EVALUATION OF FREE-RADICAL QUENCHING PROPERTIES AND DETERMINATION OF IC_{50} OF SOME EDIBLE FRUITS AND VEGETABLES SOLD ON THE GHANAIAN MARKET

By

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DECLARATION

I hereby declare that this submission is my own work towards the Master of Science in Analytical Chemistry and that, to the best of my knowledge, it contains no material previously published by another person or material which has been accepted for the award by another degree of the University, except where due acknowledgement has been made in text.

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ABSTRACT

Fruits and vegetables are good for the human system. From studies fruits and vegetables are known to have the ability to prevent diseases and sickness. This is due to the free radical scavenging abilities of the antioxidants in them. This study was carried out to investigate the free radical scavenging abilities of some selected fruits and vegetables: *Asiminia triloba*, *Mangifera indica*, *Persea americana*, *Solanum torvum*, *Xanthosoma colocasia*, *Allium ascalonicum Linn* and to determine its minimum Inhibitory concentration (IC_{50}).

The total antioxidant capacity (TAC), total phenol content (TAC), reducing power potential and the DPPH scavenging assays of the methanolic extracts of samples were determined. The result for the total antioxidant capacity of the measured concentration (0.1 - 0.3 mg/ml) had *mangifera indica* showing the highest capacity with 0.274 mg Ascorbic Acid Equivalence (AAE) with the lowest being 0.085 mg AAE for *Xanthosoma colocasia*. *Mangifera indica* again showed the highest total phenol content with 0.348 mg Tannic Acid Equivalence (TAE). There was a perfect correlation between Total Antioxidant Capacity (TAC) and Total phenol content (TPC) of the extracts with r =1 and $p < 0.0001$ for all the correlation graphs.

Highest absorbance was shown at concentration 0.1 mg/ml by *solanum torvum* with 0.1272 and *mangifera indica* with 1.4967 at 3 mg/ml with a Gallic Acid Equivalence (GAE) of 0.013919 mg GAE and 0.193267 mg GAE respectively for the ferric reducing antioxidant power assay (FRAP).

Solanum torvum showed the highest scavenging ability with IC_{50} of 1.0676 mg GAE and *Persea americana* with 2.5759 GAE mg as the minimum gallic equivalence concentration that can reduce free radicals in the system. Percentage scavenging ability was highest for *Asiminia triloba* with 80.75% and lowest for 34.25% all at the concentration of 3 mg/ml. This is the highest percentage of free radicals that can be reduced at the experimental concentrations.

The FT-IR spectroscopy (800 – 3600 cm^{-1}) was used to confirm the presence of phenol groups (3300 – 3600 cm^{-1}), benzene rings (1500 – 1700 $^{cm-1}$) and the antioxidant activity of the sample extracts. Peaks at various band lengths were obtained in both the functional group and fingerprint region to confirm the total phenols of the samples under study.

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CHAPTER ONE

1.0 INTRODUCTION

The presence of antioxidant species in fruits and vegetables is associated with certain beneficial health effects, protecting biomolecules from oxidative damage. Due to their antioxidant activity potential, the phytoconstituents of vegetables such as onions, pepper and fruits like mango, pawpaw including phenolic compounds has been researched into currently worldwide.

In Ghana, not much work has been done to investigate the free-radical quenching properties of locally consumed vegetables and fruits which are widely consumed on daily bases.

1.1 FREE RADICALS, NITROGEN AND OXYGEN SPECIES

It has been proven that free radicals play an important role in many diseases, such as cardiovascular diseases, cancer, neurodegenerative diseases, diabetes and aging (Li et al., 2010). A free radical may be defined as a molecule or molecular fragments containing one or more unpaired electrons in its outermost atomic or molecular orbital and are capable of independent existence (Halliwell, 1999).

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are free radicals and other non-radical reactive derivatives. The reactivity of radicals is generally stronger than non-radical species, though radicals are less stable (Pham-Huy, 2008).

Free radicals are formed from molecules by the homolytic cleavage of a chemical bond and via redox reactions, once formed these highly reactive radicals can start a chain reaction (Bahorun, 2006 and Valko, 2006). ROS and RNS include radicals such as superoxide (O_2•−), hydroxyl (OH•), peroxyl (RO_2•), hydroperoxyl (HO_2•), alkoxyl (RO•),

nitric oxide (NO•), nitrogen dioxide ($NO_2^{\bullet}$) and lipid peroxyl (LOO•); and non-radicals like hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), ozone (O_3) and singlet oxygen.

Non radicals are also termed as oxidants and likely lead free radical reactions in living organisms easily. Radicals derived from oxygen are characterize as the most important class of radical species generated in living systems (Valko et al, 2006).

Free radicals including reactive oxygen species (ROS) and Reactive Nitrogen Species (RNS) are generated during metabolism and other activities beyond the antioxidant capacity of a biological system and they give rise to oxidative stress (Zima et al, 2001). The body obtains energy by the oxidation of carbohydrates, fats and proteins through both aerobic and anaerobic process leading to the generation of free radicals and its overproduction can cause tissue injury.

1.2 OXIDATIVE STRESS

Oxidative damage can lead to a breakdown or even hardening of lipids, which is a composition of all cell walls. This is due to lipid peroxidation that cause possibility of the cell to properly get its nutrients or signals to one another. In addition, other biological molecules including RNA, DNA and protein enzymes are also susceptible to oxidative damage.

Oxidative stress, resulting from these free-radicals plays an important role in manifesting various disorders, including ageing and diseases like cancer, Parkinson's and in living beings, cell tumour (prostate and Colon cancers) and coronary heart diseases (Jagadish et al, 2009)

1.2.1 Oxidative Stress and Human Health

Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and our metabolism. They are continuously produced by the body via enzymatic and non-enzymatic reactions like respiratory chain reaction, the phagocytosis, prostaglandin synthesis, cytochrome P_{450} system and oxidative phosphorylation (i.e. aerobic respiration) in the

mitochondria (Tiwari, 2004, Halliwell, 2007 and Parcher et al., 2007).

ROS and RNS are the products of normal cellular metabolism, having both deleterious and beneficial effect in the body (Valko et al., 2004). At low or moderate concentration some of the free radicals play beneficial physiological role *in vivo.* These include defence against infectious agents by phagocytosis, energy production, cell growth, and function in different cellular signaling systems and the induction of a mitogenic response at low concentrations (Poli et al., 2004).

Free radicals occur continuously in all cells as part of normal function. Oxygen free radicals can cause damage of biological tissue and lead to their injury. The mechanism of damage involves lipid peroxidation, which destroys cell structures, lipids, proteins and nucleic acids. They cause damage to cell membranes with the release of intracellular components, leading to further tissue damage (Poli et al., 2004). Antioxidant enzymes and non-enzymatic defense system minimizes the harmful effect of ROS by various antioxidant mechanism.

Oxidative stress is a harmful condition that occurs when there is an excess of ROS and/or a decrease in antioxidant levels. This may cause tissue damage by physical, chemical, psychological factors that lead to tissue injury in human and causes different diseases (Tian et al., 2007). Living creatures have gone through complicated defense systems and the body has acts against free radical-induced oxidative stress by different defense

mechanisms like preventative mechanisms, repair mechanisms, physical defenses and antioxidant defenses (Valko et al., 2007)

Oxygen derived free radical reactions have been implicated in the pathogenesis of many human diseases (Pham-Huy et al., 2008; Valko et al., 2007 and Sen et al., 2009) including:

- Neurodegenerative disorder like Alzheimer's disease, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis, memory loss and depression;
- Cardiovascular disease like atherosclerosis, ischemic heart disease, cardiac hypertrophy, hypertension, shock and trauma;
- Pulmonary disorders like inflammatory lung diseases such as asthma and chronic obstructive pulmonary disease;
- Diseases associated with premature infants, including bronchopulmonary, dysplasia, periventricular leukomalacia, intraventricular hemorrhage, retinopathy of prematurity and necrotizing enterocolitis.
- Gastrointestinal diseases like peptic ulcer, inflammatory bowel disease and colitis.
- Tumors and cancer like lung cancer, leukemia, breast, ovary, rectum cancers etc.
- Ageing process, diabetes, skin lesions, liver disease, pancreatitis and Infertility.

1.2.1.1 Heart Disease

While several factors, such as high cholesterol levels, hypertension, cigarette smoking, and diabetes, are believed to promote atherosclerosis, a growing body of evidence suggests a critical step in its development is the oxidation of low-density lipoprotein (LDL) within the arterial wall (Jialal and Fuller, 1993) and this theory is supported by many epidemiological studies which link low intakes of dietary antioxidants to an increased frequency of heart disease (Hennekens and Gaziano, 1993). Antioxidants have been shown to prevent LDL oxidation *in vitro* and retard the progression of

atherosclerosis in animal models (Keaney and Frei, 1998). Several human studies found supplemental vitamin E increased vitamin E levels in LDL, increased the resistance of LDL oxidation, and decreased the rate of LDL oxidation. It has been estimated that dietary increases in antioxidant vitamins may reduce the risk of heart disease by 20-30% (Hennekens and Gaziano, 1993).

1.2.1.2 Cancer

Epidemiological evidence consistently relates low antioxidant intake or low blood levels of antioxidants with increased cancer risk. It is known that low intake of fruits and vegetables everyday can increase the tendency of getting cancer (Block et al., 1992).

Oxidants are capable of stimulating cell division, which is a critical factor in mutagenesis. When a cell with a damaged DNA strand divides, cell metabolism and duplication becomes deranged. Thus, a mutation can arise which in turn is an important factor in carcinogenesis. It is believed that antioxidants exert their protective effect by decreasing oxidative damage to DNA and by decreasing abnormal increases in cell division. Cigarette smoking and chronic inflammation, two of the major causes of cancer have strong free radical components in their mechanisms of action.

Although antioxidant activity is believed to be responsible for much of the protection against tumorgenesis, additional anticancer activities have been observed from several plant-derived substances (Keaney and Frei, 1998).

1.2.2 Pulmonary Disorders

Because of its large surface area, the respiratory tract is a major target for free radicals, with air pollution as a major source of ROS. Recent studies suggest that free radicals may be involved in the development of pulmonary disorders such as asthma ROS (Kehrer, 1994 and Bland, 1995). Cellular damage caused by free radicals is thought to be partly

responsible for the bronchial inflammation characteristic of this disease. It has been suggested that increasing antioxidant intake may help to reduce oxidant stress and help to prevent or minimize the development of asthmatic symptoms (Greene and Asthma, 1995). Vitamin C, vitamin E, and beta carotene supplementation has been associated with improved pulmonary function (Hatch, 1995 and Bendich, 1994).

Under normal conditions, the balance between the generation and scavenging of ROS is controlled by the antioxidant defense system, which includes both enzymatic antioxidant systems and non-enzymatic factors. Antioxidant vitamins, including vitamin A, carotenoids, vitamin C, and vitamin E reduce lipid peroxidation by increasing antioxidant power in a system.

In addition to the vitamin antioxidants, fruits and vegetables also contain non-nutrient antioxidants such as the flavonoids, polyphenols, and terpenes. Flavonoids such as catechin, chrysin, cyanidin, myricetin, and quercetin are found in fruits, onions, tea, and wines and have attracted much interest as dietary antioxidants (Hertog et al, 1994).

1.3 ANTIOXIDANT ACTIVITY

1.3.1 What are Antioxidants?

Antioxidants are substances which slow down or stop the oxidation reaction. Antioxidants cause protective effect by neutralizing free radicals, which are toxic byproducts of natural cell metabolism. The human body naturally produces antioxidants but the process is not 100 percent effective in case of overwhelming production of free radicals and that effectiveness also declines with age (Goldfard, 1993).

Increasing the antioxidant intake can prevent diseases and lower the health problems. Research is increasingly showing that antioxidant-rich foods like herbs reap health benefits. These foods may possibly enhance antioxidant levels because foods contain a lot

of antioxidant substances. Fruits and vegetables contain many important antioxidants such as vitamin A, C, E, β-carotene and important minerals, including selenium and zinc. Fruits, vegetables and medicinal herbs are the richest sources of antioxidant compounds. All phytoconstituents are also important source of antioxidants and are capable of terminating the free radical chain reactions (Oluwaseum et al, 2008).

