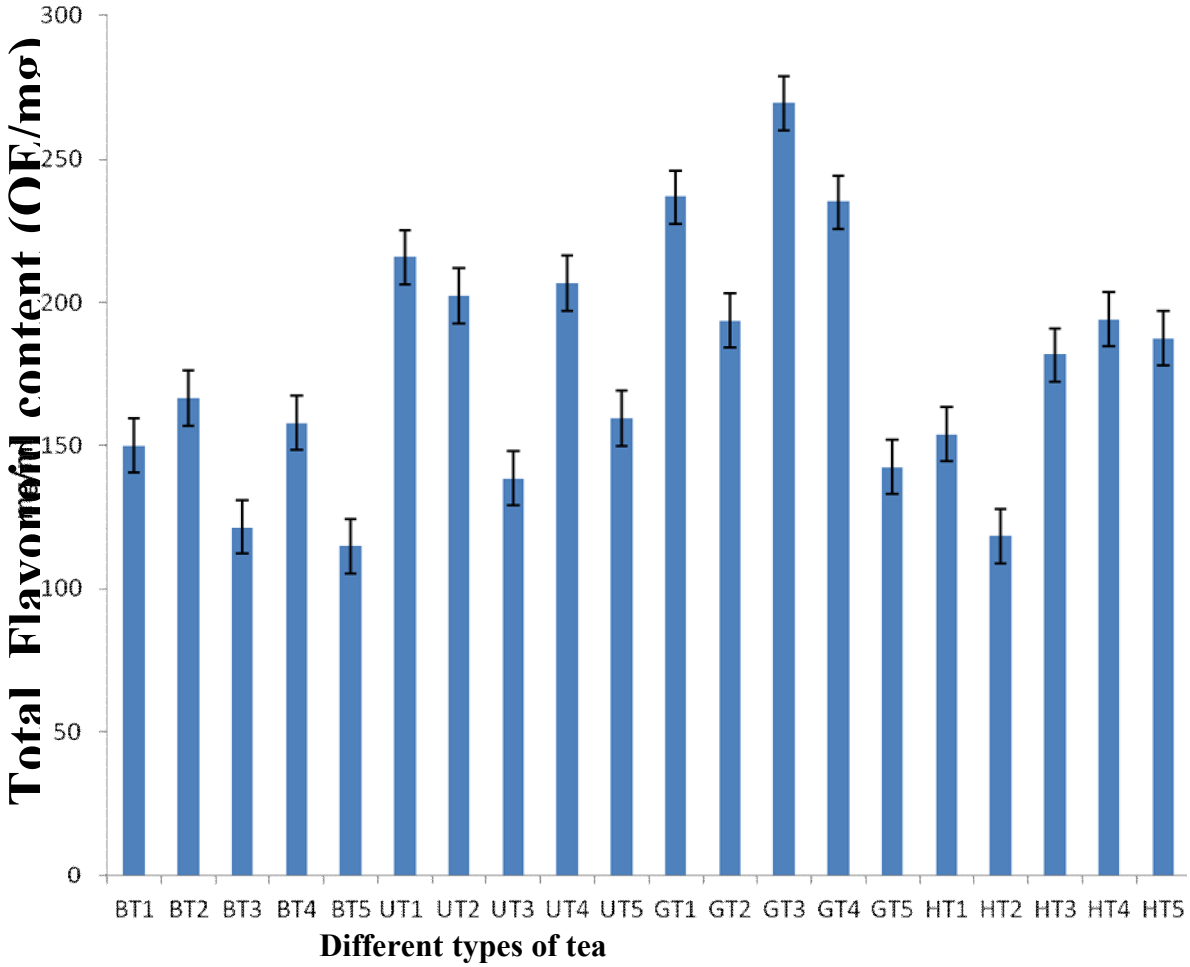


**Fig. 7: The total flavonoid content of different types of tea**

BT1=Ahmad black tea, BT2 = LOYD lemon black tea, BT3 = Lipton black tea, BT4 = Dilmah black tea and BT5 = Shenghai Bozehng black tea

UT1 = Tetley un-caffeinated tea, UT2 = Chamommile un-caffeinated tea, UT3 = Lemon and Ginger un-caffeinated tea, UT4 = Pepermint un-caffeinated tea and UT5 = Red bush un-caffeinated tea).

GT1 = Hillway green tea, GT2 = Moringa green tea, GT3 = Green life CRT green tea, GT4 = Legend green tea and GT5 = Tianshi green tea.

HT1 = Slim Dieters herbal tea, HT2 = Ginseng herbal tea, HT3 = Typhoid herbal tea, HT4 = Fat Burner herbal tea and HT5 = Anti-Diabetes herbal tea.

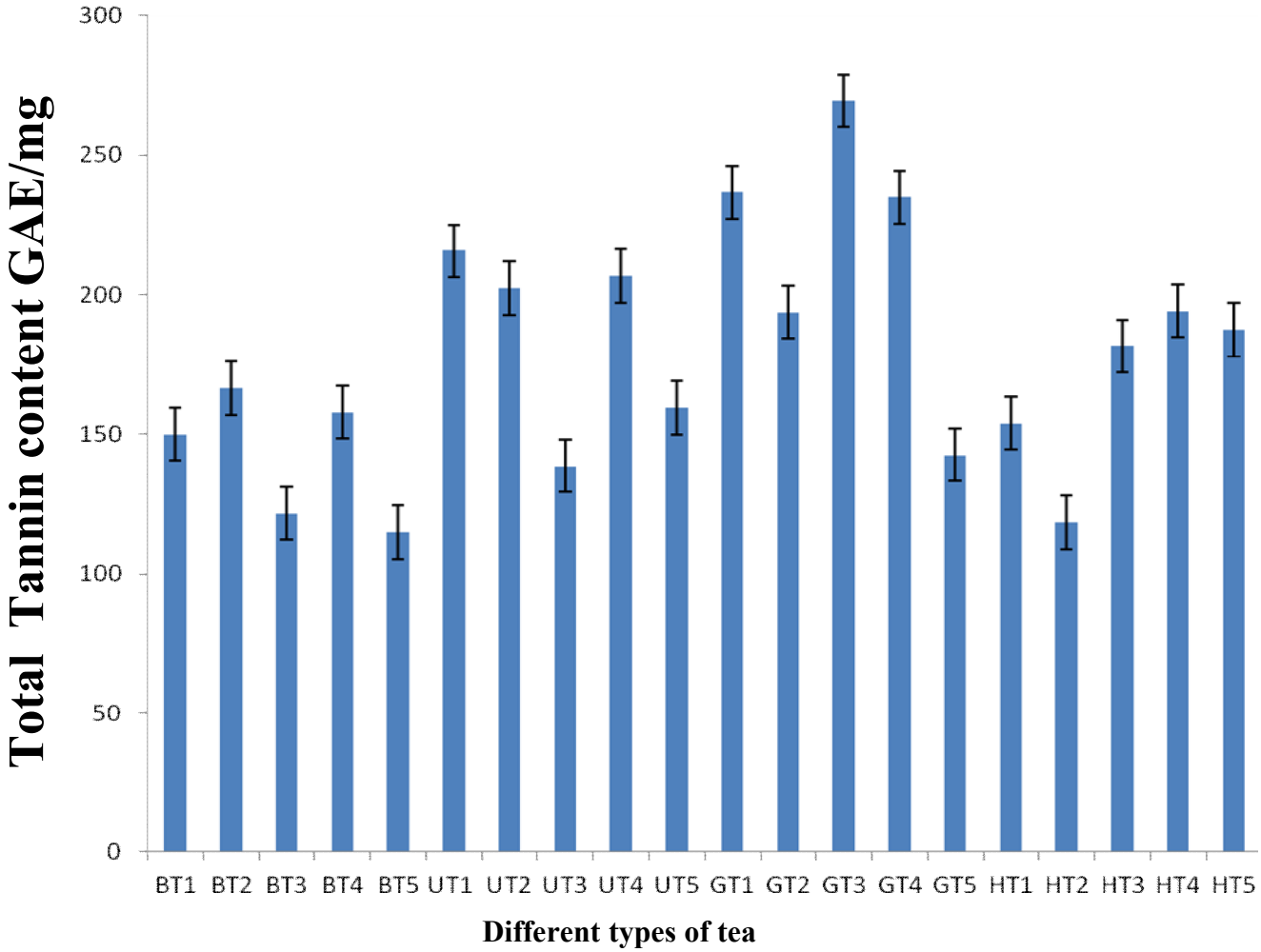


**Fig. 8: The total tannin content of Different types of tea**

BT = Back tea; UT = Un-caffeinated tea; GT = Green tea; HT = Herbal tea

BT1=Ahmad black tea, BT2 = LOYD lemon black tea, BT3 = Lipton black tea, BT4 = Dilmah black tea and BT5 = Shenghai Bozehng black tea

UT1 = Tetley un-caffeinated tea, UT2 = Chamommile un-caffeinated tea, UT3 = Lemon and Ginger un-caffeinated tea, UT4 = Pepermint un-caffeinated tea and UT5 = Red bush un-caffeinated tea).

GT1 = Hillway green tea, GT2 = Moringa green tea, GT3 = Green life CRT green tea, GT4 = Legend green tea and GT5 = Tianshi green tea.

HT1 = Slim Dieters herbal tea, HT2 = Ginseng herbal tea, HT3 = Typhoid herbal tea, HT4 = Fat Burner herbal tea and HT5 = Anti-Diabetes herbal tea.

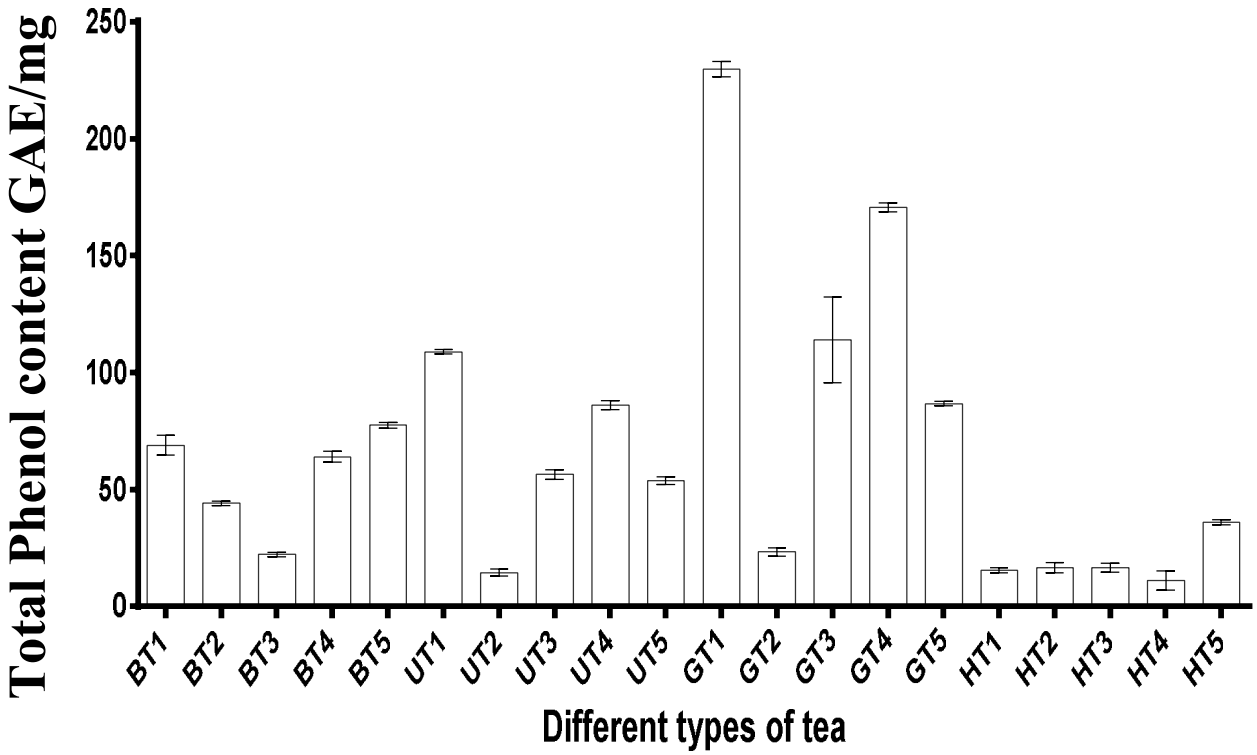


**Fig. 9: The total phenol content of different types of tea**

BT1=Ahmad black tea, BT2 = LOYD lemon black tea, BT3 = Lipton black tea, BT4 = Dilmah black tea and BT5 = Shenghai Bozehng black tea

UT1 = Tetley un-caffeinated tea, UT2 = Chamommile un-caffeinated tea, UT3 = Lemon and Ginger un-caffeinated tea, UT4 = Pepermint un-caffeinated tea and UT5 = Red bush un-caffeinated tea).

GT1 = Hillway green tea, GT2 = Moringa green tea, GT3 = Green life CRT green tea, GT4 = Legend green tea and GT5 = Tianshi green tea.

HT1 = Slim Dieters herbal tea, HT2 = Ginseng herbal tea, HT3 = Typhoid herbal tea, HT4 = Fat Burner herbal tea and HT5 = Anti-Diabetes herbal tea.