Antioxidants can be categorized into primary and secondary antioxidants. Primary antioxidants stop the oxidation process by terminating the radical chain reaction by conversion of radicals into more stable compounds (Szukalska, 2003; Leclercq *et al.*, 2007). The antioxidant activity can also be attributed to polyphenol substances appearing in rapeseed seeds as well as phenolic acids (Siger *et al.*, 2005), whose high content protects olive oil from oxidation.

The main mechanism of their actions depends on the ability to bind certain metal ions (chelating compounds, such as EDTA), and oxygen, as well as on the absorption of UV rays, regeneration of primary antioxidants (such as ascorbic acid), creation of a protective border surface between oil and air (such as phospholipids), causing also a decomposition of peroxide to non-radical products or deactivation; in other words "scavenging" or "quenching" of a singlet oxygen such as β-carotene and Pseudomonas aeruginosia (Szukalska, 2003). Antioxidants can deactivate radicals by three major mechanisms: hydrogen atom transfer (HAT), electron transfer (ET) and combination of both h hydrogen atom transfer and electron transfer (Xiaonan et al, 2011).

Hydrogen atom transfer measures the ability of an antioxidant to quench free radicals by hydrogen donation. ET detects the ability of antioxidant to transfer one electron to reduce radicals, metals and carbonyls (Prior et al., 2005 and Huang et al., 2005). Ferric reducing antioxidant power (FRAP) is an electron transfer assay and the F–C assay measures the total phenolic content using an electron transfer mechanism. Oxygen radical absorbance

capacity (ORAC) assay is a common Hydrogen atom transfer based competitive assay. TEAC and DPPH assays combine both hydrogen atom transfer and electron transfer mechanisms. The Total phenolic content and Total antioxidant capacity of vegetables have been studied extensively using the various antioxidant assays mentioned (Stratil et al., 2006) which has been stated already. However, these assays are time consuming and developing an alternative method to substitute, or at least validate the traditional ‘‘wet chemistry’’ methods is important if a large number of samples are to be screened. Infrared spectroscopy (IR) provides a unique advantage of simple sample preparation while retaining satisfactory precision and sensitivity (Movasaghi et al., 2001).

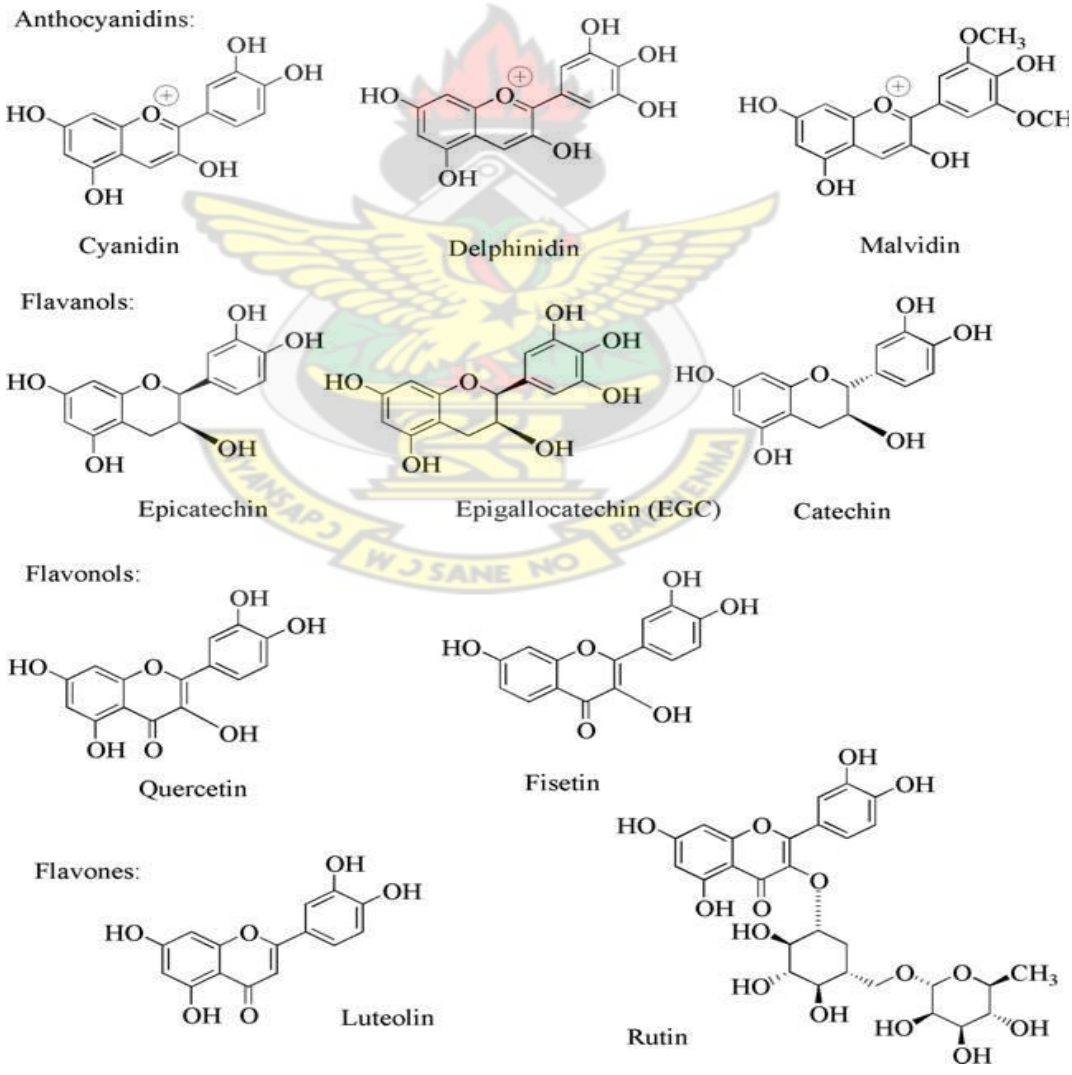


Figure 1.1 Chemical structure of some dietary antioxidants

1.4 DIETRY ANTIOXIDANTS

Vitamin C, vitamin E, and beta carotene are among the most widely studied dietary antioxidants. Vitamin C is considered the most important water-soluble antioxidant in extracellular fluids. It is capable of neutralizing ROS in the aqueous phase before lipid peroxidation is initiated. Vitamin E, a major lipid-soluble antioxidant, is the most effective chain-breaking antioxidant within the cell membrane where it protects membrane fatty acids from lipid peroxidation.

Beta carotene and other carotenoids are also believed to provide antioxidant protection to lipid-rich tissues. Research suggests beta carotene may work synergistically within vitamin E. A diet that is excessively low in fat may negatively affect beta carotene and vitamin E absorption, as well as other fat-soluble nutrients. Fruits and vegetables are major sources of vitamin C and carotenoids, while whole grains and high quality, properly extracted and protected vegetable oils are major sources of vitamin E.

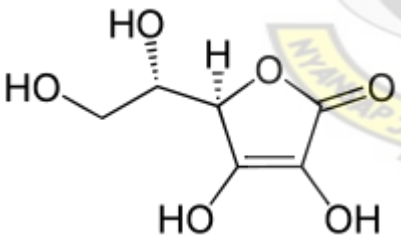


Figure 1.2 structure of vitamin C

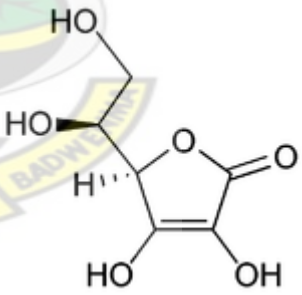


Figure 1.3 Ascorbic acid (reduced form)

1.4.1 Phytonutrients

A number of other dietary antioxidant substances exist beyond the traditional vitamins discussed above. Many plant derived substances, collectively termed phytonutrients, or phytochemicals, are becoming increasingly known for their antioxidant activity. Phenolic

compounds such as flavonoids are abundant within the plant kingdom: approximately 3,000 flavonoid substances have been described (Briviba and Sies, 1994). In plants, flavonoids serve as protectors against a wide variety of environmental stresses while, in humans, flavonoids appear to function as "biological response modifiers." Flavonoids have been demonstrated to have anti-inflammatory, antiallergenic, anti-viral, anti-aging, and anti-carcinogenic activity (Coldy et al., 1986).

1.5 ANTIOXIDANT PROTECTION

To protect the cells and organ systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system. It involves a variety of components, both endogenous and exogenous in origin, that function interactively and synergistically to neutralize free radicals (Jacob, 1995).

These components include:

- Nutrient-derived antioxidants like ascorbic acid (vitamin C), tocopherols and tocotrienols (vitamin E), carotenoids, and other low molecular weight compounds such as glutathione and lipoic acid.
- Antioxidant enzymes, such as superoxide dismutase, glutathione peroxidase, and glutathione reductase, which catalyze free radical quenching reactions.
- Numerous other antioxidant phytonutrients present in a wide variety of plant foods.

1.5.1 Endogenous Antioxidants

- Thiols such as glutathione, lipoic acid
- NADPH and NADH
- Ubiquinone (coenzyme Q10)
- Uric acid

- Enzymes:
    - copper/zinc and manganese-dependent superoxide
    - dismutase (SOD)
    - iron-dependent catalase
    - selenium-dependent glutathione peroxidase

1.5.2 Dietary Antioxidants

- Vitamin C, E
- Beta carotene and other carotenoids and oxycarotenoids, such as lycopene and lutein
- Polyphenols, such as flavonoids, flavones, flavonols, and proanthocyanidins

1.5.3 Metal Binding Proteins

- Albumin, Ceruloplasmin, Metallothionein (copper)
- Ferritin, Myoglobin, Transferrin (iron)

The body produces different antioxidants (endogenous antioxidants) to neutralize free radicals and protect the body from different disease leads by the tissue injury. Exogenous antioxidants externally supplied to the body through food also play important roles to protect the body. The body has developed several endogenous antioxidant defense systems classified into two groups such as enzymatic and non-enzymatic. The enzymatic defense system includes different endogenous enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and non-enzymatic defense system included vitamin E, vitamin C and reduced glutathione (GSH) (Jacob, 1995 and Harris, 1994).

SOD is an important endogenous antioxidant enzyme that acts as the first line of defense system against ROS which scavenges superoxide radicals to H_2O_2. GPx present in the cytoplasm of the cells removes H_2O_2 by coupling its reduction to H_2O with oxidation of GSH.

Vitamins C and E which are non-enzymatic endogenous antioxidant also exists within normal cells and react with free radicals to form radicals themselves which are less reactive than the radicals. They break radical chain reactions by trapping peroxyl and other reactive radicals (Willcox et al, 2005).

Non-enzymatic antioxidants also can be divided into metabolic antioxidants and nutrient antioxidants. Metabolic antioxidants are the endogenous antioxidants which are produced by metabolism in the body, like lipoid acid, melatonin, uric acid, bilirubin, metal-chelating proteins, transferrin (Shinde et al, 2005; Willcox et al, 2005; Droge, 2002) whiles nutrient antioxidants belonging to exogenous antioxidants, cannot be produced in the body but provided through diet or supplements via trace metals (selenium, manganese, and zinc), flavonoids, δ-3 and δ-6 fatty acids etc. (Pham-Huy, et al., 2008).

Vitamin E and C are the non-enzymatic antioxidants which exist within normal cells and can also be supplied through diet (Tiwari et al., 2001). Biosynthesis of other antioxidants or defense enzymes are induced by some antioxidants (Tiwari 2004). Therefore, antioxidants synthesized in the body or supplied from outside like phytoconstituents play important roles to protect the body from free radical induced injury.

1.6 FRUITS

Fruits are normally referred to as the edible seeds or ovary of a plant. Examples are mango, pea, and pineapple. This research seeks to look at the free radical quenching properties of pawpaw (*Asiminia triloba*), mango (*mangifera indica*) and Avocado (*persea americana*).