### 3.2.4 A Comparison of the Mean Values of the Quantity of Phytochemicals in the Tea Samples

Table 2 shows the mean values of the quantity of phytochemicals present in the tea samples: The total flavonoid content (TFC) of green tea (215.61±48.83 QE/mg) was significantly ($p < 0.05$) higher than that of black tea (142.32±22.73 QE/mg). There was no significant difference ($p > 0.05$) among the TFC of green tea (215.61±48.83 QE/mg), un-caffeinated tea (184.69±33.62 QE/mg) and herbal tea (167.26±31.25 QE/mg). Black tea had the lowest total tannin concentration (TTC) (325.14±108.08 GAE/mg). There was no significant ($p > 0.05$) difference among the TTC of un-caffeinated tea (411.55±9.21GAE/mg), green tea (406.83±22.71 GAE/mg) and herbal tea (402.74±7.95 GAE/mg). The total phenol content (TPC) of green tea (124.81±79.05 GAE/mg) was significantly ($p < 0.05$) higher than that of un-caffeinated tea (63.87±35.76 GAE/mg), black tea (51.81±18.90 GAE/mg) and herbal tea (15.78±13.02 GAE/mg). Herbal tea has the least TPC value (Table 2).

**Table 2: A Comparison of the mean values of the Quantity of phytochemicals in the tea samples**

| TEAS | TFC (QE/mg) | TTC(GAE/mg) | TPC(GAE/mg) |
|---|---|---|---|
| Black Teas | 142.32±22.73[a] | 325.14±108[a] | 51.81±18.90 [b] |
| Un-caffeinated Tea | 184.69±33.[62ab] | 411.55±9.21[b] | 63.87±35.76 [b] |
| Green Tea | 215.61±48.83[b] | 406.83±22.71[b] | 124.81±79.05[c] |
| Herbal Tea | 167.25±31.25[ab] | 402.74±7.95[b] | 15.78±13.02[a] |

Data are expressed as means ± SD. Values with different superscript down the group (or across the column) are considered statistically different at $p < 0.05$.
Abbreviations

TFC = Total flavonoid content,
TTC = Total tannin content,
TPC = Total phenol content,

### 3.3 Result of The Evaluation of Antioxidant Activity of Tea Samples Based on DPPH Assay

#### 3.3.1 Effects of Black Tea Extracts on DPPH Radical

The results of the evaluation of the antioxidant capacities of the various black tea samples are presented in Fig. 10. The result revealed that, black tea 2 (BT2) had the least $IC_{50}$ value of 538 µg/ml, while black tea 5 (BT5) had the highest 825 µg/ml.

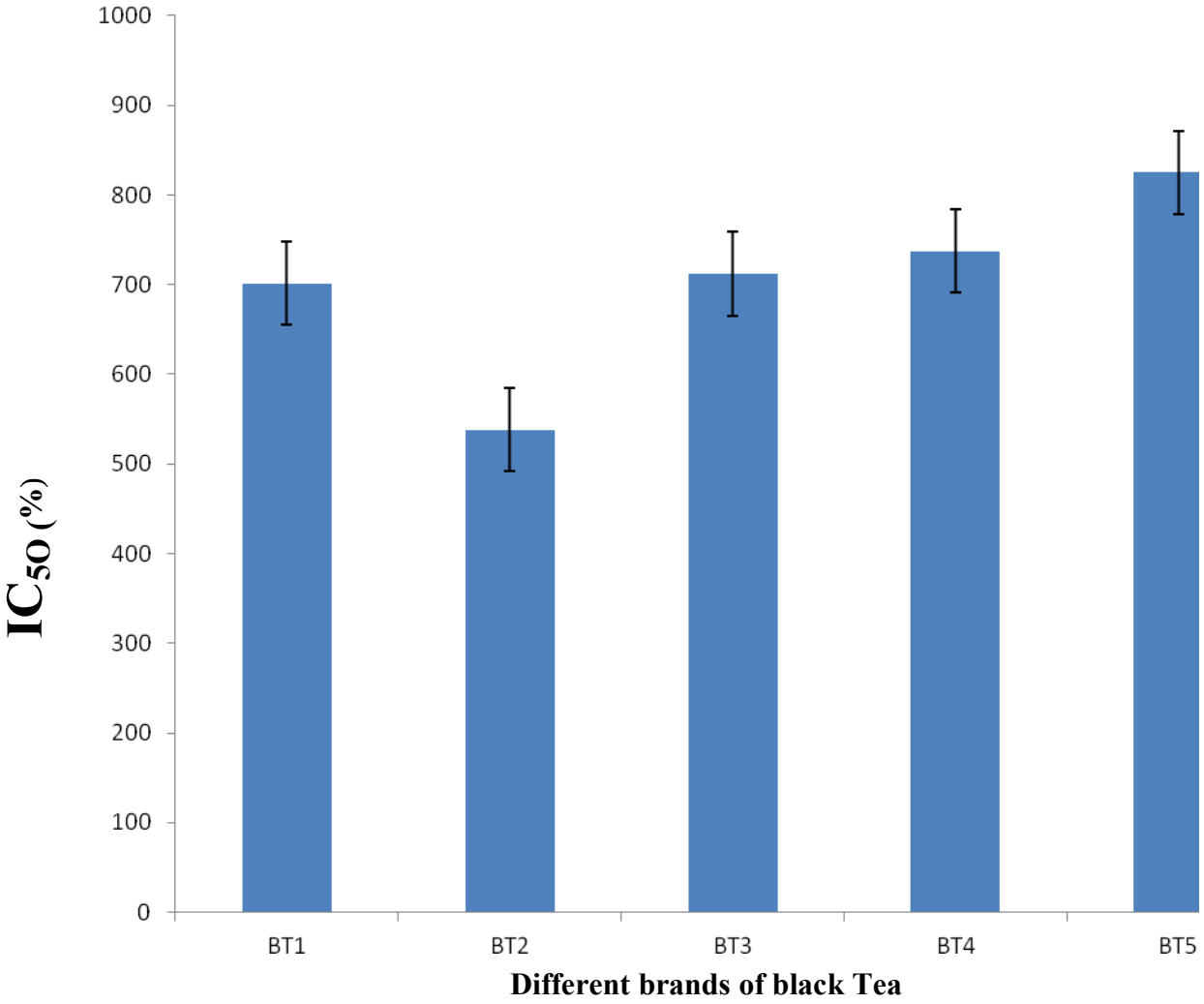


**Fig. 10: The antioxidant activity of different types of black tea extracts against DPPH radical**

**BT1=Ahmad black tea, BT2 = LOYD lemon black tea, BT3 = Lipton black tea, T4 = Dilmah black tea and BT5 = Shenghai Bozehng black tea.**

### 3.3.2 Effects of Un-Caffeinated Tea Extracts on DPPH Radical

The results of the evaluation of the antioxidant capacities of the extracts of various un-caffeinated tea samples are shown in Fig. 11. The results revealed that un-caffeinated tea 3 (UT3) had the least $IC_{50}$ value of 598 µg/ml while un-caffeinated tea 2 (UT2) had the highest, 773 µg/ml.

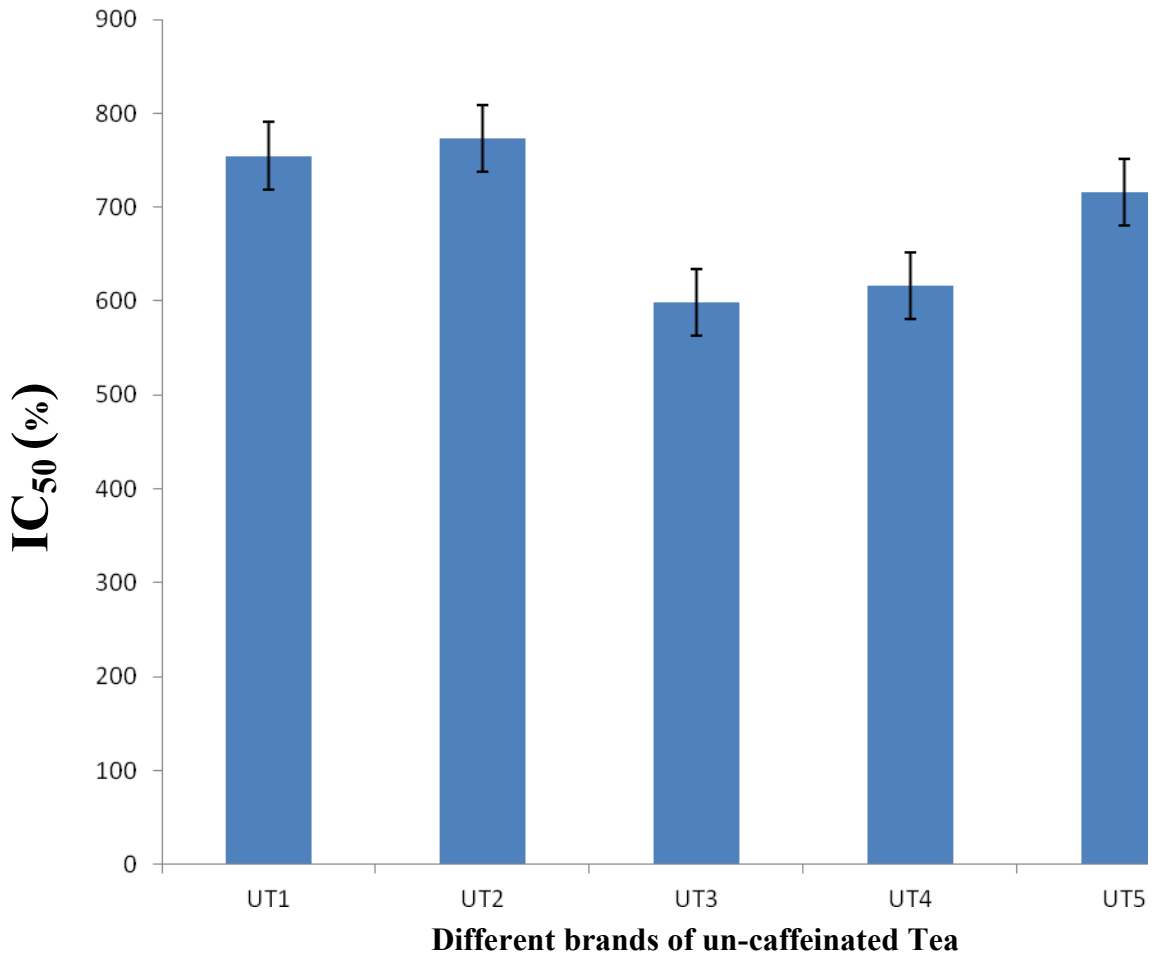


**Fig. 11: Antioxidant activity of extracts of un-caffeinated teas against DPPH radical**

**UT1 = Tetley un-caffeinated tea, UT2 = Chamommile un-caffeinated tea, UT3 = Lemon and Ginger un-caffeinated tea, UT4 = Pepermint un-caffeinated tea and UT5 = Red bush un-caffeinated tea).**

### 3.3.3 Effects of Green Tea Extracts on DPPH Radical

The results of the evaluation of the antioxidant capacities of the extracts of the various green tea samples are shown in Fig. 12. The results revealed that green tea 3 (GT3) had the least $IC_{50}$ value of 471 µg/ml while green tea 1 (GT1) had the highest, 894 µg/ml.

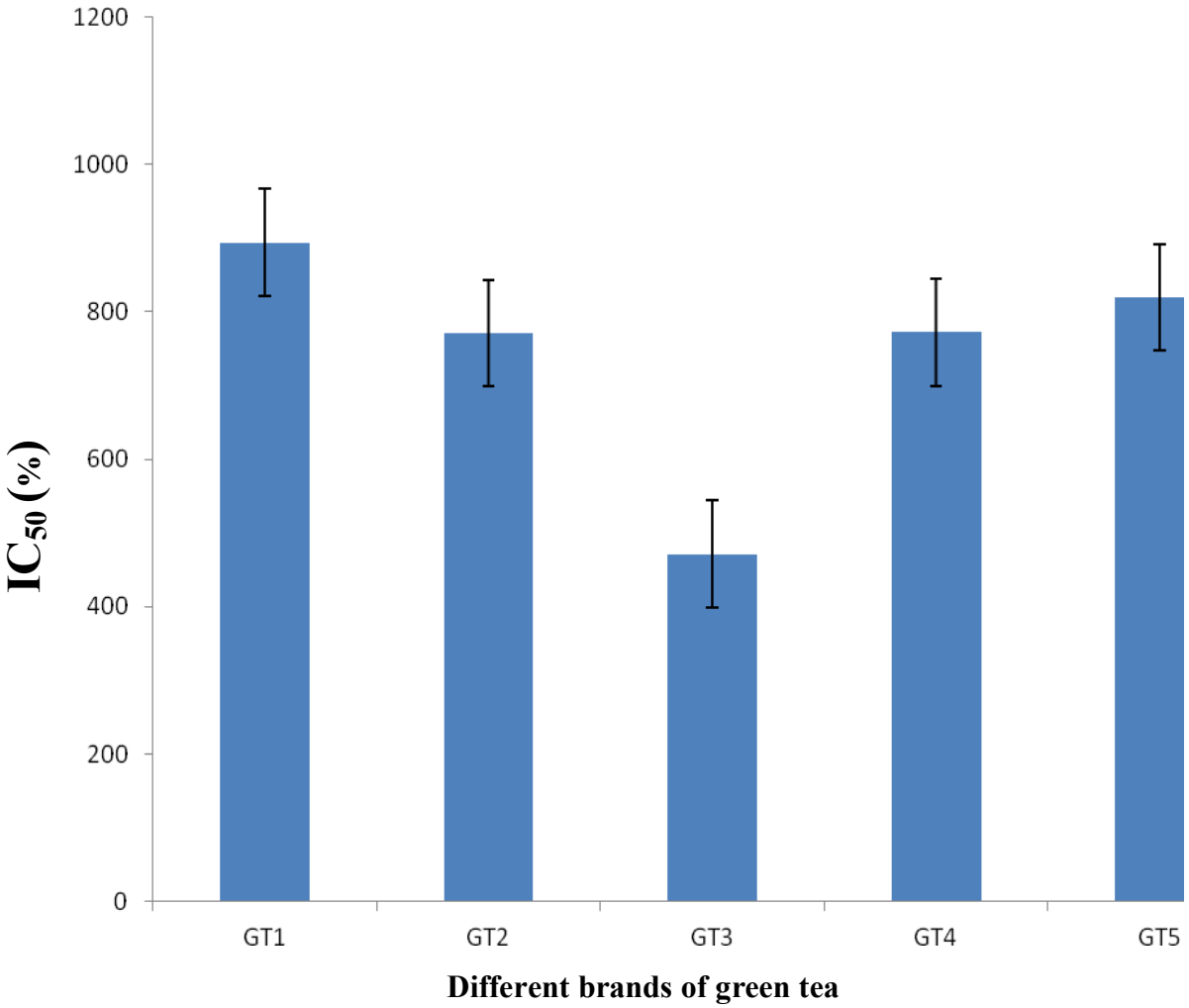


**Fig. 12: The antioxidant activity of extract of Green teas against DPPH radical**

**GT1 = Hillway green tea, GT2 = Moringa green tea, GT3 = Green life CRT green tea, GT4 = Legend green tea and GT5 = Tianshi green tea.**

### 3.3.4 Effects of Herbal Tea Extracts on DPPH Radical

The results of the evaluation of the antioxidant capacities of the extracts of the various herbal tea samples are shown in Fig. 13. Herbal tea 1 (HT1) had the least $IC_{50}$ value of 343 µg/ml while herbal tea 5 (HT5) had the highest 824 µg/ml.

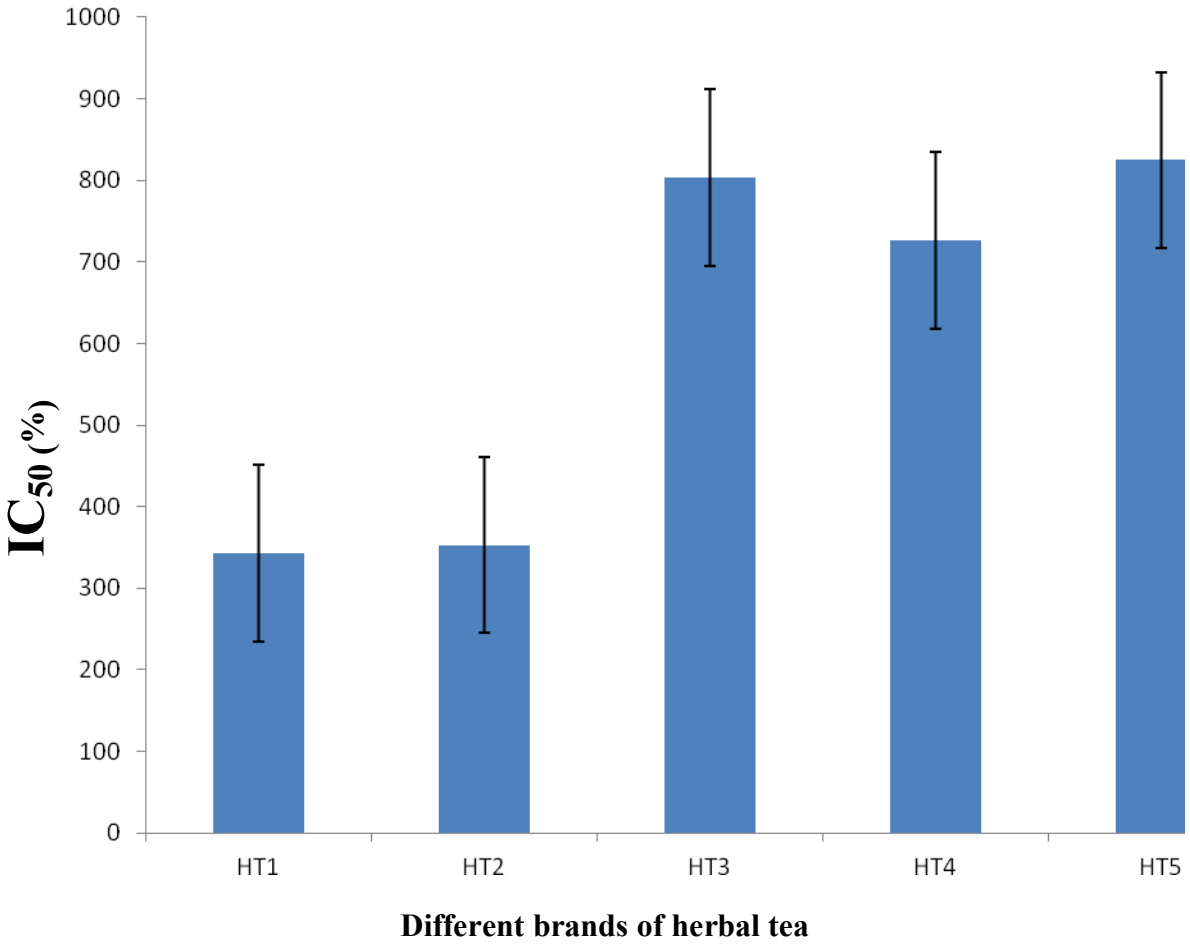


**Fig. 13: Antioxidant activity of extracts of herbal teas against DPPH radical**

**HT1 = Slim Dieters herbal tea, HT2 = Ginseng herbal tea, HT3 = Typhoid herbal tea, HT4 = Fat Burner herbal tea and HT5 = Anti-Diabetes herbal tea.**

### 3.4 Results of The Evaluation of The Antioxidant Activity of Tea Samples Based On Ferric Reducing Antioxidant Power

#### 3.4.1 Effect of Black Tea Extracts on Ferric ($Fe3^{+}$) ions

The results of the evaluation of the antioxidant capacities of the extracts of various black tea samples are shown in Fig. 14. The $IC_{50}$ of the black tea samples were black tea 3 (BT3) (491.22µg/ml), black tea 2 (BT2) (493.17 µg/ml), black tea 5 (BT5) (494.71 µg/ml), black tea 1 (BT1) (496.57 µg/ml) and black tea 4 (BT4) (497.68 µg/ml). The BT4 had the highest $IC_{50}$ and hence, least potent as ferric ion reducer.

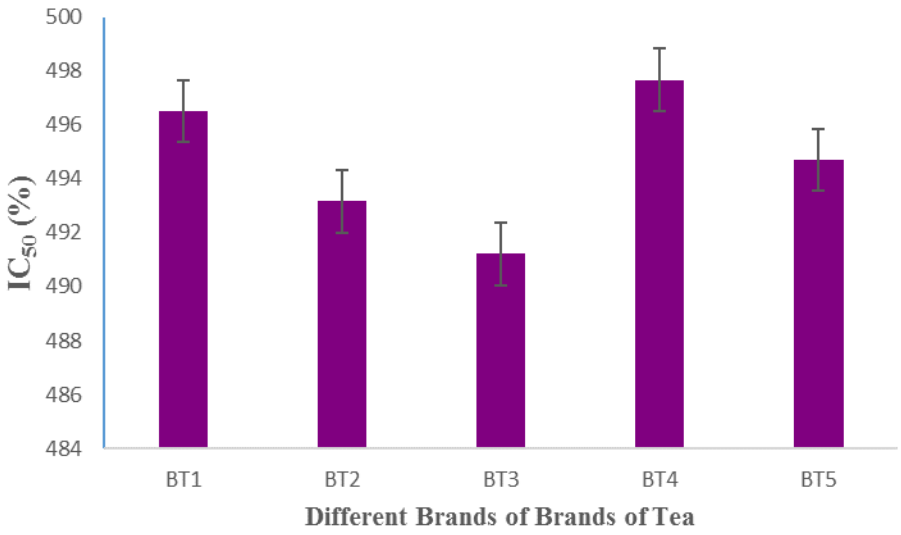


**Fig. 14 Ferric Reducing-Antioxidant Power of un-caffeinated Teas Extracts.**

**BT1=Ahmad black tea, BT2 = LOYD lemon black tea, BT3 = Lipton black tea, BT4 = Dilmah black tea and BT5 = Shenghai Bozehng black tea**

### 3.4.2 Effect of Un-Caffeinated Tea Extract on Ferric ($Fe3^{+}$)

The results of the evaluation of the antioxidant capacities of the extracts of various un-caffeinated tea samples based on their action on $Fe3^{+}$ ion as shown in Fig. 15. The results revealed their $IC_{50}$ to be 598.14µg/ml for un-caffeinated tea 1 (UT1), 593.39µg/ml for un-caffeinated tea 2 (UT2), 597.71µg/ml for un-caffeinated tea 3 601.04µg/ml for un-caffeinated tea 4 (UT4) and 602.20µg/ml un-caffeinated tea 5 (UT5).

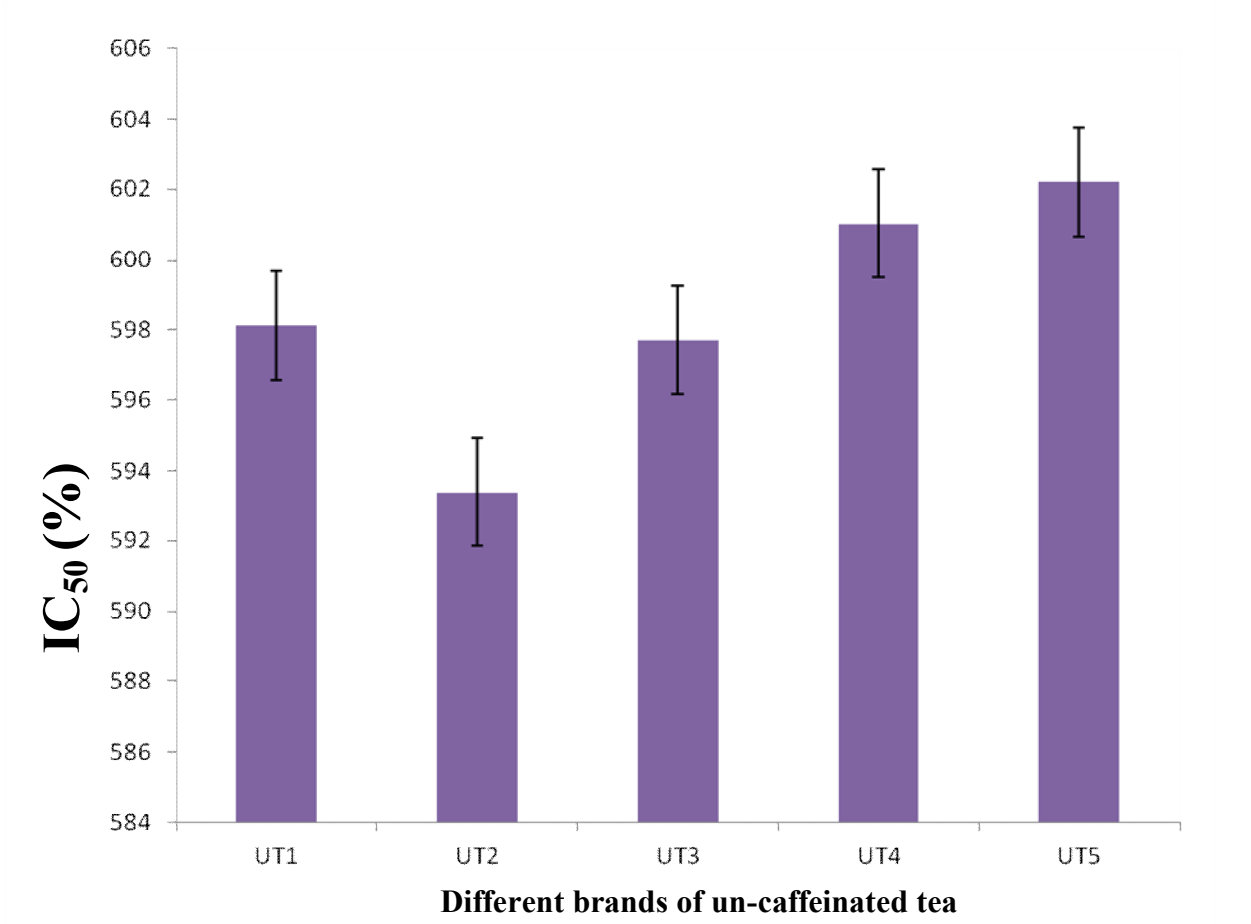


**Fig. 15: Ferric Reducing-Antioxidant Power of un-caffeinated Teas Extracts.**

**UT = Un-caffeinated tea UT1 = Tetley un-caffeinated tea, UT2 = Chamommile un-caffeinated tea, UT3 = Lemon and Ginger un-caffeinated tea, UT4 = Pepermint un-caffeinated tea and UT5 = Red bush un-caffeinated tea.**

### 3.4.2 Effect of Green Tea Extracts on Ferric ($Fe^{3+}$) ions.

The results of the evaluation of the antioxidant capacities of the extracts of various green tea samples based on their action on $Fe^{3+}$ ions are presented in Fig. 16. The results revealed that green tea 1 (GT1) had the least $IC_{50}$ value (614.83µg/ml). Green tea 5 (GT5) had an $IC_{50}$ value of 615.29 µg/ml, green tea 4 (GT4) 616.00µg/ml while green tea 2 (GT2) had 624.02µg/ml. The Green tea 3 (GT3) had the highest $IC_{50}$ value of 624.02µg/ml.