1.6.1 Mango (mangifera indica)

1.6.1.1 Taxonomy

Kingdom: Plantae

Division: Angiospermae

Class: Rosids

Order: Sapindales

Family: Anacardiaceae

Genus: Mangifera

Species: *Mangifera specie*

1.6.1.2 Botany

Mango belongs to the family Anacardiaceae, genus Mangifera, and is known to have originated in Southeast Asia. Mango as a crop is grown in about 87 countries. Several varieties exist in India, which contributes to about 64% of the world's production (FAO, 1990). However most of these fruits cultivated are not commercialized according to the preferences of different regions of the country. Most countries upon production feed their local industries to produce finished products such as drinks. Other notable producing countries are Brazil, Pakistan, Mexico, the Philippines and Thailand.

Over the couple of years, many countries have produced mango and exported it to many countries. The Philippines, over 12 years ago could produce 10,000 tonnes of fresh fruit export, was leading producers of mango producing to North American and European countries.

1.6.1.3 Morphology

Mature trees can attain a height of 40 m or more, and can survive for several hundred years. Mango trees that have been domesticated by selection from openly pollinated seedling populations show variation in tree architecture (i. e. shape and size). Leaves are simple and alternate; with petioles that range in length from 1 to 12.5 cm. Leaf morphology is highly variable, depending on the cultivar: leaves can be lanceolate, oblong, ovate and intermediate types involving these forms. Leaf length ranges from 12 to 38 cm and width can be between 2 to13 cm. The root system consists of a long, vigorous taproot and abundant surface feeder roots.

Chemical composition

Ripe mango fruits can be separated in three parts, skin (peel), pulp, and stone. The pulp is consumed by humans mainly. Composition of mango however varies with cultivation, variety, and stage of maturity. The major constituents of the pulp are water, carbohydrates, organic acids, fats, minerals, pigments, tannins, vitamins, and flavour compounds. The ripe fruit pulp contains about 11.8% carbohydrates, 4800 IU of vitamin A, and 13 mg/100g of ascorbic acid. The pulp is a rich source of β-carotene. Sucrose, glucose, and fructose constitute the bulk of carbohydrates and most of the soluble solids in the pulp.

1.6.1.4 Uses

In Ghana, many of the mangoes produced are on private basis, mostly at the backyard of houses initially to produce shade for families. The productions of mango beverages are done by a few companies with most of the fresh mango fruits finding its way onto the local markets for sale and consumption by the locals.

The leaves and barks are used as herbal medicines and it is either boiled or ground into powdered forms for treatment of various ailment.

The total amount of world production of 15.7 million metric tonnes produced makes the total amount of mango trade insignificant (FAO, 1990).

1.6.2 Pawpaw (*Asimina triloba*)

1.6.2.1 Taxonomy

Kingdom: *Plantae*

Division: *Magnoliophyta*

Class: *Magnoliopsida*

Order: *Magnoliales*

Family: *Annonaceae*

Genus: *Asimina Adans*

Species: *Asimina triloba (L.) Dunai*

1.6.2.2 Botany

The North American native pawpaw (*Asimina triloba*) is a temperate tree fruit in the mostly tropical custard apple family, Annonaceae.

The two fruits are very different from each other, but some pawpaw do have a papaya-like flavor. The pawpaw fruit has both fresh market and processing appeal, with a tropical like flavor that resembles a combination of banana, mango, and pineapple.

1.6.2.3 Morphology

A native deciduous tree to the people of India, the coarse-textured pawpaw ultimately reaches 30 feet in height (more commonly 15 to 20 feet) with an equal spread, and creates an upright, wide pyramidal silhouette. The large, dark green leaves, 6 to 12 inches in

length and three to five inches wide, seem to droop from their weight at branch tips, giving the plant a distinctive, almost wilted appearance. The fruits are popular with man and wildlife, especially raccoons and birds.

1.6.2.4 Uses

When fully ripe, the edible flesh becomes soft, almost custard-like, has a sweet, rich taste similar to bananas, and is very nutritious.

Locally, its leaves are used in the preparation of concoctions and widely used in the treatment of malaria cases.

1.6.3 AVOCADO (*persea americana*)

1.6.3.1 Taxonomy

Kingdom: Plantae

Division: *Angiosperms*

Class: *Magnoliids*

Order: *Laurales*

Family: *Lauraceace*

Genus: *Persea*

Species: *P. americana*

1.6.3.2 Botany

Avocado (*persea americana*) belongs to the family Lauraceace, together with laurel and cinnamon.

Avocado is one of many important crops grown worldwide with particular mention of countries like Brazil, Mexico, Nigeria, Colombia, Congo, South Africa, and many countries in the Central and southern regions of the Americas. It is consumed mostly as a

fresh fruit. Much export of this fruit has been on the significant rise mainly due to its dietary value. Worldwide, the 2005 figures for papaya fruits show that 6,634,580 tonnes were produced in 54 countries (FAO, 2007). Ghana is no exception of this rise as many of the fruits are turned into drinks sold on the local markets.

Avocado is a high-fat fruit containing rare sugars of high carbon number and very rich in certain vitamins, minerals, and nitrogenous substances. Considering its high oil and low sugar content, it is highly recommended food for diabetic patients.

1.6.3.3 Morphology

The avocado is an evergreen tree; it grows up to 20m in height and may be equally wide. The hollow green or deep purple trunk can grow to 10 m, is straight and cylindrical with prominent leaf scars, and can be 30-40 cm thick at the base, thinning to 5-7.5 cm at the crown (FAO, 2007).

The leaves emerge directly from the upper part of the stem in a spiral on nearly horizontal petioles 25-100 cm long and form a loose open crown. The leaf blade, deeply divided into 5 to 9 main lobes, varies from 25-75 cm in width, and has prominent yellowish ribs and veins.

The life of its leaf is 2.5 to 8 months and new leaves arise at the rate of 1.5-4 week.

Chemical composition

The avocado is one of the most nutritive among fruits. The vitamin A and C content of one medium persea americana fruit (approximately 359 g edible portion) exceeds the dietary Reference Intakes established by the US Food and Nutrition Board for adult minimum daily requirements (OECD, 2005).

However, the major fatty acid is always oleic, myristic, stearic, linolenic, and arachinodic. Lipids of avocado seeds contain less oleic acid and more linoleic and linolenic acids than avocado pericarp.

Immature avocado flesh possesses a bitter flavour with a prolonged after taste (Ahmed and Barmore, 1980). In ripe Feurte avocados, about 75% of total volatile compounds are dominated by C_6 alcohols and aldehyde. Major components include trans-hex-3-en-1-ol (25.6%), trans-hex-2-en-1-ol (19.1%), hexan-1-ol (17.9%), cis-hex-2-enal (8.7%), and hexanal (4.5%). Most of the volatile compounds are derived from lipid oxidation or degradation (Nogalingnam, 1993).

Heating of avocado paste, results in the development of off-flavour in the heated product. This precludes thermal processing as a means of processing or preserving avocado. Heating treatments ranging from 60^{o}C for 20 min to 90^{o}C for 1.4 min were the dividing line between off-flavour and non-off-flavour formation (Benet et al, 1972) identified the compounds contributing to heat-induced (100^{o}C for 15 mins) bitter off-flavour in avocado slices to be 1-acetoxy-2,4-dihydroxy *n*-heptadeca-16-en (melting point at 56^{o}C) and 1,2,4-trihydroxy-*n*-heptadeca-16-en (melting point at 56.5^{o}C). Comparison between heated and non-heated avocado slices showed that the concentrations of these chemicals were significantly increased by the heat treatment.

1.6.3.4 Uses

In Ghana, consumption of this fruit is either with bread, or with local dishes like "ampesi", "gari and beans" and rice. Oil extracts from avocado are used in the making of cosmetics and hence are in high demand in this sector of the economy in western European countries.

The papaya fruit, as well as all other parts of the plant, contain a milky juice in which an active principle known as papain is present. Aside from its value as a remedy in

dyspepsia and kindred ailments, it has been utilized for the clarification of beer. The juice has been in use on meat to make it tender (Wilson, 1994).

1.7 VEGETABLES

Vegetables are literally defined as the edible part of a plant asides the fruit or seed and may include the leaves, stem, or roots of a plant. The term vegetable comes from the Latin *vegetabilis* (animated) and from *vegetare* (enliven), which is derived from *vegetus* (active), in reference to the process of a plant growing. Taro leaves or kontomire (*Xanthosoma colocasia*), "kwawu nsusuaa" (*solanum torvum*) and shallot (*allium cepa var. aggregatum*) are the species that are studied in this project.

1.7.1 "Kwawu nsusua" (*solanum torvum*)

1.7.1.1 Taxonomy

Kingdom: *Plantae*

Division: *Angiospaerms*

Class: *Asterids*

Order: *Solanales*

Family: *Solanaceae*

Genus: *Solanum*

Species: *S. torvum*

1.7.1.2 Botany

Solanum torvum Sw. belongs to the family Solanaceae. It is a small shrub distributed widely in Thailand and commonly known as 'Turkey berry''. In Ghana, it is commonly known as "Kwawu nsusua" based on its first original cultivation in the kwawu area.

1.7.1.3 Morphology

Turkey berry or "kwawu nsusua" is an erect spiny shrub of about 4 meters tall, evergreen and widely branched. It is native and cultivated in Africa and the West Indies (Adjanohoun et.al, 1996). The plant is usually 2 or 3 m in height and 2 cm in basal diameter, but may reach 5m in height and 8 cm in basal diameter. The shrub usually has a single stem at ground level, but it may branch on the lower stem. The twigs are grey-green and covered with star-shaped hairs. The spines are short and slightly curved and vary with thickness throughout the plant, including the leaf midrib, to entirely absent in some parts. The leaves are opposite or one per node, broadly ovate with deeply lobed. The petioles are 1 to 6 cm long and the blades are 7 to 23 by 5 to 18 cm and covered with short hairs. The flowers are white, tubular with 5 pointed lobes, and grouped in corymbiform cymes. They are shed soon after opening. The fruits are berries that grow in clusters of tiny green spheres (ca. 1 cm in diameter) that look like green peas.

Chemical constituents

Solanum torvum contains a number steroidal glycosides viz. Torvoside A-L (Yahara et al., 1996, Iida et al., 2005), among these torvoside A, torvoside B and torvoside E, torvoside F, torvoside G, torvoside H, are considered to be furostanol glycosides (Gus – Mayer et al., 1994).

Torvoside M and N have antimicrobial activity (Iida et al., 2005) and show cytotoxic activity with cell lines (Lida et al., 2005). Non alkaloidal constituents like tetratriacontanoic acid, sitosterol, stigmasterol and campesterol have also been isolated and identified from *S. torvum* leaves (Nakamura et al., 1996). The 26-O-β-glycosidase also known as torvosidase (Arthan et al., 1992) is present in the young leaves of *Solanum torvum*. Plant β - glycosidase play an important role in many biological processes such as

phytoharmone activation lignin synthesis cell wall degradation and defense mechanism (Esen, 1993).

1.7.1.4 Uses

Its fruit and leaves, which are rich in alkaloids can be used for medicinal or ritual purposes. The plant is cultivated in the tropics for its sharp tasting immature fruits. The fruits of *Solanum torvum* are used commonly in traditional medicine as anti-hypertensive (Fui, 1992). It has antioxidant (Sivapriyan & Srinivas, 2007), Cardiovascular, anti-platelet aggregation activities (Nguelefack et al., 2008) anti-microbial activity against human and clinical isolates (Wiart et al., 2004) and sedative, digestive, hemostatics and diuretic activities (Zhu, 2003).

1.7.2 Shallot (*Allium ascalonicum* Linn.)

1.7.2.1 Taxonomy

Kingdom: Plantae

Division: *Angiosperms*

Class: *Monocots*

Order: *Asparagales*

Family: *Liliaceae*

Genus: *Allium*

Species: *A. cepa var. aggregatum*

1.7.2.2 Botany

Allium ascalonicum Linn (Shallot) is an annual herbaceous plant of the family Liliaceae that grows in many parts of the world. However shallot is probably of Asiatic origin. *Allium ascalonicum* is a mildly aromatic herb.

1.7.2.3 Uses

Like onions it is used to flavour food, particularly meats and sauces. Shallot has been used worldwide as a spice, food, and folk medicine and there is a long-held belief in their health- enhancing properties. The bulb of shallot is of considerable importance in African cooking and in salads (Adeniyi and Anyiam, 2004). Many authors have studied the antimicrobial (Adeniyi and Anyiam, 2004), anti-viral (Ashrafi et al., 2004) and anti-parasite activities (Azadbakht et al., 2002) of *A. ascalonicum*. Antioxidant (Leelarungrayub et al., 2006), anti-diabetic (Adeniyi and Anyiam, 2004) and haematological effects (Owoyele et al., 2004) of *A. ascalonicum* were also reported.