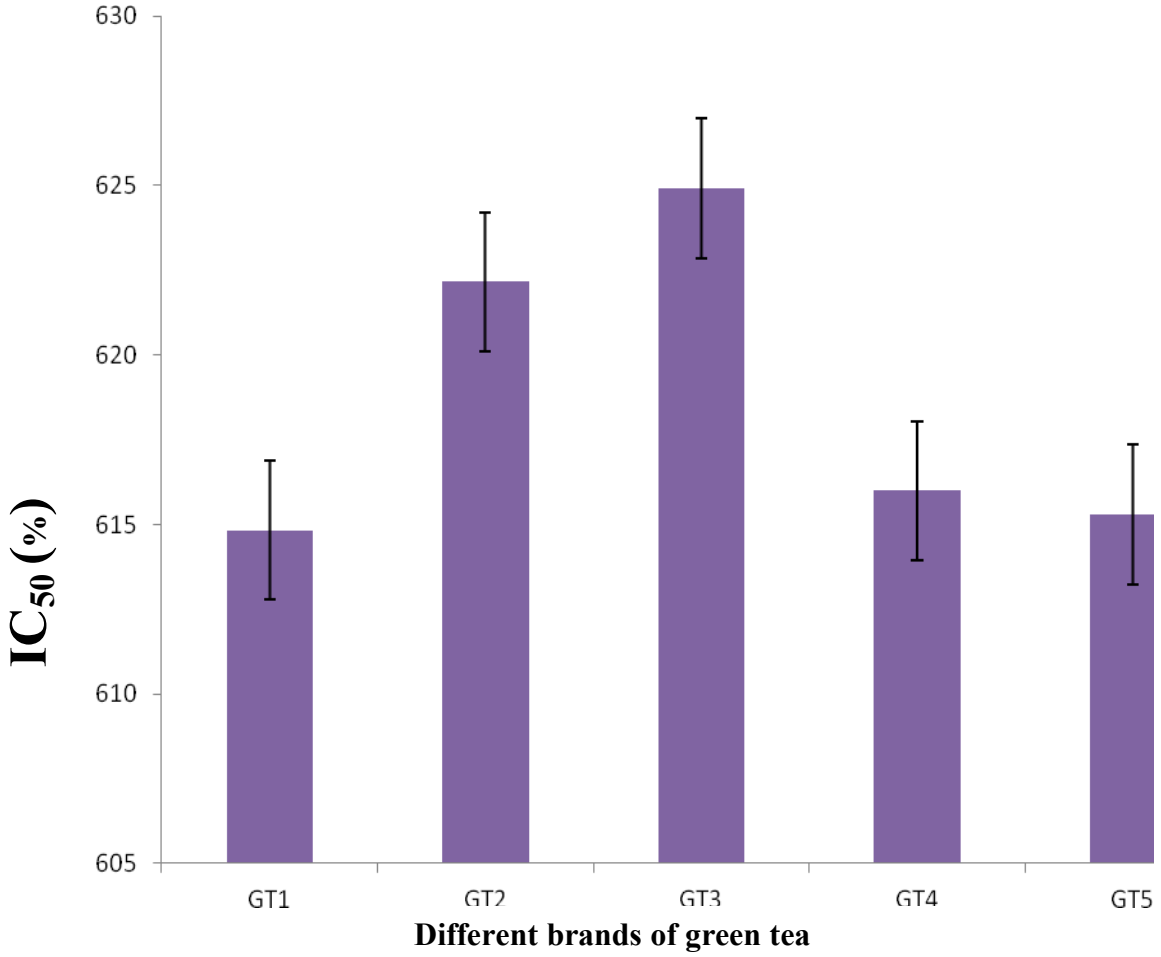


**Fig. 16: Ferric Reducing-Antioxidant Power Green Tea Extracts**

**GT1 = Hillway green tea, GT2 = Moringa green tea, GT3 = Green life CRT green tea, GT4 = Legend green tea and GT5 = Tianshi green tea.**

### 3.4.3 Effects of herbal tea extracts on Ferric ($Fe^{3+}$) ions.

The results of the evaluation of the antioxidant capacities of the extracts of various herbal tea samples based on their action on their action on $Fe^{3+}$ ions are presented in Fig. 17. The results revealed that herbal tea 1 (HT1) had the least $IC_{50}$ with a value of 576.19 µg/ml The $IC_{50}$ values of other herbal tea samples were, herbal tea 5 (HT5) 578.00 µg/ml, herbal tea 2 (HT2), 580.62 µg/ml, herbal tea 3 (HT3) 585.11 µg/ml. Herbal tea 4 (HT4) had the highest $IC_{50}$ value of 587.68 µg/ml.

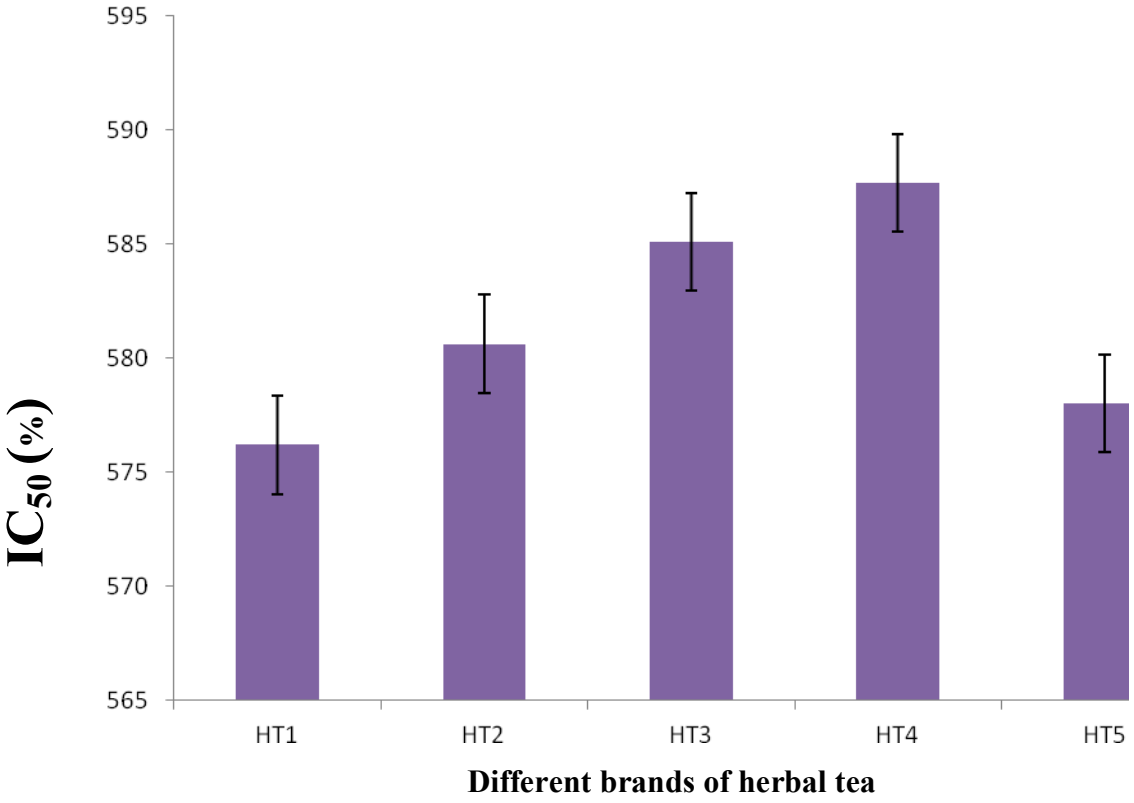


**Fig. 17: Ferric Reducing-Antioxidant Power of herbal Tea Extracts**

**HT1 = Slim Dieters herbal tea, HT2 = Ginseng herbal tea, HT3 = Typhoid herbal tea, HT4 = Fat Burner herbal tea and HT5 = Anti-Diabetes herbal tea.**

### 3.5 A Comparison of the Phytochemicals and Antioxidant Activities of Different Types of Tea

A comparison of the phytochemical constituents and antioxidant activities of different brands of tea is shown in Table 3. The total flavonoid content (TFC) of green tea (215.61±48.83 QE/mg) was significantly ($p < 0.05$) higher when compared to black tea (142.32±22.73 QE/mg), herbal tea (167.25±31.25 QE/mg) and un-caffeinated tea (184.69±33.62 QE/mg). However, there was no significant ($p > 0.5$) difference between the TFC of herbal tea and un-caffeinated tea. The total tannin content (TTC) of black tea (325.14±108.01 GAE/mg) was significantly ($p < 0.05$) lower than un-caffeinated tea (411.55±9.21 GAE/mg), green tea (406.83±22.71 GAE/mg) and herbal tea (402.74±7.95 GAE/mg). However, there was no significant ($p > 0.5$) difference among the TTC of un-caffeinated tea (411.55±9.21 GAE/mg), green tea (406.83±22.71 GAE/mg) and herbal tea (402.74±7.95 GAE/mg). The total phenol content (TPC) of green tea (124.81±79.05 GAE/mg) was significantly ($p < 0.05$) higher than black tea (51.81±8.90 GAE/mg), un-caffeinated tea (63.87±35.76 GAE/mg) and herbal tea (15.78±13.02 GAE/mg). However, there was no significant ($p > 0.5$) difference between the TPC of black tea, un-caffeinated tea and herbal tea. The results of the ferric ion reducing power (FRAP) of green tea ($IC_{50}$ = 618.64±233.63 µg/ml) was significantly ($p < 0.05$) higher than that of un-caffeinated tea ($IC_{50}$ = 598.50±378.25 µg/ml), herbal tea (581.52±271.47 µg/ml) and black tea (494.66±208.82 µg/ml). The DPPH radical scavenging activity of herbal tea ($IC_{50}$ = 610.22±242.02 µg/ml) was the least. There was no significant ($p > 0.05$) difference in the DPPH radical scavenging activity of black tea ($IC_{50}$ = 702.88±104.10 µg/ml), un-caffeinated tea ($IC_{50}$ = 691.72±79.9 µg/ml) and green tea (745.64±161.36).

**Table 3: A comparison of the phytochemical constituents and antioxidant activities of different brands of tea**

| Teas | TFC(QE/mg) | TTC(GAE/mg) | TPC(GAE/mg) | FRAP(µg/ml) | DPPH(µg/ml) |
|---|---|---|---|---|---|
| Black Tea | 142.32±22.73[a] | 325.14±108[a] | 51.81±8.90[a] | 494.66±208.82[a] | 702.88±104.10[b] |
| Un-caffeinated Tea | 184.69±33.62[ab] | 411.55±9.21[b] | 63.87±35.76[a] | 598.50±378.25[c] | 691.72±79.94[b] |
| Green Tea | 215.61±48.83[b] | 406.83±22.71[b] | 124.81±79.05[b] | 618.64±233.63[d] | 745.64±161.36[b] |
| Herbal Tea | 167.25±31.25 [ab] | 402.74±7.95[b] | 15.78±13.02[a] | 581.52±271.47[b] | 610.22±242.02[a] |

Data are mean ± SD

Values with different superscript within a column are significantly different from each other $p < 0.05$.

Abbreviations:
TFC = Total flavonoid content
TTC = Total tannin content
TPC = Total phenol content
FRAP = Ferric reducing antioxidant power
DPPH = 2,2-Diphenyl-2-picrylhydrazyl

### 3.6 The Correlation of the Phytochemical Constituents with Antioxidant Activities

Table 4 shows the correlation of the phytochemical constituents with antioxidant activities of different brands of the teas studied. There was a positive correlation between the antioxidant capacity and the phytochemical constituent of the tea samples. A positive correlation was observed between the TFC and the DPPH radical scavenging activity of the tea samples (with correlation coefficient, r = 0.347). There was a positive correlation between the TPC and the DPPH radical scavenging activity of the tea samples (with correlation coefficient, r = 0.457). Same was observed between TPC and FRAP of the tea samples with correlation coefficient, r = 0.622. Also, a positive correlation was observed between the TFC and FRAP of the tea samples (with a correlation coefficient, r = 0.448), and between the TTC and FRAP (with correlation coefficient, r = 0.060). On the other hand, there was a negative correlation between the TTC and the DPPH radical scavenging activity of the tea samples (with correlation coefficient r = -0.137).