1.7.3 Taro leaves or Kontomire (*Xanthosoma colocasia*)

1.7.3.1 Taxonomy

Kingdom: Plantae

Division: *Angiosperms*

Class: *Monocots*

Order: *Alismatales*

Family: *Araceae*

Genus: *Colocasia*

Species: *Xanthosoma sp*

1.7.3.2 Botany

Cocoyam (*Xanthosoma* spp.) is one of the six most important root and tuber crops world-wide (Onwueme & Charles, 1994). The corm, cormels, and leaves of cocoyam are an important source of carbohydrates for human nutrition, animal feed (Nyochembeng & Garton, 1998) and of cash income for farmers. The crop is mainly cultivated by small-

scale farmers (Onwueme & Charles, 1994) in Asia, Africa and Latin America. In spite of its importance as a staple food in many countries, cocoyam has received very little research attention (Goenaga & Heperly, 1990), and is regarded as an under exploited, and insufficiently studied crop (Giacometti & León, 1994 and Watanabe, 2002).

1.7.3.3 Morphology

Cocoyam is an herbaceous, monocotyledonous crop. The main stem is a starch-rich underground structure called corm from which offshoots, termed cormels, develop. The leaves are between 1-2 m long and arise directly from the corm, with long ribbed petioles. The leaves have a marginal vein and two large basal lobes with variable pigmentation. The inflorescence of *Xanthosoma* is protogynous, and the pistillate flowers are normally receptive 2 to 4 days before pollen is shed (Wilson, 1984). The growth and development cycle can be divided into three main periods. During the first two months the growth is slow.

1.7.3.4 Uses

The corms are roasted, baked or boiled and the natural sugars give a sweet nutty flavour. The starch is easily digestible and grains are fine and small and often used for baby food. The leaves are a good source of vitamins A and C and contain more protein than the corms**.**

Fruit and vegetables are an important component of a healthy diet and, if consumed daily in sufficient amounts, could help prevent major diseases such as CVDs and certain cancers. According to The World Health Report 2002, low fruit and vegetable intake is estimated to cause about 31% of ischemic heart disease and 11% of stroke worldwide. Overall it is estimated that up to 2.7 million lives could potentially be saved each year if fruit and vegetable consumption was sufficiently increased.

Consuming vegetables and fruits may reduce the risk of chronic diseases, including cardiovascular disease, stroke, neuro-degeneration, and type II diabetes. Substantial recent research has been performed to investigate the potential health benefits of antioxidants in food. Antioxidants can inhibit oxidative reactions *in vivo*, and aid in functional performance of enzyme systems for self-defense mechanisms within cells (Lee et al, 2004).

1.8 GENERAL OBJECTIVES

This project seeks to evaluate the free-radical scavenging properties and the IC_{50} of some selected edible fruits and vegetables sold on the Ghanaian market.

1.8.1 Specific Objectives

- To determine the total polyphenol contents present in mango, pawpaw, avocado, "Kontomire", "kwawu nsusua" and shallot commonly consumed in Ghana.
- To establish the free-radical scavenging activity of the extracts from mango, pawpaw, avocado, "Kontomire", "kwawu nsusua" and shallot using three free-radical scavenging assays.
- To estimate the half maximal inhibitory concentrations of the extracts from the selected vegetables and fruits on the Ghanaian market.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 FREE RADICALS

Free radicals (the antioxidants' arch enemy), as a causative factor in the disease and aging process, have become popular focal points of clinical research. The increased interest in the negative health effects of free radicals has given rise to the term Free Radical Pathology. Free radicals have been implicated as a pathological factor in the following health problems: arthritis, atherosclerosis, ischemic heart disease, cancers, cataracts, emphysema and retinopathy. The main target of free radicals is the polyunsaturated fats (PUFA) that make up the major part of the cell membranes.

Free radical attacks cause the PUFA to undergo a process called peroxidation (becoming rancid in nature). This rancidity causes further free radical propagation which leads to cellular damage, enzyme deactivation, and a foothold for the health problems listed above. With the growth in knowledge of free radical pathology has renewed interest in the roles of the antioxidant micronutrients. The effectiveness of vitamin E, vitamin C, and beta-carotene as potential weapons against the dangers of free radicals has been most frequently investigated. Much evidence points to their effectiveness in fighting atherosclerosis, cataracts, and enhancing the immune system.

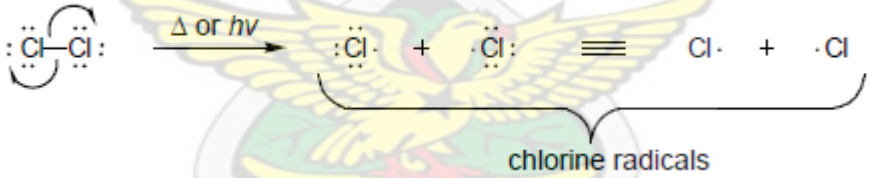
More recently, the roles of certain trace minerals in the maintenance of the body's antioxidant defense system have produced increased interest. Selenium, iron, copper, zinc, and manganese have all exhibited antioxidant involvement. A major player in the body's antioxidant defense system is an enzyme known as superoxide dismutase (SOD). Many supplements reportedly offer superoxide dismutase as an ingredient (Valko et al, 2006). Unfortunately, the molecular weights of enzymes are quite high. The copper-zinc

SOD has a molecular weight in excess of 54,000,000 me, while the manganese SOD weighs more than 109,000,000 me. In either case, both structures have molecular weights that far exceed what the body can absorb intact without digestion which, in this case, results in the destruction of the SOD enzyme.

2.1.1 Free Radical Reactions

Free radicals generally involved in chain reactions, a series of reactions leads to regeneration of a radical that can begin a new cycle of reactions. Free radical reactions take three distinct identifiable steps (Manavalan, 2001).

Initiation step: formation of radicals. E.g. Cl_2 absorbs energy and the bond is homolytically cleaved.



The first-formed reactive intermediate is chlorine radical.

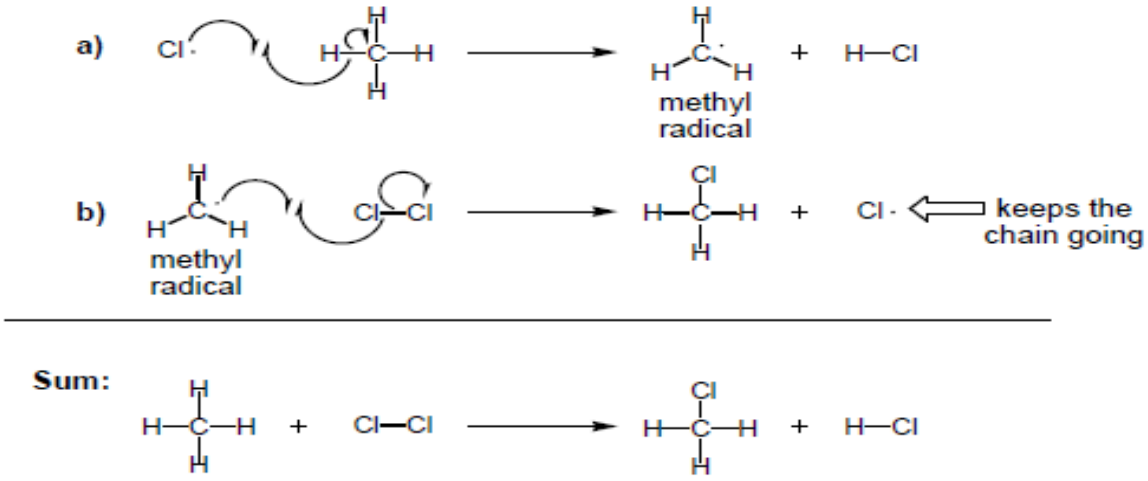
Reactive intermediate: short-lived species that react quickly as they are formed

Radical**:** species with an unpaired electron.

Propagation step: In this step, the required free radical is regenerated repeatedly as a result of a chain reaction, which would take the reaction to completion.

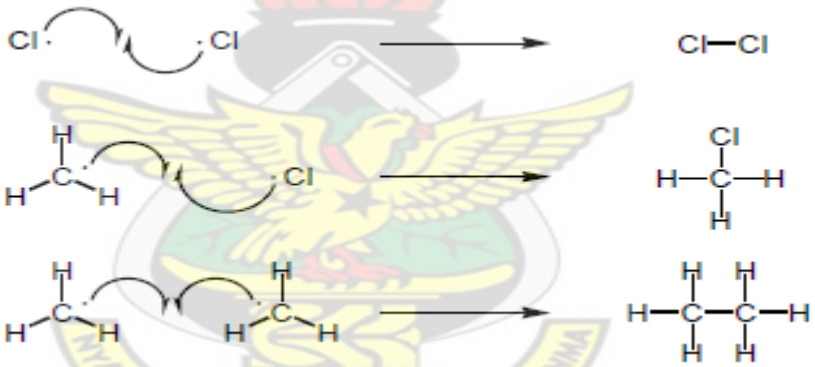
a) Chlorine radical abstracts hydrogen from methane to form a methyl radical.

b) Methyl radical abstracts chlorine from Cl_2 to form product and chlorine radical.



The sum of the propagation steps is the overall chlorination reaction.

Termination step: Destruction of radicals, that is, consumption of reactive intermediates (radicals) without generation of new ones.



• Termination steps slow down and eventually stop the chain reaction.

• Termination reactions become most important at the end of a reaction, when there are very few reactant molecules left.

2.2 GENERATION AND SOURCES OF FREE RADICALS

Free radicals can be formed from both endogenous and exogenous substances. They are continuously forming in cells and in the environment. Different sources of free radicals are as follows (Valko et al., 2006 and Nagendrappa, 2005)**:**

- UV radiations, X-rays, gamma rays and microwave radiation.

- Metal-catalyzed reactions.
- Oxygen free radicals in the atmosphere considered as pollutants.
- Inflammation initiates neutrophils and macrophages to produce ROS and RNS.
- Neutrophils stimulated by exposure to microbes.
- In mitochondria-catalyzed electron transport reactions, oxygen free radicals produced as by product.
- ROS formed from several sources like mitochondrial cytochrome oxidase, xanthine oxidases, and neutrophils and by lipid peroxidation.
- ROS generated by the metabolism of arachidonic acid, platelets, macrophages and smooth muscle cells.
- Interaction with chemicals, automobile exhausts fumes, smoking of cigarettes, cigars.
- Burning of organic matter during cooking, forest fires, volcanic activities.
- Industrial effluents, excess chemicals, alcoholic intake, certain drugs, asbestos, certain pesticides and herbicides, some metal ions, fungal toxins and xenobiotics.

2.2.1 Radical Reactions

$O_2 + e <=> O_2\bullet^-$ Equation 1

$O_2 + 2e + 2H+ \rightarrow H_2O_2$ Equation 2

$O_2^{\bullet-} + O_2^{\bullet-} + 2H+ \rightarrow SOD \rightarrow H_2O_2 + O_2$ Equation 3

$2H_2O_2 + Catalase \rightarrow 2H_2O + O_2$ Equation 4

$O^{2}\bullet^- + NO\bullet \rightarrow ONOO-$ Equation 5

$Fe^{2+} + H_2O_2 \rightarrow HO\bullet + HO- + Fe^{3+}$ Equation 6

Free radicals are atomic or molecular species with unpaired electrons on an otherwise open shell configuration. These unpaired electrons are usually highly reactive, so they are likely to take part in chemical reactions. Radicals play an important role in combustion, atmospheric chemistry, polymerization, plasma chemistry, biochemistry, and many other chemical processes, including human physiology. Some of these are discussed in the subsections below.

2.2.2 Hydroxyl radical (OH)

In saturated compounds, a hydroxyl radical abstract a hydrogen atom from the weakest C-H bond to yield a free radical. The resulting radicals can react with oxygen and generate other free radicals. Hydroxyl radicals also react with lipid, polypeptides and DNA and also especially with thiamine and gaunosine (Ashok and Ali, 1999).

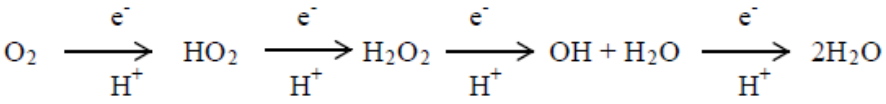
Fenton's reaction for production of hydroxyl radicals by oxidation of Fe^{2+} ions is well known (Matysik et al., 2002)

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

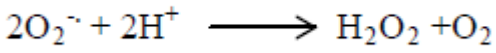
2.2.3 Superoxide anions

With one unpaired electron, the superoxide ion is a free radical, and, like dioxygen, it is paramagnetic ($\dot{O}_2^-$).

Superoxide anion is a reduced form of molecular oxygen created by receiving one. It is formed from a mitochondrial electron system. Mitochondria generates energy using four electron chain reaction, reducing oxygen to water. Some of the electrons escaping from the chain reaction of mitochondria directly react with oxygen and form superoxide anions (Gulam and Haseeb, 2006).



The superoxide plays an important role in the formation of other reactive oxygen species such as hydrogen peroxide, hydroxyl radical or single oxygen in living systems.



The superoxide anion can react with nitric oxide ($NO^{\cdot}$) and form peroxynitric oxide ($OONO^{-}$), which can generate toxic components such as hydroxyl radical and nitric dioxide.

2.2.4 Hydrogen peroxide

Hydrogen peroxide (H_2O_2) is a very pale blue liquid which appears colourless in a dilute solution, slightly more viscous than water. It is a weak acid. It has also found use as a disinfectant, as an oxidizer, and in rocketry (particularly in high concentrations as high-test peroxide (HTP) as a monopropellant), and in bipropellant systems

Hydrogen peroxide can be generated through a dismutation reaction from superoxide anion by superoxide dismutase. Enzymes such as amino acid oxidase and xanthine oxidase also produce hydrogen peroxide from superoxides. Hydrogen peroxide is highly diffusible and crosses the plasma membrane easily (Gulam and Haseeb, 2006).

Hydrogen peroxide is the least reactive among the reactive oxygen species and is stable under physiological pH and temperature in the absence of metal ions. It is a weak oxidizing and reducing agent and thus regarded as being poorly reactive. Hydrogen

peroxide can generate the hydroxyl radical in the presence of metal ions and superoxide anion.

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

It can produce singlet oxygen through reaction with superoxide anion or with HOCl or chloroamine in living system (Steif, 2003). Hydrogen peroxide can degrade certain proteins such as haemoglobin, to release iron ions.

2.2.5 Singlet Oxygen

Singlet oxygen is a non-radical and excited species, with the electrons in the π-antibonding orbitals paired. Singlet oxygen is rather mild and non-toxic for mammalian tissues compared with other ROS (Steif, 2003). However, it has been known to be involved in cholesterol oxidation, which results in the formation of 5α-OOH (3-β-hydroxy-5α-cholest-6-ene-5-hydroxyperoxide).

2.2.6 Peroxyl and Alkoxyl radicals

Peroxyl radicals ($ROO^{\cdot}$) are formed by a direct reaction of oxygen with alkyl radicals ($R^{\cdot}$), for example, the reaction between lipid radicals and oxygen. Decomposition of alkyl peroxides (ROOH) also result in peroxyl ($ROO^{\cdot}$) and alkoxyl ($RO^{\cdot}$) radicals. Irradiation of UV light or the presence of metal can cause decomposition of peroxides to produce peroxyl and alkoxyl radicals.

$$ROOH \xrightarrow{uv} ROO^{\cdot} + H^{\cdot}$$

$$ROOH + Fe^{3+} \longrightarrow ROO^{\cdot} + Fe^{2+} + H^+$$

Peroxyl and alkoxyl radicals are good oxidizing agents, having more than 1000 mV of standard potential. They can abstract hydrogen from other molecules with lower standard potential. This is frequently observed in the propagation state of lipid peroxidation. Very

often, the alkyl radical formed from this reaction can react with oxygen to form another peroxyl radical, resulting in chain reaction. Some peroxyl radicals break down to liberate superoxide anion or can react with each other to generate singlet oxygen (Halliwell, 1997). Aromatic alkoxyl and peroxyl radicals are less reactive than respective open chain radicals because of the delocalization of electrons in the ring.

2.2.7 Nitric oxide and nitric dioxide

Nitric oxide ($NO^{\cdot}$) is a free radical with a single unpaired electron. It is formed from L-arginine (Fang, 2002). Nitric oxide itself is not a very reactive free radical, but the over production of $NO^{\cdot}$ in ischemia reperfusion causes neurodegenerative and chronic inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease.

2.2.8 Peroxynitrite

Reactions of nitric oxide and superoxide generate peroxynitrite.

$$O_2^{\cdot} + NO \longrightarrow OONO^{-}$$

Peroxynitrite is a cytotoxic species and oxidizes low-density lipoprotein (LDL). It appears to be an important tissue-damaging species generated at the sites of inflammation and has been shown to be involved in various neurodegenerative disorders and several kidney diseases. Peroxynitrite can cause direct protein oxidation and DNA base oxidation and modification acting as a "hydroxyl radical-like" oxidant. The significance of peroxynitrite as a biological oxidant comes from its high infusibility across cell membrane (Gulam and Haseeb, 2006).

2.3 ANTIOXIDANTS

2.3.1 The Antioxidant Process

Antioxidants prevent the process of oxidation by neutralizing free radicals. In the cause of prevention, they self-oxidize. That is why there is a constant need to replenish our antioxidant resources, which could be in the form of eating fruits and vegetables.

Their working ability can hence be classified accordingly:

• Chain-breaking: When a free radical releases an electron, a second radical is formed. This molecule thus affected in turn releases an electron and forms a third molecule, continuing to generate more unstable products. Continuously this process happens until termination occurs. The radical is stabilized by a chain-breaking antioxidant such as beta-carotene and vitamins C and E, or it simply decays into a harmless product to end this process.

• Preventive: Antioxidant enzymes like superoxide dismutase (SOD), catalase and glutathione peroxidase prevent oxidation by reducing the rate of chain initiation. That is, by scavenging initiating radicals, such antioxidants can thwart an oxidation chain from ever setting in motion (Holliday, 1995). They can also prevent oxidation by stabilizing transition metal radicals such as Cu^{2+} and Fe^{3+}.

There are antioxidants that react with oxygen free radicals, quench or reduce their effects to the minimum effects possible. These include α-tocopherol (vitamin E), tocotrienol, ascorbic acid (vitamin C), uric acid, sulphydryl containing compounds such as cystein and glutathione, bilirubin, ubiquinol and carnosine (Gulam and Haseeb, 2006).

L-Ascorbic acid is a 6-carbon lactone ring structure with 2, 3-enediol moiety. The antioxidant activity of ascorbic acid comes from the 2, 3-enediol. L-ascorbic acid first

changes to semi-dehydroascorbic acid through donating one hydrogen and three electrons (Holliday, 1995).

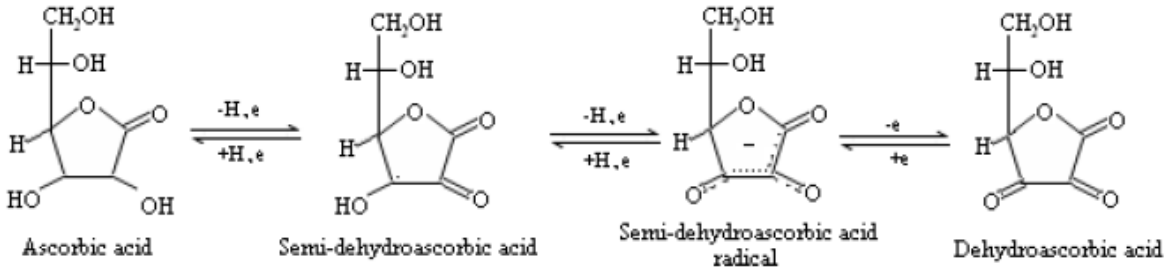


Figure 2.1 Sequential 1-electron oxidation of L-ascorbic acid

The antioxidant mechanisms of ascorbic acid are based on hydrogen atom donation to lipid radicals, quenching of singlet oxygen, and removal of molecular oxygen. Scavenging of aqueous radicals and regenerating of α-tocophenols from the tocopheroxyl radical species are also known antioxidant mechanisms of ascorbic acid (Emmanuel, 2008).

Figure 2.2 General structure of tocopherols

Carotenoids are the most efficient singlet oxygen quenchers in biological systems. The rate at which singlet oxygen are quenched by carotenoids is dependent on the number of conjugated double bonds and on the type of functional group on the ring structure of the molecule. Achieving a high level of efficiency in singlet oxygen quenching requires at least seven conjugated bonds and as the number of conjugation increases, quenching

efficiency also increases (Boff and Min, 2002). Singlet oxygen mechanisms by carotenoids are physical quenching without generating oxidizing products.

$$^1O_2 + {}^1\text{Carotenoid} \longrightarrow {}^3O_2 + {}^3\text{Carotenoid}$$

2.3.2 Determination of antioxidant properties

The antioxidant activities of antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical scavenging (Yildirim et al., 2001). Various methods are used in determining the antioxidant properties of plant samples. These include measurement of total phenolic content, reducing power, hydrogen peroxide decomposition/consumption, DPPH scavenging and Fe^{2+} chelation. Yildirim et al (2001) has suggested non-linear correlation between total antioxidant activity and the individual measurement and that the antioxidant activity of any species is a cumulative effect of most of the measurements.

2.3.3 Reducing power determination

Heavy metals like Fe^{3+} and Cu^{2+} are known to catalyze oxidative process in living organisms. Fe^{3+}, for instance, is reduced to Fe^{2+} in the process. It follows that if the +2 state does not aid the oxidative process, then the process does occur. A species ability to reduce Fe^{3+} to the +2 state is known as it reducing power and it is an indication of its antioxidant property (Yildirim et al., 2001). The Fe^{3+} reducing power of the samples is determined based on the chemical reaction of

$$\text{Fe (III)} \rightarrow \text{Fe (II)}.$$

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Yildirim et al., 2001). Oyaizu (1986) has described a dose-dependent

method (which was modified by Yildirim *et al* in 2001) for the determination of the reducing capacity of samples. In his method, various concentrations (100-1000 μg/ml) of the plant samples are prepared and mixed with phosphate buffer and 1% w/v of potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture is incubated at 50^oC for 30 minutes, after which 10% w/v trichloroacetic acid is added and centrifuged at 3000 rpm for 10 minutes. To about 2.5ml of the supernatant layer of the solution is added 2.5 ml distilled water and 0.5 ml of 0.1% w/v FeCl3. The sample's ability to reduce the Fe (III) to Fe (II) is determined by measuring the amount of the Fe (II) spectroscopically; the absorbance of the reaction mixture is measured at 700 nm. Increased absorbance indicates increased reducing power (Blázovics et al., 2003).

2.3.4 DPPH scavenging ability

The antioxidant ability of a sample can also be estimated be determining the hydrogen donating ability of the samples in the presence of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical at 517 nm on the basis of the method of Hatano *et al* (1988) . The determination is based on the discolouration of the purple coloured methanolic solution of DPPH free to yellow by free radical scavengers.

Figure 2.3 structure of 1, 1-diphenyl-2-picrylhydrazyl (DPPH)

Representing the DPPH radical by Z• and the donor molecule by AH, the primary reaction is

$$Z^{\bullet} + AH \rightarrow ZH + A^{\bullet}$$

Where ZH is the reduced form and $A^{\bullet}$ is free radical produced (Molyneux, 2004).

2.3.5 Chelation of Fe^{2+}

By ascertaining an iron chelating property of a sample, the antioxidant ability of a test sample can be determined. 1, 10 - phenanthroline is used as the chelating agent for the determination of free irons ions (Fe^{2+}) using a standard Fe^{2+} solution containing about 1 ml conc. H_2SO_4.