**Table 4: The correlation between the phytochemical constituents and antioxidant activity of different brands of tea**

| Phytochemicals | DPPH | FRAP |
|---|---|---|
| TTC | -0.137 | 0.060 |
| TFC | 0.347 | 0.448 |
| TPC | 0.457 | 0.622 |

TFC = Total flavonoid content,
TTC = Total tannin,
TPC = Total phenol content
FRAP = Ferric reducing antioxidant power
DPPH = 2,2-Diphenyl-2-picrylhydrazyl

# CHAPTER FOUR

# DISCUSSION

In this study, the phytochemical constituents and antioxidant potential of herbal tea, black tea, un-caffeinated tea and green tea samples were determined. The results of the qualitative phytochemical screening, as shown in Table 1, showed that flavonoids and tannins were present in all the tea samples. This observation is in line with that of Funmilayo *et al*. (2012), who also detected the presence of flavonoids and tannins in some of these tea types. Flavonoids and phenolic acids are the most important groups of secondary metabolites of plants and their presence in tea is to be expected (Kim *et al*., 2011). Flavonoids were detected in all the tea types. The total flavonoid content (TFC) of green tea (215.61±48.83 QE/mg) was significantly ($p < 0.05$) higher than un-caffeinated tea (184.69±22.73 QE/mg), herbal tea (167.25±31.25 QE/mg) and black tea (142.32±22.73 QE/mg). The types and amounts of flavonoids present in tea will differ dependent on the variety of leaf, soil condition, processing, particle size of ground tea leaves and infusion preparation. The major polyphenolic compounds in green tea are flavan-3-ols, a class of flavonoid which remains intact even after processing. Processing of black tea involves the condensation of catechin and orthoquinones (oxidation of the B ring di- and trihydroxylated catechins) by polyphenol oxidase to form thea-flavins (TFs) (Karori *et al.,* 2007; Sen and Bera 2012). This could be responsible for the lower TFC observed in the present study. Reports showed that un-caffeinated tea is not processed from a single plant but a blend of herbs that have flavonoid (Diane *et al*., 2006; Kopjar *et al*., 2015). Flavonoids being ubiquitous have been reported to be present in chamomile, redbush and peppermint from which un-caffeinated tea is produced (Kopjar *et al*., 2015). This may be responsible for the higher TFC observed in un-caffeinated tea compared to black tea. Also, for the flavonoid content of tea to be absorbed, the flavonoid must be accessed by hot water infusion and the subsequent action of digestive juices. The absorption of the flavonoid liberated from tea will depend on its physicochemical properties such as molecular size and configuration, lipophilicity, solubility, and pKa (Hollman, 2001). Most flavonoids, except catechins, are usually present in the diet as ɓ-glycosides. Tea catechins and galloylated epigallocatechins gallate (EGCG) occur as aglycones and are quite rapidly absorbed; suggesting absorption from the small intestine. Bioavailability of the various catechin monomers seems to be quite similar. However, dimerization reduces bioavailability. Donovan *et*

*al*. (2001) reported that ethanol could increase their absorption by improving their solubility. Antioxidant activity of flavonoid is concentration dependent; hence, their health benefits are dependent on the amount that is bioavailable after consumption. Tea flavonoids are beneficial to health. They have anti-proliferative, anti-inflammatory, anti-thrombogenic and anti-bacteria properties (Fuchs *et al*., 2014).

Tea ñtanninsò are chemically distinct from other types of plant tannins such as tannic acid. Tannic acid is absent in tea extract (Khasnabis *et al*., 2015). The results of the total tannin content (TTC) of the tea samples showed no significant ($p > 0.05$) difference among the TTC of un-caffeinated tea (411.55±9.21 GAE/mg), green tea (406.83±22.71 GAE/mg) and herbal tea (402.74±7.95 GAE/mg). However, the TTC of these three tea samples were significantly ($p < 0.05$) higher than that of black tea (325.14±108 GAE/mg). This could be because of the differences in the fermentation processes of the various tea types. Processing of black tea converts the tea polyphenols to theaflavin and thearubigin. Obanda *et al*. (2001) reported the presence of these polyphenolic compounds (theaflavin and thearubigin) in black teas. Tannins do not function solely as primary antioxidants (*i.e.*, they donate hydrogen atom or electrons); they also function as secondary antioxidants. They have the ability to chelate metal ions such as Fe(II) and interfere with one of the reaction steps in the Fenton reaction and thereby retard oxidation (Karamac *et al*., 2006). Tannins contribute an astringent or bitter taste to teas, in addition to the antioxidant property; tannins have been reported to possess anti-carcinogenic, anti-mutagenic potential and antimicrobial properties (Amarowicz 2007).

The total phenolic content (TPC) of the aqueous tea extract, as shown in Table 2, revealed that the TPC of green tea (124.81± 79.05 GAE/mg) was significantly ($p < 0.05$) higher than those of un-caffeinated tea (63.67±35.76 GAE/mg), black tea (51.81±8.90 GAE/mg) and herbal tea (15.78±13.02 GAE/mg). This could be attributed to the differences in the processing methods. Processing of black tea, un-caffeinated tea and herbal tea involves the oxidation of their polyphenols to other compounds. In contrast, green tea processing does not affect the polyphenols, hence, high polyphenols content in green tea. Quan *et al.* (2010) reported that green tea polyphenols make up to 30% of the leafôs dry weight. Tea polyphenols are rich natural source of antioxidant. It has broad-spectrum and specific creative effects in antioxidant, anti-atherosclerosis, resistance to dental caries, antitumor, anti-radiation, ant-aging, antimicrobial and

in reducing blood pressure, hematic fat and blood sugar and even in anti-HIV (Khasnabis *et al*., 2015).

The radical scavenging activity of the extract is expressed in $IC_{50}$ (the percentage of the extract that was able to scavenge half the concentration of a radical). The lower the $IC_{50}$ of a substance against a radical the better the radical scavenging power of that substance. The 2,2 Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the tea samples, as shown in Figures 10-14, indicated that the antioxidant potential of a herbal tea (610.22±8.79%) was significantly ($p<0.05$) higher than un-caffeinated tea (691.72±79.94%), black tea (702.88±3.79.94%) and green tea (745.64±4.93%). In this study it was observed from the manufactures description that some of the brands of herbal tea were made by the combinations of tea types, from different plant such as *Camellia Sinensis*, cassia tea, ginseng, apigenin, and tangerine and this could be the reason for the observed higher antioxidant capacity. Furthermore the low radical scavenging activity observed in green tea may be due to the pro-oxidant activity of flavonoid, which was detected in high concentration in green tea. Yen *et al.* (2003) and Procházkováa *et al*. (2011) also reported that flavonoids pro-oxidant activity is concentration dependent. The possible pro-oxidant effects of flavonoids may be important *in vivo* if free transition metal ions are involved in oxidation processes (Procházkováa *et al*., 2011). Flavonoids are capable of reducing Cu(II) to Cu(I) and thus initiate the formation of radicals (Cao *et al*., 1997). In healthy human body, metal ions appear largely sequestered in forms that are unable to catalyse free radicals (e.g in ferritin or caeruloplasmin) (Halliwell, 1998). However, injury to tissues may release iron or copper and catalytic metal ions have also been measured in atherosclerosis lesions (Stadler *et al*., 2004). In this case, the potential for flavonoids to act as pro-oxidants cannot be ignored (Croft, 2006). However, there was no significant ($p > 0.05$) difference between the DPPH radical scavenging power of un-caffeinated tea, black tea and green tea. All the tea samples demonstrated good radical scavenging activity. This could be due to the presence of the secondary metabolites found in the tea samples.

The results of the ferric reducing antioxidant power (FRAP) assay revealed that the $IC_{50}$ of green tea (618.64±4.60%) was significantly ($p < 0.05$) higher than black tea (494.66±2.58 %), un-caffeinated tea (598.50±3.43%) and herbal tea (581.52±4.81%). The black tea had the least $IC_{50}$ and thus, more potent in ferric reducing antioxidant power. Catechin oxidation involved in black

tea preparation leads to the formation of catechin dimers, known as theaflavins and thearubugins. These compounds are responsible for the colour and taste and also key factors in the antioxidant activity of processed teas (Yashin *et al*., 2011). The ability to reduce ferric iron to their ferrous state shows effective radical scavenging characteristics of the various teas. Herbal and un-caffeinated tea contains apigenin and other polyphenolic compounds which may be responsible for the antioxidant activity of the tea samples (Liu and Hu, 2004).

A positive correlation between the antioxidant capacity and the phytochemical constituent of the tea samples was observed. There was a positive correlation between the TTC and FRAP of the tea samples. Tannins have the ability to chelate metal ions such as Fe(II) and interfere with one of the reaction steps in the Fenton reaction and thereby retard oxidation (Karamac *et al*., 2006). However, a negative correlation between tea TTC and DPPH radical scavenging activity was observed. This could be because at certain concentration, tannin acts as pro-oxidant other than antioxidant. A positive correlation was also observed between the TFC and FRAP, and DPPH radical scavenging activity of the tea samples. Similarly, a positive correlation was observed between the total phenol content (TPC) and DPPH radical scavenging activity of the tea samples. Same was observed between TPC and FRAP. The antioxidant activities of tea samples are due to the polyphenol content. Though catechins are oxidised to theaflavins and thearubugin during processing of some tea types, the products also possess radical scavenging activity. Korari *et al.* (2007) reported that conversion of catechins to theaflavins during black tea processing does not affect the radical scavenging potency of black tea. The findings from this study agree with this opinion. These observations are consistent with those of Leung *et al.* (2001) that black tea possesses relatively same antioxidant potency due to catechins present in green tea. This is because theaflavins and thearubugin can donate hydrogen electrons from the hydroxyl groups in their structure to stabilize free radicals. They have been found to have excellent antioxidant activities, more powerful than vitamin C, vitamin E, and ϐ-carotene (Vuong *et al*., 2010). In addition to the capturing (quenching) of free radicals, the tea catechins can chelate metal ions such as iron and copper, preventing their participation in Fenton and Haber-Weiss reactions (Higdon and Frei 2003).

## 4.2 Conclusion

These findings in this study show that the green tea, black tea, un-caffeinated tea and herbal tea samples are rich in important phytochemicals such as flavonoids and tannins, and possess antioxidant potentials. However, the tea types vary in their contents of antioxidants and in their antioxidant potentials. Based on the FRAP assay, black tea had the highest antioxidant potential while green tea had the least. Conversely, based on the DPPH assay, black tea, un-caffeinated tea and green tea had equal antioxidant potential while herbal tea had the highest antioxidant potential.

## 4.3 Recommendation

- The radical scavenging action of antioxidant is concentration-dependent; hence, further studies should be done to validate the dose at which the bioactive constituent of tea will have optimal antioxidant activity.

## REFERENCES


Aggio, A., Grassi, D. and Onori, E. (2012). Endothelium/nitric oxide mechanism mediates vasorelaxation and counteracts vasoconstriction induced by low concentration of flavanols. *European Journal of Nutrition,* **172**: 239ï 262.

Ahmad, N. and Mukhtar, H. (2001). Cutaneous photochemoprotection by green tea: A brief review. *Skin Pharmacology and Applied Skin Physiology*, **14**(2): 69-76.

Amarowicz, R. (2007). Tannins: the new natural antioxidants. *European Journal of Lipid Science and Technology*, **109**: 549-551.

American Diabetes Association (ADA) (2012). Diagnosis and classification of diabetes mellitus. *Diabetes Care*, **35**(1): S64ï S71.

Amie, D., Amie, D.D., Beslo, D. and Trinajstie, N. (2003). Structure-radical scavenging activity relationships of flavonoids. *Croatica Chemica Acta*, **76**: 55-61.

Anderson, R.A. and Polansky, M.M. (2002). Tea enhances insulin activity. *Journal of Agriculture and Food Chemistry,* **50**(24): 7182-7186.

Aqil, F., Ahmad, I. and Mehmood, Z. (2006). Antioxidant and free radical scavenging properties of twelve traditionally used Iranian medicinal plants. *Turkey Journal of Biology*, **30**: 177-183.

Aruoma, O. (1999). Free radicals, antioxidants and international nutrition. *Asia Pacific Journal of Clinical Nutrition*, **8**: 55-63.

Ashok, P.K and Upadhyaya, K. (2012). Tannins are Astringent. *Journal of Pharmacognosy and Phytochemistry*, **1**(3): 45-50.

Bahorun, T., Soobrattee, M.A., Luximon-Ramma, V. and Aruoma, O.I. (2006). Free radicals and antioxidants in cardiovascular health and disease. *International Journal of Medicine,* **1**: 1-17.

Bassiouny, M.A., Kuroda, S. and Yang, J. (2008). Topographic and radiographic profile assessment of dental erosion. Part III: Effect of green and black tea on human dentition. *General Dentistry,* **56**(5): 451-561.

Basu, A., Betts, N.M., Mulugeta, A., Tong, C., Newman, E. and Lyons, T.J. (2013). Green tea supplementation increases glutathione and plasma antioxidant capacity in adults with the metabolic syndrome. *Nutrition Research,* **33**: 180-187.

Bate-Smith, E.C. and Swein T. (1962). Flavonoid compounds. In: Comparative Biochemistry. Mason, H.S. and Florkin, A.M. (Eds.). Academic Press, New York (USA). pp. 755-809.

Bhattacharyya, A., Mandal, D., Lahiry, L., Sa, G. and Das, T. (2004). Black tea protects immunocytes from tumor-induced apoptosis by changing Bcl-2/Bax ratio. *Cancer Letter,* **209**(2): 147-154.

Bohm, H. (1998). Flavonols, flavone and anthocyanins as natural antioxidants of foods and their possible role in the prevention of chronic disease. *Ernahrungswiss,* **2**: 147-163.

Bors, W. (1990). Flavonoids as antioxidants: Determination of radical-scavenging efficiency. *Methods in Enzymology,* **186**: 343-355.

Bourne, L. and Rice-Evans, C. (1998). Bioavailablity of ferulic acid. *Biochemical and Biophysical Research Communication,* **253**: 222-227.

Bravo, L. (1998). Poliphenol: Chemistry, dietary sources, metabolism and nutritional significance. *Nutrition Review,* **56**(11): 317-333.

Brigelius-Flohe, R. (2009). Vitamin E: The shrew waiting to be tamed. *Free Radical Biology and Medicine*, **46**: 543-554.

Bronner, W.E and Beecher, G.R., (1998)**.** Method for determining the content of catechins in tea infusions by high-performance liquid chromatography. *Journal of Chromatography A*. 1;805 1-2:137-142.

Bruneton, J. (1999), Pharmacognosy, Phytochemistry and Medicinal Plants. 2nd Edition. France: Lavoisier Publishing; Pp1072ï 1080.

Bruneton, J. (2001). Pharmacognosie. Phytochimie. Plantes Meôdicinales Paris: Technique Documentation-Lavoisier. Pp 452-462.

Bryan, J., Tuckey, M. and Einöther, S.J.L. (2012). The relationship between tea and other beverage consumption, work performance and mood. *Appetite*, **58**(1): 339ï 346.

Cabrera, C., Gimenez, R. and Lopez, C.M. (2003). Determination of tea components with antioxidant activity. *Journal of Agriculture and Food Chemistry*, **51**: 4427-4435.

Cao, G., Soýc, E. and Prior, R.L. (1997). Antioxidant and prooxidant behaviour of þavonoids: Structureï activity relationships. *Free Radical Biology and Medicine*, **22**: 749ï 60.

Carr, A.C., Zhu, B.Z. and Frei, B. (2000). Potential antiatherogenic mechanisms of ascorbate (vitamin C) and alpha-tocopherol (vitamin E). *Circulation Research,* **87:** 349-354.

Chacko, S.M., Thambi, P.T., Kuttan, R., and Nishigaki, I., (2010). Beneficial effects of green tea: A literature review. *Chinese Medicine*, **5**:13 1-9.

Conney, A.H., Lu, Y.P., Lou, Y.R., Xie, J.G. and Huang, M.T. (1999). Inhibitory effect of green and black tea on tumor growth. *Proceedings of the Society for Experimental Biology and Medicine*, **220**: 229-233.

Costa, L.M., Gouveia, S.T. and Nobrega, J.A. (2002). Comparison of heating extraction procedures for Al, Ca, Mg and Mn in tea samples. *Annals of Science Journal*, **18**: S313-S318.

Croft, K.D. (2006). The chemistry and biological effects of þavonoids and phenolic acids. *Annals of the New York Academy of Sciences*, **854**: 435ï 442.

De Bruin, E.A., Rowson, M.J., Van Buren, L., Rycroft, J.A. and Owen, G.N. (2011). Black tea improves attention and self-reported alertness. *Appetite*, **56**: 235-240.

De Felice, L., S. (1995). The nutraceutical revolution, its impact on food industry. *Trends in Food Science and Technology*, **6**:59-61.

DeMan, J.M. (1999). Principles of Food Chemistry. Aspen Publishers, Gaithersburg. pp. 239-62.

Deng, Z., Tao, B., Li, X, He, J. and Chen, Y. (1998). Effect of green tea and black tea on the metabolisms of mineral elements in old rats. *Biological Trace Element Research*, **65**: 75-86.

Diane L.M. and Jeffrey B.B. (2006). A Review of the Bioactivity of South African Herbal Teas: Rooibos (*Aspalathus linearis*) and Honeybush (*Cyclopia intermedia*). *Phytotheraphy Research.* **21** 1-6.

Donovan, J.L., Crespy, V., Manach, C., Morand, C., Besson, C., Scalbert, A. and Remesy, C. (2001). Catechin is metabolized by both the small intestine and liver of rats. *Journal of Nutrition*, **131**: 1753-1757.

Droge, W. (2002). Free radicals in the physiological control of cell function. *Physiological Review*, **82**: 47-95.

Duarte, T.L. and Lunec, J. (2005). When is an antioxidant not an antioxidant? A review of novel actions and reactions of vitamin C. *Free Radical Research,* **39**(7): 671-686.

Dulloo, A.G., Duret, C., Rohrer, D., Girardier, L., Mensi, N., Fathi, M., Chantre, P. and Vandermander, J. (1999). Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans. *American Journal of Clinical Nutrition*, **70**(6): 1040-1045.

Egashira, N. and Ishigami, N. Pu, F., Mishima, K., Iwasaki, K., Orito, K., Oishi, R., Fujiwara, M. (2008). Theanine prevents memory impairment induced by repeated cerebral ischemia in rates. *Phytotherapy Research*, **22**(1): 65-68.

Embola, C.W., Weisburger, J.H. and Weisburger, M.C. (2001). Urinary excretion of N-OH-2-amino-3-methylimidazo [4,5-f]quinoline-N-glucuronide in F344 rats is enhanced by green tea. *Carcinogenesis,* **22**: 1095-1098.

Erickson, L. (2003). Rooibos tea: Research into antioxidant and anti-mutagenic properties. *Herbal Gram*, **59**: 34-45.

Evans, W.C. (2002). Trease and Evans Phamacognosy. 15th Edition. W.R. Sauders, London. pp. 137-140.

Farbstein, D., Kozak-Blickstein, A. and Levy, A.P. (2010). Antioxidant vittamins and their use in preventing cardiovascular disease. *Molecules*, **15**(11): 8098-8110.

Fuchs, D., Great, Y., Kerckhoven, R. and Draijer, R. (2014). Effect of tea theaflavins on microvascular function. *Nutrient,* **6**(12): 5772-5785.

Funmilayo, O.O., Kamaldeen A. and Buhari a.m. (2012). Phytochemical screening and ant-microbial properties of a common brand of black tea (*Camellia Sinensis*) marketed in Nigerian environment. *Advanced Pharmaceutical Bulletin* **2:** 259-263.

Ganguly, C., Saha, P., Panda, C.K. and Das, S. (2005). Inhibition of growth, induction of apoptosis and alteration of gene expression by tea polyphenols in the highly metastatic human lung cancer cell line NCI-H460. *Asian Pacific Journal of Cancer Preview*, **6**(3): 326-331.

Geleijnse, J.M., Launer, L.J, Hofman, A., Pols, H.A.P. and Witteman, J.C.M. (1999). Tea flavonoids may protect against atherosclerosis: The Rotterdam Study. *Archives of Internal Medicine*, **159**: 2170-2174.

Genestra, M. (2007). Oxyl radicals, redox-sensitive signalling cascades and antioxidants. Review. *Cell Signal*, **19**: 1807-1819

Ghasemzadeh, A., Jaafar, H.Z. and Rahmat, A. (2011). Effects of solvent type on phenolics and flavonoids content and antioxidant activities in two varieties of young ginger (*Zingiber officinale Roscoe*) extracts. *Journal of Medicinal Plant Research*, **5**(7): 1147-1154.

Gholivand, M.B. and Piryaei, M. (2014). Total phenols, flavonoids, anthocyanins, ascorbic acid ccontents and antioxidant activity of *Rhamnus Kardica* Boiss for flowering and pre-flowering stages. *African Journal of Biochemistry,* **13**(10): 1131-1135.

Glaser, T.S., Doss, L.E., Shih, G., Nigam, D., Sperduto, R.D., Agrón, E., Clemons, T.E. and Chew, E.Y. (2015). The association of dietary lutein plus zeaxanthin and B vitamins with cataracts in the age-related eye disease study. *Ophthalmology,* **122**(7): 1471-1479.

Graham, H.N. (1992) Green tea composition, consumption, and polyphenol chemistry. *Preventive Medicine,* **21**: 334-350.

Grassi, D., Mulder, T.P. and Draijer, R. (2009). Black tea consumption dose-dependently improves flow-mediated dilation in healthy males. *Journal of Hypertension*, **27**: 774ï 781.

Groot, H. and Rauen, U. (1998). Tissue injury by reactive oxygen species and the protective effects of flavonoids. *Fundamental and Clinical Pharmacology,* **3**: 249-255.

Habu, T., Flath, R.A., Mon, T.R., and Morton, J.F. (1985). Volatile components of rooibos tea (*Aspalathus linearis*). *Journal of Agriculture and Food Chemistry,* **33**: 249ï 254.

Hagerman, A. E., Zhao, Y. and Johnson, S. (2005). Methods for determination of condensed and hydrolysable tannins. *ACS Symposium Series*, **662**: 209ï 222.

Hakim, I.A, Harris, R.B. and Weisgerber, U.M. (2000). Tea intake and squamous cell carcinoma of the skin: Influence of type of tea beverages. *Cancer Epidemiological Biomarkers Preview,* **9**(7): 727-731.

Hakim, I.A. and Chow, S.H. (2004). Green tea, polyphenol and cancer prevention. In: Proceedings of International Conference on Ocha (Tea) Culture and Science. Shizuoka. Japan.

Hakim, I.A. and Harris, R.B. (2001). Joint effects of citrus peel use and black tea intake on the risk of squamous cell carcinoma of the skin. *BMC Dermatology*, **1**(1): 3-7.

Hakim, I.A., Alsaif, M.A., Alduwaihy, M., Al-Rubeaan, K., Al- Nuaim, A.R. and Al-Attas, O.S. (2003). Tea consumption and the prevalence of coronary heart disease in Saudi adults: results from a Saudi national study. *Preventive Medicine,* **36**(1): 64-70.

Hakim, I.A., Chow, H.H.S. and Harris, R.B. (2008). Green tea consumption is associated with decreased DNA damage among GSTM1 positive smokers regardless of their hOGG1 genotype. *Journal of Nutrition*, **138**: S1567ï S1571.

Hakim, I.A., Harris, R.B., Brown, S., Chow, H.H., Wiseman, S., Agarwal, S. and Talbot, W. (2003). Effect of increased tea consumption on oxidative DNA damage among smokers: A randomized controlled study. *Journal of Nutrition*, **133**(10): S3303- S3309.

Halliwell, B. (1994). Free radicals, antioxidants, and human disease: Curiosity, cause, or consequence. *The Lancet,* **344**: 721-724.

Halliwell, B. (2006). Reactive species and antioxidants. *Plant Physiology*, **141**: 312-322.

Halliwell, B. (2007). Biochemistry of oxidative stress. *Biochemical Society Transactions*, **35**: 1147-1150.

Halliwell, B. and Gutteridge, J.M.C. (1998). Free radicals in biology and medicine. Third Edn. Oxford University Press, Oxford.

Hamdaoui, M.H., Chabchob, S. and Heidhili, A. (2003). Iron bioavailability and weight gains to iron-deficient rats fed a commonly consumed Tunisian meal ñbean seeds ragoutò with or without beef and with green or black tea decoction. *Journal of Trace Element in Medicine and Biology*, **17**: 159-164.

Hamilton-Miller, J.M. (1995). Antimicrobial properties of tea (*Camellia sinensis* L.). *Antimicrobial Agents and Chemotherapy*, **39**(11): 2375-2377.

Hang, L., Meng, X., Chuvan, I., Sang, S., Patten, C., Sheng, S., Hung, J., Winnik, B. and Yang, S. (2003). Glucoronides of tea catechins: Enzymology of biosynthesis and biological activities. *Drug Metabolism Disposition,* **31**: 452-461.

Hanneken, A., Lin, F.F., Johnson, J. and Maher, P. (2006). Flavonoids protect human retinal pigment epithelial cells from oxidative stress-induced death. *Investigative Ophthalmology and Visual Science*, **47**: 3164-3177.

Hansen, H.V. and Christensen, K.I. (2009). The common chamomile and the scentless may weed revisited. *Taxonomy*, **58**: 261-264.

Higdon, J.V. and Frei, B. (2003). Tea catechins and polyphenols: Their impact on health, metabolism and antioxidative functions. *Critical Review of Food Science and Nutrition*, **43**: 89-143.

Hilal, Y. and Engelhardt, U. (2007). Characterization of white tea comparison to green and black tea, *Journal für Verbraucherschutz und Lebensmittelsicherheit*, **2:** 414-421.

Hollman, P.C., Van, H. H.K.H., Tijburg, L.B. and Katan, M.B. (2001). Addition of milk does not affect the absorption of flavanols from tea in man. *Free Radical Research,* **34** (3): 297-300.

Hu, G. and Bidel, S. (2007). Coffee and tea consumption and the risk of Parkinsonôs disease. *Journal of Movement Disorder*, **22**(15): 2242-2248.

Huang, K. (1999). The Pharmacology of Chinese Herbs. 2nd Edition. CRC Press, Boca Raton, FL. pp. 209-213.

Huber, L.G. (2003). Green tea catechins and L-theanine in integrative cancer care: A review of alternative complement therapy. *Archives of Internal Medicine*, **9**: 294ï 298.

Hurrell, R.F. Reddy, M. and Cook, J.D. (1999). Inhibition of non-haem iron absorption in man by polyphenolic-containing beverages. *The British Journal of Nutrition*, **81**(4): 289-295.

Hursel, R., Viechtbauer, W. and Dulloo, A.G. (2011). The effects of catechin rich teas and caffeine on energy expenditure and fat oxidation: A meta-analysis. *Obesity Review,* **7**: 573-581.

Hursel, R., Viechtbauer, W. and Westerterp-Plantenga, M.S. (2009). The effects of green tea on weight loss and weight maintenance: A meta-analysis. *International Journal of Obesity,* **33**(9): 956-961.

José, M. Barbosa-Filho A.A., Alencar, X.P., Nunes, A.C., de Andrade, T. José, G., Sena-Filho, P.F., Athayde-Filho, M.S., Silva, M.F., Vanderlei de Souza, E.V. Leitão da-Cunha (2008). Sources of alpha-, beta-, gamma-, delta- and epsilon-carotenes. *Revistra. Brassilera. Farmacogn.* 18:1

Kabel, A.M. (2014). Free radicals and antioxidants: Role of enzymes and nutrition. *Journal of Nutrition and Health,* **2**(3): 35-38.

Kakuda, T., Yanase, H., Utsunomiya, K. and Nozawa, A. (2000). Protective effect of gamma-glutamylethylamide (theanine) on ischemic delayed neuronal death in gerbils. *Neuroscience Letters*, **289**: 189-192.

Kamath, A.B., Wang, L., Das, H., Li, L., Reinhold, V.N. and Bukowski, J.F. (2003). Antigens in tea-beverage prime human V gamma 2Vdelta 2 T cells *in vitro* and *in vivo* for memory and non-memory antibacterial cytokine responses. *Proceedings of the National Academy of Sciences*, **100(**10): 6009-6014.

Kao, Y.H., Hiipakka, R.A. and Liao, S. (2000). Modulation of endocrine systems and food intake by green tea epigallocatechin gallate. *Endocrinology*, **141**:980ï 987.