An example is the chelating of 1, 10 - phenanthroline with Fe^{2+} through donation of the lone pair on the nitrogen

Figure 2.4, 10-phenanthroline chelation of Fe^{2+}

2.4 PHYTOCHEMICAL CONSTITUENTS

2.4.1 Flavonoids

Flavonoids are polyphenolic compounds that are abundant in nature and are categorized, according to chemical structure. The flavonoids have aroused considerable interest since 1990 because of their potential beneficial effects on human health. They have been reported to have antiviral, antiallergic, antiplatelet, anti-inflammatory, anti-tumour and antioxidant activities.

Flavonoids (flavonols and flavanols) are most commonly known for their antioxidant activity *in vitro*. At high experimental concentrations that would not exist *in vivo*, the antioxidant abilities of flavonoids *in vitro* may be stronger than those of vitamin C and E, depending on concentrations tested (Bagchi et al., 1999)

Flavonoids represent a very wide group of water-soluble derivatives of the basic compound shown in fig 2.6. Many of them are coloured – red, crimson purple or yellow (Goodwin and Mercer, 1983). They are polymeric compounds possessing fifteen carbon atoms, with two benzene rings joined by a linear three-carbon (3-C) chain as its basic structure (Ikan R, 1991); the variation is the state of oxidation of the connecting 3-C moiety, which determines the properties and class of each compound.

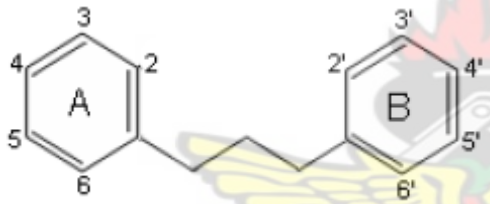
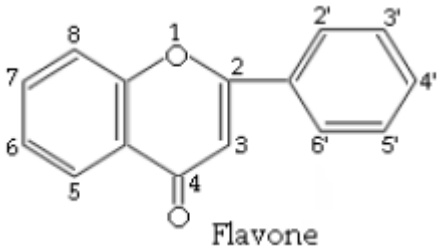


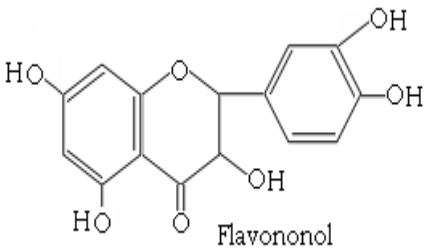
Figure 2.5 General structure of flavonoid

2.4.1.1 Classes of Flavonoids

Flavonoids are classified into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones according to their chemical structure. The substituents on positions on the rings differentiate each group from the other as shown in fig 2.6.



Flavone



Flavononol

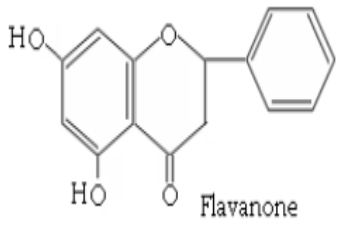
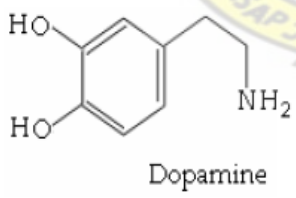


Figure 2.6 Chemical structures of some classes of flavonoids

2.4.2 Alkaloids

Many of the alkaloids known contain a basic nitrogen atom. An alkaloid containing plant almost never contains one alkaloid but rather a whole range of closely related components (Mann et al., 1994). Alkaloids are very difficult to define. The term alkaloid is commonly applied to basic nitrogenous compounds that are physiologically active. They nearly contain their nitrogen as part of a heterocyclic system (Goodwin and Mercer, 1983) and are often quite complex in structure. Alkaloids usually show specific pharmacological activity. Amongst the pharmacologically active ingredients found in plants, alkaloids are arguably most important (Ikan, 1991).



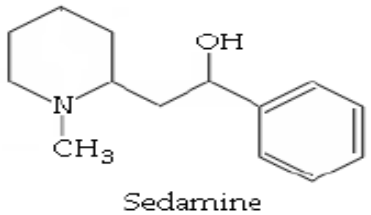


Figure 2.7 some examples of alkaloids

Some alkaloids have further use; quinine (commonly found in *chinchona* spp), for example, is used in the food industry for bitter flavouring and as an anti-malaria drug (Schmid, 1996). They are known to have antioxidant and antimicrobial activities (Akinyemi et al., 2006 and Pitzshchke et al., 2006)

The classification of alkaloids is usually based on the chemical structure from which they are derived. The biological functions of alkaloids in plants are not well understood. Many alkaloids are extremely toxic. They evolved as a defensive mechanism that protects plant against predators (Mann et al., 1994).

Some examples of alkaloids are nicotine (from *Nicotiana tabaccum*), quinine (from *Cinchona officinalis*), dopamine, anabasine (from *Anabasis aphylla*), edamine, cocaine, morphine (from *Papaver samniferum*), strychnine and conetine (Ikan, 1991).

2.4.3 Saponins

They can be said to be natural detergents found in plants because they contain both water-soluble and lipid-soluble components. They consist of a lipid-soluble nucleus, having either a steroid or triterpenoids, saponins with one or more side chain of water-soluble carbohydrate (sugar). Their physiological action depends on the fact they break up the red blood cells – haemolysis. Saponins have a bitter and acidic taste. They are highly toxic to cold-blooded animals because of their haemolytic properties (Cobbinah, 2008).

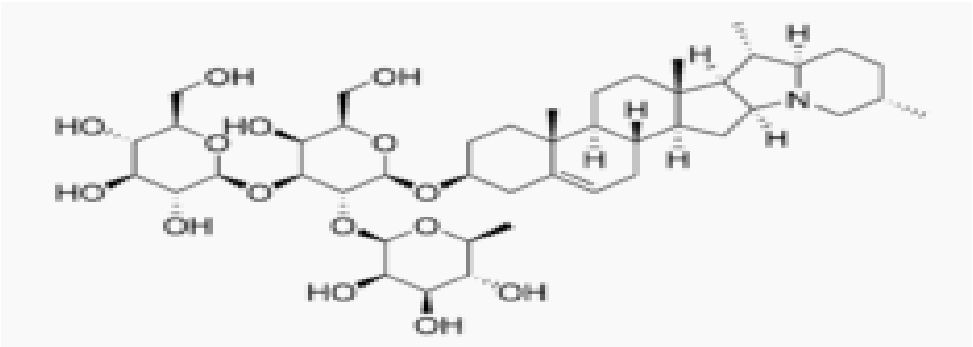


Figure 2.8 Solanine, an example of saponin

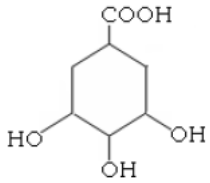
They are, however, comparatively harmless when taken by mouth. Upon hydrolysis of saponins a product known as sapogenin is produced. The highly toxic sapogenins are called sapotoxins. A simple test for sapotoxins in saponins is to shake up an aqueous alcoholic extract in a test tube. The formation of a persistent foam above the alcoholic extract in test tubes is a reliable evidence to show their presence (Cobbinah, 2008). Two kinds of saponins are recognized, the steroidal (commonly tetracyclic triterpenoids) and pentacyclic triterpenoids according to the structure of aglycone.

Among the chemical properties of saponins, their polarity, hydrophobicity and nature of the reactive groups seem to be important determinants of their biological properties. They are known to have antibacterial, antitumour and cytotoxic, fungucidal and molluscicidal activities (Akinyemi et al., 2006).

2.4.4 Tannins

Tannins are secondary metabolites of plants, non-nitrogenous, phenolic in nature and are used for various types of polyphenols obtained from plants and also to convert animal skins to leather. The term "tannins" or tannin compounds are also used for the polyphenols that combine with or precipitate the protein of skins to increase their stability to water, micro-organisms, heat, and abrasion. Tannins are one of the many types of secondary compounds found in plants. They are oligomeric compounds with multiple structure units with free phenolic groups. Their molecular weight ranges from 500 to 20,000 or more (Caret et al., 1997). They are soluble in water, with exception of some high molecular weight structures, and are able to bind proteins forming insoluble or soluble tannin-protein complexes.

There are two main types of tannins which are distributed unevenly throughout the plant kingdom. These are: hydrolysable tannins (HT) and proanthocyanidins (PA) (often called Condensed Tannins).



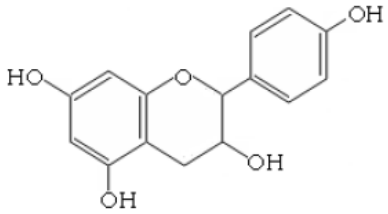


Figure 2.9 Gallic acid (hydrolysable tannin)

Figure 2.10 Flavan-3-ol (Condensed tannins)

2.4.5 Coumarins

Coumarins are phytochemicals widely distributed in several plants, including: Tonka beans, lavender, liquorice, strawberries, apricots, cherries, cinnamon, and sweet clover. They are unsaturated aromatic lactones, which occur either in the free-state or combined with the sugar glucose that is Coumarin glycoside (Mann et al., 1994).

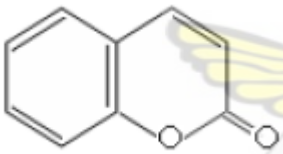


Figure 2.11 General structure of Coumarins

Coumarins have blood-thinning, anti-fungal and anti-tumour activities. They are known to increase the blood flow in the veins and to decrease capillary permeability. Coumarins can be toxic when used at high doses for a long period. Coumarin seems to work as a pesticide in the plants that produce it. Plants also use them as growth inhibitors (anti-auxins) as well as defensive compounds.

2.4.6 Anthraquinones

Anthraquinones occur in various types of plant materials and may occur as free anthraquinones, or as glycosides. Natural products have also been found to contain

reduced derivatives of anthraquinones. They are oxanthrones (an ether form), anthranols and anthrones and compounds formed by union of two conthrone molecules.

Anthraquinone derivatives are sometimes orange-red compounds that may be obscured in sight. They are usually soluble in hot water and dilute alcohol and are known to have antibacterial and antifungal properties (Babu et al., 2003). Bomtrager's test is used for their detection (Trease and Evans, 1983).

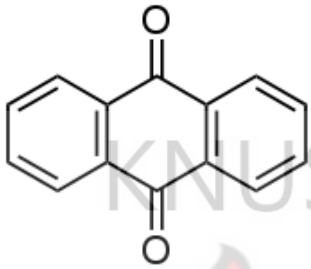


Figure 2.12 General structure of structure of anthraquinones

A large industrial application of anthraquinones is for the production of hydrogen peroxide. 2-Ethyl-9, 10-anthraquinone or a related alkyl derivatives is used, rather than anthraquinone itself (Gutaaf et al., 2007).

Figure 2.13 Catalytic hydrogen peroxide productions with Anthraquinone process

2.4.7 Glycosides

Glycosides are compounds that yield one or more sugars among the products of hydrolysis. They are acetals in which the hydroxyl group of sugars is condensed with a hydroxyl group of a non-sugar component, and a secondary hydroxyl is condensed within

the molecule itself to form an oxide ring. More simply, glycosides can be considered as sugar-ethers (Tyler eta l., 1995) consisting of non-sugar and a component sugar in the same molecule (Trease, 1983).

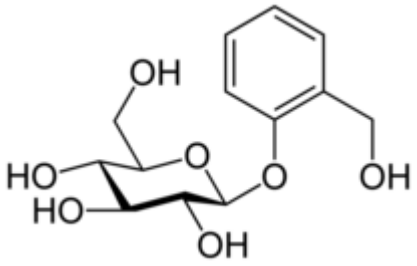


Figure 2.14 Salicin, a glycoside related to aspirin

The sugar components are referred to as aglycone and the non-sugar components are known as glycone. Depending on the stereo-configuration of the glycosidic linkage, sugars exist in isomeric α-forms and β-forms, both α-glycosides and β-glycosides are possible theoretically. However, all natural glycosides are practically of the β-type, even though the α-linkage is found in some carbohydrates like sucrose, glycogen and starch. The type of glycosides described above (involving oxygen linkages) occurs most in nature and are normally known as O-glycosides. Other glycosides do, however, occur in which the linkage is through sulphur (S-glycoside), nitrogen (N-glycoside) or carbon i.e. C-glycoside (Gulam and Haseeb, 2006).