Karamac, M., Kosinska, A. and Amarowicz, R. (2006). Chelating of Fe(II), Zn(II) and Cu(II) by tannin fractions separated from hazelnuts, walnuts and almonds. *Bromat Chem Toksikol*, **39:** 257-260.

Karori, S.M., Wachira, F.N., Wanyoko, J.K. and Ngure, R.M. (2007). Antioxidant capacity of different types of tea product. *African Journal of Biochemistry*, **6(**19): 2287-2296.

Katiyar, S.K., Bergamo, B.M., Vyalil, P.K. and Elmets, C.A. (2001). Green tea polyphenols: DNA photodamage and photoimmunology. *Journal of Photochemistry Photobiology,* **65**(2-3): 109-114.

Katiyar, S.K., Perez, A. and Mukhtar, H. (2000). Green tea polyphenol treatment to human skin prevents formation of ultraviolet light B-induced pyrimidine dimers in DNA. *Clinical Cancer Research*, **6**(10): 3864-3869.

Kawakami, M., Kobayashi, A. and Kator, K. (1993). Volatile constituents of rooibos tea (*Aspalathus linearis*) as affected by extraction process. *Journal of Agriculture and Food Chemistry,* **41**: 633-636.

Kawashima, A., Madarame, T., Koike, H., Komatsu, Y. and Wise, J.A. (2007). Four week supplementation with mixed fruit and vegetable juice concentrates increased protective serum antioxidants and folate and decreased plasma homocysteine in Japanese. *Asia Pacific Journal of Clinical Nutrition,* **16**(3): 411-421.

Kelly, S.P., Gomez-Ramirez, M., Montesi, J.L. and Foxe, J.J. (2008). L-Theanine and caffeine in combination affect human cognition as evidenced by oscillatory alpha-band activity and attention task performance. *Journal of Nutrition*, **138**: S1572- S1577.

Khasnabis. J., Rai, C. and Roy, A. (2015). Determination of tannin content by titrimetric method from different types of tea. *Journal of Chemical and Pharmaceutical Research*, **7**(6): 238-241.

Kim, Y., Goodner, K.L., Park, J.D., Choi, J. and Talcott, S.T. (2011). Changes in antioxidant phytochemicals and volatile composition of *Camellia sinensis* by oxidation during tea fermentation. *Food Chemistry*, **129**(4): 1331ï 1342.

Kokate, C.K., Purohit, A.P. and Gokhale, S.B. (2002). Nutraceutical, Cosmaceutical and Pharmacognosy. 21st Edition. Nirali Prakashan, Pune, India. pp. 542-549.

Kopjar, M., Tadic, M., and Pilizota., V. (2015). Phenol content and antioxidant activity of Green, Yellow and Black Tea. *Chemical and Biological Technology in Agriculture*. **2**:1, 1-6.

Kumar, N., Titus-Ernstoff, L., Newcomb, P.A., Trentham-Dietz, A., Anic, G. and Egan K.M. (2009). Tea consumption and risk of breast cancer. *Cancer Epidemiological Biomarkers Preview*, **18**(1): 341-345.

Kunwar, A. and Priyadarsini, K.I. (2011). Free radicals, oxidative stress and importance of antioxidant in human health. *Journal of Medical and Allied Sciences,* **1**(2): 53-60.

Kushiyama, M., Shimazaki, Y., Murakami, M. and Yamashita, Y. (2009). Relationship between intake of green tea and periodontal disease. *Journal of Periodontology,* **80**(3): 372-377.

Langseth, L. (1993). From the Editor: Antioxidants and diseases of the brain. *Antioxidant Vitamins Newsletter*, **4**: 3.

Larsson, S.C. and Wolk, A. (2005). Tea consumption and ovarian cancer risk in a population-based cohort. *Archives of Internal Medicine*, **165**(22): 2683-2686.

Leung, L.K, Su, Y., Chen, R. and Zhang, Z. (2001). Theaflavins in black tea and catechins in green tea are equally effective antioxidants. *Journal of Nutrition*, **131**: 2248-2251.

Li, N., Zheng, S., Han, C., and Chen, J. (1999). The Chemoprotective Effects of Tea on Human Oral Precancerous Mucosa Lesions. *Proceedings of The Society for Experimental Biology and Medicine,* **220**:218-224.

Liang, J., Puligundla, P., Ko, S. and Wan, X-C. (2014). A Review on Selenium-enriched Green Tea: Fortification Methods. *Biological Activities and Application Prospect,* **43**(11): 1685ï 1692.

Lindahl, M. and Tagesson, C. (1997). Flavonoids as phospholipase $A_2$ inhibitors: Importance of their structure for selective inhibition of group II phospholipase $A_2$. *Inflammation,* **21**: 347-356.

Linke, H.A. and LeGeros, R.Z. (2003). Black tea extract and dental caries formation in hamsters. *International Journal of Food Science and Nutrition*, **54**(1): 89-95.

Liu, T. and Hu, Y.Z., (2004). Synth. Commun. *Total synthesis of apigenin* 34, 3209-3217

Loizzo, M.R., Pugliese, A. and Menichini, F. (2012). Radical scavenging antioxidant and ferric reducing activities of commercial mineral water enriched with fruit and ready to drink flavoured teas. *Open Nutraceuticals Journal,* **5**: 160ï 168.

Lu, Y.P., Lou, Y.R., Lin, Y., Shih, W.J., Huang, M.T., Yang, C.S. and Conney, A.H. (2001). Inhibitory effects of orally administered green tea, black tea, and caffeine on skin carcinogenesis in mice previously treated with ultraviolet B light (high-risk mice): Relationship to decreased tissue fat. *Cancer Research,* **161**(13): 5002-5009.

Mandel, S., Packer, L., Moussa, Y.B.H. and Weireb, O. (2006). Proceedings from the 3rd international conference on mechanism and action of nutraceuticals. *Journal of Nutritional Biochemistry,* **16:** 513-520.

Mandel, S.A, Amit, T., Kalfon, L., Reznichenko, L. and Youdim, M.B.H. (2008). Targeting multiple neurodegenerative diseases etiologies with multimodal-acting green tea catechins. *Journal of Nutrition*, **138**: S1578ï S1583.

Mark. P. (1996). Antioxidant Review. *Clinical Nutrition Insight*. **6:** 342-352

Mensor, L.L, Menezes, F.S., Leitão, G.G., Reis A.S., Santos, T.C. and Coube, C.S. (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy Research*, **15**(2): 127-130.

Meyer-Buchtela E. (1999). Tee-Rezepturen: Ein Handbuch für Apotheker und Ärzte. Stuttgart, Germany: Deutscher Apotheker Verlag. Pp 234-264.

Miketova, P., Schram, K. and Whitney, J. (1998). Mass spectrometry of selected components of biological interest in green tea extracts. *Journal of Natural Products,* **61**(4): 461-467.

Miller, A.L. (1996). Antioxidant flavonoids: Structure, function and clinical usage. *Alternative Medicine Review,* **1**: 103-111.

Mira, L., Fernandez, M.T., Santos, M., Rocha, R., Florencio, M.H. and Jennings, K.R. (2002). Interactions of flavonoids with iron and copper ions: A mechanism for their antioxidant activity. *Free Radical Research*, **36**: 1199-1208.

Miura, S., Watanabe, J., Sano, M., Tomita, T., Osawa, T. and Hara, Y. (1995). Effects of various natural antioxidants on the $Cu^{2+}$-mediated oxidative modification of low density lipoprotein. *Biological and Pharmaceutical Bulletin,* **18**: 1-4.

Morton, J.F. (1983). Rooibos tea, *Aspalathus linearis,* a caffeineless, low-tannin beverage. *Economic Botany,* **37**: 164-173.

Murase, T., Haramizu, S., Shimotoyodome, A. and Tokimitsu, I. (2005). Reduction of diet-induced obesity by a combination of tea-catechin intake and regular swimming. *International Journal of Obesity,* **30**(3): 561-568.

Murase, T., Haramizu, S., Shimotoyodome, A., Tokimitsu, I. and Hase, T. (2006). Green tea extract improves running endurance in mice by stimulating lipid utilization during exercise. *American Journal of Physiology- Regulatory, Integrative and Comparative Physiology,* **290**(6): R1550-1556.

Murase, T., Nagasawa, A., Suzuki, J., Hase, T. and Tokimitsu, I. (2002). Beneficial effects of tea catechins on diet-induced obesity: Stimulation of lipid catabolism in the liver. *International Journal of Obesity and Related Metabolic Disorders,* **26**(11): 1459-1464.

Mythili, K., Reddy, C. U., Chamundeeswari, D., and Manna, P.K. (2014). Determination of total phenol, alkaloid, flavonoid and tannin in different extracts of *Calanthe triplicata*. *Journal of Pharmacognosy and Phytochemistry,* **2**(2): 40-44.

Nagao, T., Hase, T. and Tokimitsu, I. (2007). A green tea extract high in catechins reduces body fat and cardiovascular risk in humans. *Obesity,* **15**: 1473-1483.

Nagao, T., Komine, Y., Soga, S., Meguro, S., Hase, T., Tanaka, Y. and Yokimitsu I. (2005). Ingestion of a tea rich in catechins leads to a reduction in body fat and malondialdehyde-modified LDL in men. *American Journal of Clinical Nutrition*, **81**(1): 122-129.

Narotzki, B., Reznick, A.Z., Aizenbud, D. and Levy, Y. (2012). Green tea: A promising natural product in oral health. *Archives of Oral Biology*, **57**: 429-435.

Nelson, M. and Poulter, J. (2004). Impact of tea drinking on iron status in the UK: A review. *Journal of Human Nutrition and Diet,* **17**: 43-54.

Niki, E (2001). Antioxidants in relation to lipid peroxidation. *Chemistry and Physics of Lipids*, ***44***, 227-253.

Niki, E. (2001). Antioxidants in relation to lipid peroxidation. *Chemistry and Physics of Lipids*, **44**: 227-253.

Nugala, B., Namasi, A., Emmadi, P. and Krishna, P.M. (2012). Role of green tea as an antioxidant in periodontal disease. The Asian paradox. *Journal of Indian Society of Periodontology*, **16**(3): 313-316.

Obanda, M., Owuor, P. and Mangôka, R. (2001) Changes in the chemical and sensory quality parameters of black tea due to variations of fermentation time and temperature. *Food Chemistry*, **75**:395-404.

Okushio, K., Suzuki, M., Matsumoto, N., Nanjo, F. and Hara, Y. (2001). Methylation of tea catechins by rat liver homogenates. *Bioscience Biotechnology and Biochemistry*, **63**: 430-432.

Olaniyi, O.O., Odeyemi, O. A., Adewale, B. D., Oloyede, A.A., Anagbogu, C.F., Adeigbe, O.O. and Adenuga, O.O. (2014). Tea (*Camellia Sinensis*) Breeding in Nigeria: Past and Present Status. *International Journal of Scientific and Research Publications*. 4:9

Omolaja S.S. and Esan E.B. (2005). Yield evaluation of high altitude tea [Camellia sinensis (L.) O. Kunze] in lowland ecologies of Nigeria. *Nigeria Journal of Horticultural Science*. 10: 87-93.

Pack, S., Jung, I.C. and Lee, W.K. (2011). A Combination of green tea extract and L-theanine improves memory and attention in subjects with mild cognitive impairment: A double-blind placebo-controlled study. *Journal of Medicine and Food,* **14**(4): 334-343.

Paola, R.D., Mazzon, E., Muia, C., Genovese, T., Menegazzi, M., Zaffini, R., Suzuki, H. and Cuzzocrea, S. (2005). Green tea polyphenols attenuates lung injury in carrageean-induced pleurisy injury in mice. *Respiratory Research,* **6**: 1465-9921.

Pham-Huy, N.L.A., He, H. and Pham-Huy, C. (2008). Green tea and health- An overview. *Journal of Food Agriculture and Environment,* **6**: 6-13.

Pourmorad, F., Husseinimehr, S.J. and Shahabimajd, N. (2006). Antioxidant, phenol and flavonoid contents of some selected Iranian medicinal plants. *African Journal of Biotechnology,* **5**: 1142-1145.

Procházkováa, D., Bouġováb, I. and Wilhelmováa, N. (2011). Antioxidant and pro-oxidant properties of þavonoids. *Fitoterapia,* **82**: 513ï 523.

Rashida, A., Mohammad, A., Umer A. S., Abudiat, A., and Manzar Q., (2009). Review Nutraceuticals as natural healers: Emerging evidences. *African Journal of Biotechnology,* **8** (6):891-898.

Raymond, C., James, D.M., and Dorothy, M.M., (2005). Medicinal Benefits of Green Tea: Part I. Review of Noncancer Health Benefits. *The Journal of Alternative and Complementary Medicine.* 11:3, 521ï 528.

Record, I.R., McInerney, J.K. and Dreosti, I.E. (1996). Black tea, green tea, and tea polyphenols: Effects on trace element status in weanling rats. *Biological Trace Element Research*, **53**: 27-43.

Reeves, S.G., Owuor, P.O. and Othieno, C.O. (1987). Biochemistry of black tea manufacture. *Tropical Science*, **27**: 121-133.

Rezai-Zadeh, K., Shytle, D., Sun, N., Mori, T., Hou, H., Jeanniton, D., Ehrhart, J., Townsend, K., Zeng, J., Morgan, D., Hardy, J., Town, T. and Tan, J. (2005). Green tea epigallocatechin-3-gallate (EGCG) modulates amyloid precursor protein cleavage and reduces cerebral amyloidosis in Alzheimer transgenic mice. *Journal of Neuroscience,* **25**(38): 8807-8814.

Rhodes, L.E., Darby, G., Massey, K.A., Clarke, K.A. and Dew, T.P. (2013). Oral green tea catechin metabolites are incorporated into human skin and protect against UV radiation-induced cutaneous inflammation in association with reduced production of pro-inflammatory eicosanoid 12-hydroxyeicosatetraenoic acid. *British Journal of Nutrition,* **28**: 1-10.

Rietveld, A. and Wiseman, S. (2003). Antioxidant effects of tea: Evidence from human clinical trials. *Journal of Nutrition,* **133**: 3275ï 3284.

Roberts, L.J., Fessel, J.P., and Davies, S.S., (2005). The biochemistry of the isoprostane, neuroprostane, and isofuran pathways of lipid peroxidation. *Brain Pathology*. **15**:143ï 148.

Roy, M., Chakrabarty, S., Sinha, D., Bhattacharya, R.K. and Siddiqi, M. (2003). Anticlastogenic, antigenotoxic and apoptotic activity of epigallocatechin gallate: A green tea polyphenol. *Mutation Research*, **33**(41): 523-524.

Rumsey, S.C., Wang, Y. and Levine, M. (1999) Vitamin C. In: Antioxidant Status, Diet, Nutrition, and Health. Papas, A.M. (Ed.). CRC Press, Boca Raton, Fla. pp. 479ï 496.

Saira Saeed Khan, Rahila Najam1, Humera Anser, Bushra Riaz and Nausheen Alam (2014) Chamomile tea: Herbal hypoglycemic alternative for conventional medicine. *Pakistan Journal of Pharmaceutical Science.* **27**:51509-51514

Sakamoto, Y., Mikuriya, H., Tayama, K., Takahashi, H., Nagasawa, A., Yano, N., Yuzawa, K., Ogata, A. and Aoki, N. (2001). Goitrogenic effects of green tea extract catechins by dietary administration in rats. *Archive of Toxicology*, **75**: 591-596.

Samman, S., Sandstrom, B., Toft, M.B., Bukhave, K., Jensen, M., Sorensen, S.S. and Hansen, M. (2001). Green tea or rosemary extract added to foods reduces nonheme-iron absorption. *American Journal of Clinical Nutrition,* **73**: 607-612.

Sano, M.Y., Takeuaka, R., Kojima, S.I., Saito, I., Tomita, H. and Kato, H. (1986). Effects of pu-erh tea on lipid metabolism in rats. *Chemical and Pharmaceutical Bulletin*, **34**: 221-228.

Sarkar, S., Sett, P., Chowdhury, T. and Ganguly, D.K. (2000). Effect of black tea on teeth. *Journal of Indian Society of Pedodontics Preventive Dentistry*, **18**: 139-140.

Satoh, K., Sakamoto, Y., Ogata, A., Nagai, F., Mikuriya, H., Numazawa, M., Yamada, K. and Aoki, N. (2002). Inhibition of aromatase activity by green tea extract catechins and their endocrinological effects of oral administration in rats. *Food and Chemical Toxicology*, **40**: 925-933.

Sayama, K., Lin, S., Zheng, G. and Oguni, I. (2000). Effects of green tea on growth, food utilization, and lipid metabolism in mice. *In vivo*, **14**:481ï 484.

Scarmeas, N., Luchsinger, J.A., Schupf, N., Brickman, A.M, Cosentino, S., Tang, M.X. and Stern, Y. (2009). Physical activity, diet, and risk of Alzheimer disease. *Journal of the American Medical Association*, **302**(12): 627-635.

Schmidt, M., Schmitz, H.J, Baumgart, A., Guedon, D., Netsch, M.I, Kreuter, M.H, Schmidlin, C.B. and Schrenk, D. (2005). Toxicity of green tea extracts and their constituents in rat hepatocytes in primary culture. *Food and Chemical Toxicology,* **43**: 307-314.

Scholz, E. and Bertram, B. (1995). *Camellia sinensis* (L.) O. Kuntze ï Der Teestrauch. *Z. Phytotherapy,* **16**: 235ï 250.

Sen, G. and Bera, B. (2012). Black tea as a part of daily diet: A boon for healthy living *International Journal of Tea Science.* **9**: 2-8.

Serafini, M. (2006). The role of antioxidants in disease prevention. *Medicine,* **34**(12): 533-535.

Shekelle, P., Morton, S. and Hardy, M.L. (2003). Effect of supplemental antioxidants vitamin C, vitamin E, and coenzyme Q10 for the prevention and treatment of cardiovascular disease. *Evidence Report and Technology Assessment Summary,* **83**: 1-3.

Shimotoyodome, A., Haramizu, S., Inaba, M., Murase, T. and Tokimitsu, I. (2005). Exercise and green tea extract stimulate fat oxidation and prevent obesity in mice. *Medical Science Sports Exercise*, **37**(11): 1884-1892.

Siddiqui, I.A. and Saleem, M. (2007). Tea beverage in chemoprevention and chemotherapy of prostate cancer. *Acta Pharmacological Sinica,* **28**(9): 1392-1408.

Siex, H. (1997). Physiological society symposium: Impaired endothelial and smooth muscle cell function in oxidative stress: Oxidants and antioxidants. *Experimental Physiology*, **82**: 291-295.

Singh, R., Verma, P.K. and Singh, G. (2012). Total phenolic, flavonoids and tannin contents in different extracts of *Artemisia absinthium*. *Journal of Interculture Ethnopharmacology*, **1**(2): 101-104.

Stadler, N., Lindner., R.A. and Davies, M.J. (2004).Directdetectionandquantiýcationof transition metal ions in human atherosclerotic plaques: evidence for the presence of elevated levels of iron and copper. *Arteriosclerosis Thrombosis and Vascular Biology.* **24**:949ï 54.

Stewart, A.J., Mullen, W. and Crozier, A. (2005). On-line HPLC analysis of the antioxidant activity of phenolic compounds in green and black tea. *Molecular Nutrition and Food Research*, **49**: 52-60.

Stote, K.S. and Baer, D.J. (2008). Tea consumption may improve biomarkers of insulin sensitivity and risk factors for diabetes. *Journal of Nutrition*, **138**: 1584Sï 1588S.

Su, X., Duan, J., Jiang, Y., Duan, X. and Chen, F. (2007). Polyphenol profile and antioxidant activity of brewed oolong tea at different conditions. *International Journal of Molecular Science*, **8**: 1196-1205.

Sudheesh, S. (1999). Antioxidant activity of flavonoids from *Solanum melongena*. *Phytotherapy Research,* **13**: 393-396.

Takabayashi, F., Tahara, S., Kanerko, T. and Harada, N. (2004). Effect of green tea catechins on oxidative DNA damage of hamster pancreas and liver induced by Nnitrosobis (2-oxopropyl) amine and/or oxidized soybean oil. *Biofactors,* **21**: 335-337.

Talbott SM. The Cortisol Connection. New York: Hunter House, 2002.

Thasleema, S.A. (2013). Green tea as an antioxidant. *Journal of Pharmaceutical Science and Research.* 5 **9**: 171 ï 173.

Tian, C., Ye, X., Zhang, R. and Long, J. (2013). Green tea polyphenols reduced fat deposits in high fat-fed rats via erk1/2- PPARy-adiponectin pathway. *Plos One,* **8**(1): 53796.

Traber, M.G. and Atkinson, J. (2007). Vitamin E, antioxidant and nothing more. *Free Radical Biology and Medicine:* **4:** 4-15.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. and Mazur, M. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *International Journal of Biochemistry and Cell Biology*, **39**(1): 44-84.

Vanessa, C. and Williamson, G. (2004). A review of the health effects of green tea catechins in *in-vivo* animal models. *Journal of Nutrition,* **134**: 3431-3440.

Venables, M.C., Hulston, C.J., Cox, H.R. and Jeukendrup, A.E. (2008). Green tea extract ingestion, fat oxidation and glucose tolerance in healthy humans. *American Journal of Clinical Nutrition,* **87**(3): 778-784.

Vermeer, M.A., Mulder, T.P. and Molhuizen, H.O. (2008). Theaflavins from black tea, especially theaflavin-3-gallate, reduce the incorporation of cholesterol into mixed micelles. *Journal of Agriculture and Food Chemistry,* **56**(24): 12031-12036.

Vernarelli, J.A. and Lambert, J.D. (2012). Tea consumption is inversely associated with weight status and other markers for metabolic syndrome in US adults. *European Journal of Nutrition,* **52**(3): 1039-1048.

Vuong, Q.V., Golding, J.B. and Paul, D.M.N. (2010). Roach extraction and isolation of catechins from tea. *Journal of Special Science*, **33**: 3415-3428.

Wang, H. and Halliwell, K. (2001). Determination of flavonols in green and black tea leaves and green tea infusions by HPLC. *Food Research International*, **34**: 223-227.

Willcox, J.K., Ash, S.L. and Catignani, G.L. (2004). Antioxidants and prevention of chronic disease- review. *Critical Review Food Science and Nutrition*, **44**: 275-295.

Xu, J.Z., Yeung, S.Y, Chang, Q., Huang, Y. and Chen, Z.Y. (2004). Comparison of antioxidant activity and bioavailability of tea epicatechins with their epimers. *British Journal of Nutrition,* **91**: 873-881.

Yamamoto, M.M., Inagaki, N., Kitaura, J., Chikumoto, T., Kawahara, H., Kawakami, Y., Kawakami, T. and Nagai, H. (2004). O-methylated catechins from tea leaves inhibit multiple protein kinases in mast cells. *Journal of Immunology*, **172**: 4486-4492.

Yang, G., Liu, Z. and Seril, D.N. (1997a). Black tea constituents, theaflavins, inhibit 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone NNK0-induced lung tumorigenesis in A/J mice. *Carcinogenesis*, **18**: 2361-2365.

Yang, G., Wang, Z.Y. and Kim, S. (1997b). Characterization of early pulmonary hyperproliferation and tumor progression and their inhibition by black tea in a 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumorigenesis model with A/J mice. *Cancer Research*, **57**: 1889-1894.

Yashin, A., Yashin, Y. and Nemzer, B. (2011). Determination of antioxidant activity in tea extracts, and their total antioxidant content. *American Journal of Biomedical Sciences,* **3**(**4**): 322-335.

Yen, G.C., Duh, P.D., Tsai, H., L. and Huang, S.L. (2003). Pro-oxidative properties of þavonoids in human lymphocytes. *Bioscience, Biotechnology and Biochemistry*, **67**: 1215ï 1222.

Yokogoshi, H. and Kobayashi, M. (1998). Hypotensive effect of gammaglutamyl-methylamide in spontaneously hypertensive rats. *Life Science,* **62**: 1065ï 1068.

Yokogoshi, H. and Terashima, T. (2000). Effect of theanine R-glutamylethylamide on brain monoamines, striatal dopamine release, and some kinds of behavior in rats. *Nutrition,* **16**: 776ï 777.

Yokogoshi, H., Kato, Y., Sagesaka, Y.M. and Takihara, M.T. (1995). Reduction effect of theanine on blood pressure and brain 5-hydroxyindoles in spontaneously hypertensive rats. *Bioscience Biotechnology and Biochemistry,* **59**: 615ï 618.

Young, I.S. and Woodside, J.V. (2001). Antioxidant in health and disease. *Journal of Clinical Pathology,* **3**: 176-186

Yu, S.Z., (1995) Primary prevention of hepatocellular carcinoma. *Journal of Gastroenterology and Hepatology* **10**: 674-682.

Yun, S.Y., Kim, S.P. and Song, D.K. (2006). Effects of (-)-epigallocatechin-3-gallate on pancreatic beta-cell damage in streptozotocin-induced diabetic rats. *European Journal of Pharmacology*, **541**: 115-121.

Zaveri, N.T. (2006). Green tea and its polyphenolic catechins: medicinal uses in cancer and noncancer applications. *Life Science*, **78**:2073-2080.

Zhang, G., Miura, Y. and Yagasaki, K. (2002). Effects of dietary powdered green tea and theanine on tumor growth and endogenous hyperlipidemia in hepatoma-bearing rats. *Bioscience, Biotechnology and Biochemistry,* **66**: 711-716.

Zhang, G., Wang, Y., Zhang, Y. and Wan, X. (2012). Anti-cancer activities of tea epigallocatechin-3-gallate in breast cancer patients under radiotherapy. *Current Molecular Medicine*, **12**(2): 163-176.

Zhang, M., Binns, C.W. and Lee, A.H. (2002). Tea consumption and ovarian cancer risk: A case-control study in China. *Cancer Epidemiology Biomarkers and Prevention*, **11**(8): 713-718.

Zhang, Y., Turunen, M. and Appelkvist, E.L. **(**1996**)**. Restricted uptake of dietary coenzyme Q is in contrast to the unrestricted uptake of alpha-tocopherol into rat organs and cells. *Journal of Nutrition*, **126**: 2089-2097.

Zhang, Y.J., DeWitt, D.L., Murugesan, S. and Nair, M.G. (2004). Novel lipid-peroxidation and cyclooxygenase inhibitory tannins from *Picrorhiza kurrora* seeds. *Chemistry and Biodiversity*, **1**: 426-441.

Zheng, F.J, Shi, L., Yang, J., Deng, X.H., Wu, Y.Q., Yan, X.Q. and Huang, N. (2012). Effect of tea polyphenols on the adhesion of highly metastatic human lung carcinoma cell lines to endothelial cells in vitro. *Asian Pacific Journal of Cancer Prevention*, **13**(8): 3751-3755.

Zheng, X.X., Xu, Y.L., Li, S.H., Hui, R., Wu, Y.J. and Huang, X.H. (2013). Effects of green tea catechins with or without caffeine on glycemic control in adults: A meta-analysis of randomized controlled trials. *American Journal of Clinical Nutrition,* **97**(4): 750-762.