2.4.8 Cyanogenic Glycosides

Cyanogenic glycosides are widely distributed among 100 families of flowering plants. They are also found in some species of ferns, fungi and bacteria. There are many economical important plants highly cyanogenic, including white clover, linum, almond, sorghum and the rubber tree. Cyanogenic glycosides can be found in the fruits (and wilting leaves) of the rose family (including cherries, apples, plums, almonds, peaches,

apricots, raspberries, and crab apples) and many seeds of the Rosacae (Goodwin and Mercer, 1983) cassava (Ilza, 2000).

2.4.9 Terpenoids and Steroids

Steroids are compounds possessing the skeleton of cyclopenta [a] phenanthrene or a skeleton derived therefrom by one or more bond scissions or ring expansions or contractions. Methyl groups are normally present at C-10 and C-13. An alkyl side chain may also be present at C-17 (IUPAC and IBU, 1989).

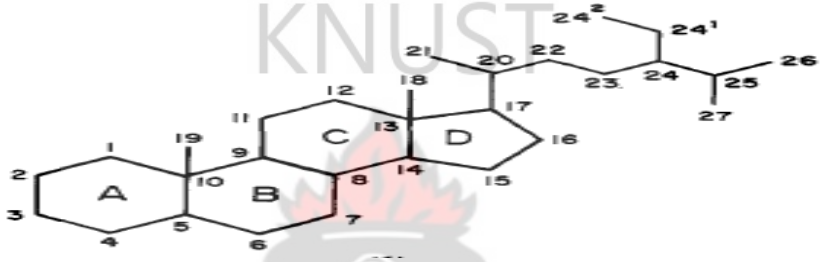


Figure 2.15 General structure of steroids

Steroids are modified triterpenoids which are also derived from squalene by cyclisation, unsaturation and substitution. The nucleus of all steroids is the tetracyclic C17 hydrocarbon 1, 2 cyclopentanoperhydrophenanthrene (gonane or sterane) substituted by methyl groups at C10 and C13, as well as alkyl side-chain at C17. Steroids may possess a nucleus derived from the former one by one or more C-C bond scissions or ring expansion or contractions (Emmanuel, 2008).

Terpenoids are relatively volatile, this has several important consequences. Firstly they are easily isolated by distillation from volatile plant materials. Secondly, they have distinctive and sometimes pleasant aromas. For this reason they have been extensively used in the flavour and fragrance industries. The biological functions of terpenoids are not

known, in some cases they seem to protect the plant from the animals through their disagreeable taste and odour, but no general role has been established (Caret et al., 1997).

Classification of Terpenoids

Most natural Terpenoids hydrocarbon have the general formula $(C_5H_8)_n$. They can be classified on the basis of value of n or number of carbon atoms present in the structure (Sameena, 2007).

Table 2.1 Classification of Terpenoids

S.No.	Number of carbon atoms	Value of n	Class
1.	10	2	Monoterpenoids($C_{10}H_{16}$)
2.	15	3	Sesquiterpenoinds($C_{15}H_{24}$)
3.	20	4	Diterpenoids($C_{20}H_{32}$)
4.	25	5	Sesterpenoids($C_{25}H_{40}$)
5.	30	6	Troterpenoids($C_{30}H_{48}$)
6.	40	8	Tetraterpenoids($C_{40}H_{64}$)
7.	>40	>8	Polyterpenoids(C_5H_8)n

Each class can be further subdivided into subclasses according to the number of rings present in the structure.

i) Acyclic Terpenoids: They contain an open structure.

ii) Monocyclic Terpenoids: They contain one ring in the structure.

iii) Bicyclic Terpenoids: They contain two rings in the structure.

iv) Tricyclic Terpenoids: They contain three rings in the structure.

v) Tetracyclic Terpenoids: They contain four rings in the structure

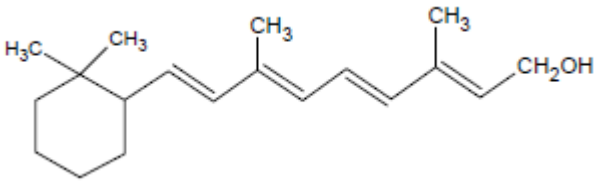


Figure 2.16 Vitamin A, an example of mono cyclic diterpenoids

2.4.10 Carotenoids

Carotenoids are a class of yellow, red natural fat-soluble pigments (Mann et al., 1994) found principally in plants, algae, and photosynthetic bacteria, where they play a critical role in the photosynthetic process. They also occur in some non-photosynthetic bacteria, yeasts, and molds, where they may carry out a protective function against damage by light and oxygen (Emmanuel, 2008).

Carotenoids are responsible for many of the red, orange, and yellow hues of plant leaves, fruits, and flowers, as well as the colors of some birds, insects, fish and crustaceans, and of many microorganisms.

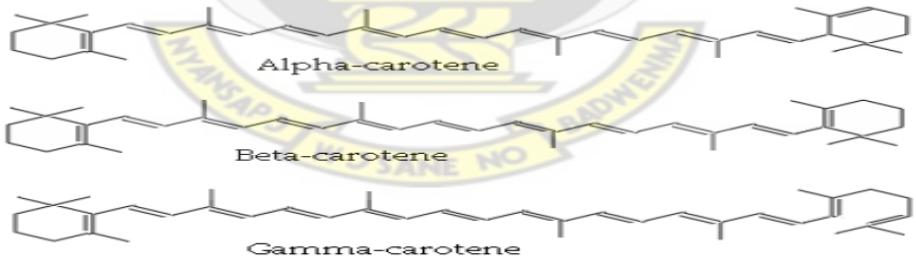


Figure 2.17 some chemical structures of carotenes

In general, when the intensity of colour is high it means there is high level of carotenoids. In green leafy vegetables, beta-carotene is the predominant carotenoid. In the orange-coloured fruits and vegetables - such as carrots, apricots, mangoes, yams- beta-carotene concentrations are high, but other pro-vitamin A carotenoids typically predominate.

Yellow vegetables have higher concentrations of yellow carotenoids (xanthophylls), hence a lowered pro-vitamin A activity; but some of these compounds, such as lutein, may have significant health benefits, potentially due to their antioxidant effects.

The red and purple vegetables and fruits - such as tomatoes, red cabbage, berries, plums, flamingoes and salmon 30 - contain a large portion of non-vitamin A–active carotenoids. Legumes, grains, and seeds are also significant sources of carotenoids

2.5 PREVIOUS RESEARCH WORKS

2.5.1 Antioxidant research Database

In 2008, Monica et al conducted a comprehensive antioxidant survey work that spun over the world. These antioxidant measurements were conducted over a period of eight years, from 2000 to 2008. The samples were procured from local stores and markets in Scandinavia, USA and Europe and from the African, Asian and South American continents. Many of the samples of plant material, like berries, mushrooms and herbs, were handpicked.

It has been hypothesized that plant antioxidants may contribute to the beneficial health effects of dietary plants. Their objective was to develop a comprehensive food database consisting of the total antioxidant content of typical foods as well as other dietary items such as traditional medicine plants, herbs and spices and dietary supplements. The database when completed was intended for use in a wide range of nutritional research, from *in vitro* and cell and animal studies, to clinical trials and nutritional epidemiological studies.

The results demonstrated that there are several thousand-fold differences in antioxidant content of foods. Spices, herbs and supplements included the most antioxidant rich

products in their study, some exceptionally high. Berries, fruits, nuts, chocolate, vegetables and products therefore constitute common foods and beverages with high antioxidant values.

When they classified the samples into the three main classes, the difference in antioxidant content between plant- and animal-based foods became apparent or visible. The results obtained uncovered that the antioxidant content of foods varied several thousand-fold and that antioxidant rich foods originated from the plant kingdom while meat, fish and other foods from the animal kingdom were low in antioxidants.

Comparing with the mean value of the 'Meat and meat products' category with plant based categories, fruits, nuts, chocolate and berries had from 5 to 33 times higher mean antioxidant content than the mean of meat products. Diets comprised mainly of animal based foods were thus low in antioxidant content while diets based mainly on a variety of plant-based foods were antioxidant rich, due to the thousands of bioactive antioxidant phytochemicals found in plants which are conserved in many foods and beverages.

In their results, most of the spices and herbs analysed had particularly high antioxidant contents. Although spices and herbs contributed little weight on the dinner plate, they may still be important contributors to our antioxidant intake, especially in dietary cultures where spices and herbs are used regularly (Monica et al., 2008). They interpreted the elevated concentration of antioxidants observed in several dried herbs compared to fresh samples, as a normal consequence of the drying process leaving most of the antioxidants intact in the dried end product. Thus, they concluded that dried herbs and fruit are potentially excellent sources of antioxidants.

Herbal and traditional plant medicines emerged as many of the highest antioxidant-containing products in this study. They therefore speculated that the high inherent

antioxidant property of many plants is an important contributor to the herb's medicinal qualities.

With their high content of phytochemicals such as flavonoids, tannins, stilbenoids, phenolic acids and lignans (Kahkonen et al., 2001) berries and berry products are potentially excellent antioxidant sources. The phytochemical content of berries varied with geographical growing condition, and between cultivars (Scalzo et al., 2005 and Wang et al., 2005) and thus explaining the variations found in their study. During the processing of berries to jams, total phenol content was reduced (Amakura et al., 2000) resulting in lower antioxidant values in processed berry products than in fresh berries. From these results, it can be expected that "kwawu nsusua" (turkey berry) would have high antioxidant levels.

One significant finding in their study was that they identified *Sangre de Grado*, the sap from the tree trunk of the species *Croton lechleri* sampled in Peru to have exceptional high antioxidant content. This sap has a long history of indigenous use in South America for wound healing and as an antifungal, antiseptic, antiviral and anti-haemorrhagic medicine.

Biochemically active phytochemicals found in plant based foods also have many powerful biological properties which are not necessarily correlated with their antioxidant capacity, including acting as inducers of antioxidant defence mechanisms in vivo or as gene expression modulators. Thus a food low in antioxidant content may have beneficial health effects due to other food components or phytochemicals executing bioactivity through other mechanisms (Monica et al., 2008).

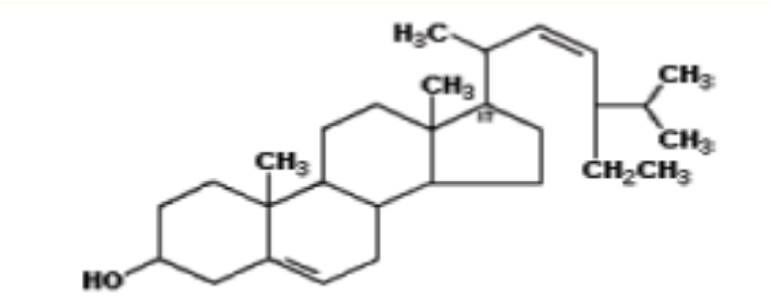


Figure 2.18 Torvoside B

2.5.1.1 Anti - ulcerogenic

Solanum torvum revealed the presence of flavanoids, sterols & triterpens which may be responsible for anti-ulcer property. It strengthens the mucosal barrier through the increase of the

mucas and bicarbonate production and reducing the volume of gastric acid secretion or by simply

neutralising the gastric activity (Nguelefacka et al., 2008).

2.5.1.2 Anti-viral activity

A new C4 - sulfated isoflavonoid (torvanol A) and steroidal glycoside (torvoside H) together with torvoside A were isolated from methanolic extract of *Solanum torvum* fruits exhibited antiviral activity that is herpes simplex virus type 1(Arthan et al., 2002).

2.5.1.3 Analgesic

The aqueous extract of *Solanum torvum* leaves exhibited peripheral analgesic activity. The pain killing effect of the plant may be due to prostaglandin synthesis inhibition (Ndebia et al., 2007).

2.5.1.4 Anti-inflammatory

S. torvum extract has anti-inflammatory activity. *Solanum torvum* extract may act by suppressing the later phase of the inflammatory process by inhibition of cyclooxygenase involved in the formation of prostaglandin (Ndebia et al., 2007).

2.5.1.5 Antioxidant activity of "kwawu nsusua"

Solanum torvum possess significant antioxidant activity in vitro. Due to antioxidant activity, solanum torvum is used for reducing oxidative stress in diabetes (Winthana et al., 2009).

Antioxidants in Turkey

Elsewhere, Karadeniz et al. (2005), while evaluating the antioxidant activity of selected fruits and vegetables grown in turkey reported that total phenolics in grape, red cabbage and onion were 2025 ± 56.6, 2166 ± 7.1 and 536 ± 113.1 mg/kg respectively. Several factors could be attributed to the differences in total phenolic content of foodstuffs of same or similar origin. They include variation in fruit cultivars, harvest and post-harvest handling and storage conditions, processing techniques during analytical determinations. It also reported that apples showed antioxidant activity attributed to total phenolic amount over a wide range of cultivars from 14.7 to 40.7% (Oviasogie et al., 2009).

Their study used all the fruits and vegetables that were obtained at the open market; the implication is that several physiochemical reaction would have taken place between the harvest time and the open market where the foodstuffs are sold which may be responsible for the relatively low levels of total phenolic determined in the fruits and vegetable.

Antioxidants in Africa (Nigeria)

In recent Nigeria, most research work has been done and published in Nigeria. Quite recently, Olajire and Azeez (2011) researched and published their work on antioxidant activities, total phenolic, flavonoid and ascorbic acid contents of different vegetables commonly consumed in Nigeria. The antioxidant activities of vegetables ranged from 22.15% (*Talinum triangulare*) to 92.30% (*Capsicum frutescens*). *Solanum macrocarpon,* with the lowest IC_{50}, was seen as the most potent vegetable of the samples analyzed, that could scavenge most free radicals; while *Cucumissativus,* with the highest IC_{50}, was the least potent. Total phenolic, flavonoid and ascorbic acid contents of vegetables ranged from 22.1 to 370.68 mg quercetin g^{-1}; 10.23 to 215.39 mg quercetin g^{-1} and between 16.67 to 150.67 mg ascorbic acid g^{-1}, respectively. A high and significant correlation existed between antioxidant activity and total phenolic content of vegetables ($r^2 = 0.861$, $p < 0.05$), indicating that total phenolic content is the major contributor to the antioxidant activity of vegetables. However, flavonoids, which belong to the phenolic compounds, were not significantly correlated with antioxidant activity ($r^2 = 0.143$, $p < 0.05$). Ascorbic acid fairly correlated ($r^2 = 0.546$, $p < 0.05$) with antioxidant and phenolic content ($r^2 = 0.591$, $p < 0.05$).

The total antioxidant activity obtained in this study were comparable with those obtained by Marinova et al., (2005) but higher than that of Odukoya et al (2007). This could be due to methods used for the analysis and the medium of extraction as pointed out by Li et al., (2008).

There was no correlation between total flavonoids and radical scavenging activity, ($r^2 = 0.143$). This lack of relationship is in agreement with other reports (Anagnostopoulou *et al.,* 2006); which indicated that flavonoids did not contribute to antioxidant activity of vegetables. *Murraya koenigii* had the highest value of 150.67 mg ascorbic acid g^{-1} and

Cucumis sativus had the lowest value of 16.67 mg ascorbic acid g^{-1}. The values are in agreement with values obtained by Sumazian *et* al., (2010) but higher than what were obtained by Okiei et al., (2009).

According to Olajire and Azeez (2011), it is normal when total ascorbic acid do not correlate with the total antioxidant activities since total ascorbic acid made little or no contribution to the total antioxidant activities of vegetables.

Ganiyu Oboh et al, 2011 conducted a research work on effect of combination on the antioxidant and inhibitory properties of tropical pepper varieties against a-Amylase and α-Glucosidase Activities *in Vitro* in the Nigerian community. They investigated the health benefits of a combination of 3 pepper varieties commonly consumed in Nigeria based on the individual preferences of using one or more types of pepper as spices in meals. Aqueous extracts (1:100 w/v) of *Capsicum annuum var. grossum, C. annuum var. abbreviatum,* and *C. annuum var. accuminatum* and a combination of the 3 varieties (1:1:1) were assayed for phenolic content (total phenol and flavonoid), antioxidant activities (reducing power and 1,1-diphenyl-2-picrylhydrazyl radical scavenging abilities), inhibitory effect on Fe^{2+} -induced lipid peroxidation in rat pancreas in vitro, and the ability of the extracts to inhibit key enzymes linked with type 2 diabetes (a-amylase and a-glucosidase) were determined. The combination of peppers showed additive effects in their phenolic content and displayed antioxidant properties. However, the inhibition of pancreatic a-amylase activity showed an additive effect, whereas a-glucosidase inhibitory activity was antagonistic with the combination. *C. annuum var. accuminatum* contributed most to the activities of the combined peppers. Strong inhibitory activities of the peppers against key enzymes linked to type 2 diabetes and Fe^{2+} -induced lipid peroxidation in rat pancreas in vitro, coupled with their antioxidant properties, suggested that pepper could

be used in the prevention and management of type 2 diabetes. The pepper combination showed additive tendencies of these properties.

Their results showed that *C. annuum var. abbreviatum* (ROA) (795.15 mg/100g) had the highest phenolic content, and *C. annuum var accuminatum* (SOC) had the lowest (545.25 mg/100g). However, the combination of the 3 pepper fruits (RST) in equal amounts had a phenolic content of 680.42 mg/100 g (Ganiyu Oboh et al, 2011).

Antioxidants in Ghana

Morrison and Twumasi (2010) performed some epidemiological studies in 2010 that indicated that consumption of fruits and vegetables has the ability to inhibit the damaging activities of free radicals in the human body. Eight edible leafy vegetables of Ghana namely: *Xanthosoma sagittifolium, Hibiscus Sabdariffa, Solanum macrocarpon, Talinum triangulare, Corchorus olitorius, Laportea aestuans, Ipomoea batatas, and Amaranthus cruentus* were assessed for their antioxidant properties. The total antioxidant capacity (TAC) and total phenol content (TPC) in the methanol extracts (METE) and hydro-ethanol extracts (HETE) from the selected leafy vegetables within the measured concentration range (0.1 - 3.0 mg/ml) decreased in the order *X. sagittifolium > I. batatas > L. aestuans > T. Triangulare > H. Sabdariffa > C. olitorius > S. macrocarpon > A. cruentus.* A high and positive correlation was observed between TPC and TAC in both the METE and HETE from all the selected leafy vegetables. The selected leafy vegetables showed strong antioxidant properties with respect to their free radical scavenging activity and Fe^{3+} reduction ability with hydro-ethanol extracts indicating higher antioxidant potential compared with their respective methanol extracts.

Mercy et al, (2012) did a study that reported *in vitro* radical scavenging and antioxidant capacity of crude methanol and ethanol-water extracts of the fruits of *Tetrapleura*

tetraptera and *Parkia biglobosa*. Total phenolic contents in *Tetrapleura tetraptera* were 147.82 ± 1.36 and 130.33 ± 1.04 mg GAE/g dry weight while that of *Parkia biglobosa* were 128.32 ± 0.49 and 127.23 ± 0.11 mg GAE/g dry weight respectively. The total antioxidant capacity of the extracts ranged from175.52 ± 4.66 (methanol) to 172.87 ± 2.15 mg/g (ethanol/water) for *Tetrapleura tetraptera* and from 160.44 ± 2.26 (methanol) to 157.31 ± 1.90 mg/g (ethanol/water) for *Parkia biglobosa*. The antioxidant activities of both fruits were determined by the 2, 2'-diphenyl-1-picrylhydrazyl radical (DPPH) and the reducing power (RPA) assays produced concentration-dependent values that were compared with that of ascorbic acid control. The results of the study showed that fruits of *Tetrapleura tetraptera* and *Parkia biglobosa* had strong radical scavenging and reducing capacities.

Antioxidants in Asia (Malaysia)

In 2009, Kho et al did some antioxidant related work in Malaysia with the *Auriculariaauricula-judae* and mycelium of *A. auricular judae* that are currently grown in Malaysia. In their study, the methanolic extracts from fruit bodies were treated as fresh, oven-dried, and freeze-dried and then evaluated for their antioxidant capacities based on 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity and ferric reducing antioxidant power (FRAP) assay. They also measured the total phenolic content in the extracts. The extract of freeze-dried fruit bodies of *A. auricula-judae* had potent DPPH free radical scavenging activity with a 50% effective concentration of 2.87 mg/mL. In the study, three extracts of *A. auricula-judae* were able to quench the DPPH free radicals at different efficiencies. The extract of freeze-dried *A. auricula-judae* fruit bodies had the strongest scavenging activity of DPPH free radicals when compared to the other extracts tested. The scavenging effect exhibited by all the extracts tested was dose dependent, and the effect reached a plateau at concentrations greater than 3 mg/mL, 6

mg/mL, and 25 mg/mL for the extracts of freeze-dried fruit bodies, oven-dried fruit bodies, and mycelium, respectively.

The scavenging effects of all the extracts of *A. auricula-judae* on DPPH radicals were quite good. However, the extract of fresh fruit bodies could not be dissolved in methanol, and this may be because of the high fibre content in fresh fruit bodies. Thus, it was not possible to estimate the DPPH radical scavenging effect of this extract.

Not only has researched been done on antioxidants in fruits but comparison has been done between antioxidants in different vegetables and fruits and the work they do in human plasma.

Antioxidants in North America (United States)

Nuttakaan et al have done some research into the antioxidant and free radical-scavenging properties of extracts from onion species and shallots in United States.

The aim of their study was to detect any antioxidant and free radical-scavenging properties of aqueous and organic solvent extracts of the red shallot and to compare them with extracts of garlic and with a range of well-known antioxidants.

In their work the total phenolic content (TPC) and total antioxidant capacity (TAC) of four onion varieties (red, white, yellow and sweet) and shallot from selected locations (Washington, Idaho, Oregon, Texas and Georgia) were determined using Fourier transform infrared (FT-IR) spectroscopy (4000–400 cm1).

The Folin–Ciocalteu (F–C) assay was used to quantify TPC and three assays were used to determine TAC, including 2,2-diphenyl-picrylhydrazyl (DPPH) assay, Trolox equivalent antioxidant capacity (TEAC) assay and ferric reducing antioxidant power (FRAP) assay. Partial least squares regression (PLSR) with cross-validation (leave-one out) was conducted on onion and shallot extracts.

In this study, the relative potencies of shallot and garlic preparations were assessed by comparison with the antioxidant properties of the common antioxidants Trolox, BHT, NAC, and gallic acid. They realized that hexane-extracted shallot produced the highest rate, followed by fresh garlic and commercial garlic powder, with the aged garlic preparation reacting at the slowest rate. The results showed a high reducing power of the pure antioxidants compared with extracts, which clearly contained much material irrelevant to their antioxidant action.

In summary, their study has demonstrated that shallot extracts have an antioxidant activity similar to that associated with garlic. In garlic and shallot, hexane extracts had higher antioxidant activity than did water extracts or direct pressings. The activity appeared to depend largely on the phenolic compounds and diallyl disulfide in the bulbs, which allow them to function as sacrificial antioxidants, thus providing support for the ancient claims of their health-enhancing properties. Their result suggested that further studies of the composition of the shallots, bioavailability of shallot-derived antioxidants, and their effectiveness in cells and animals subjected to oxidative stress should provide a useful basis for nutritional advice.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 CHEMICAL REAGENTS

DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate), Folin-Ciocalteu Phenol Reagent, Ascorbic acid, Anhydrous Sodium Carbonate, Trichloroacetic Acid, Sodium Acetate, 98% Acetic Acid; Sodium Carbonate, Sodium Acetate, Tannic acid

3.2 PLANT MATERIAL

3.2.1 Extraction

25 g of the dried powdered leaves of the Kontomire and pebbles of ‘’kwawu nsusua’’, shallot and the fruit samples were extracted with 250 ml Methanol (99%), for 5 hours using Soxhlet apparatus. The extracts were filtered and concentrated in a rotary evaporator apparatus (BUCHI Rotavapor, R-144) at approximately 60 °C. The concentrated extracts were kept in a desiccator until analyses.

3.3 SCREENING FOR PHYTOCONSTITUENTS

The following secondary metabolites were screened using the method described in Trease and Evans’ Pharmacognosy Book (Trease and Evans, 1989).

3.3.1 Test for saponins

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

3.3.2 Test for general glycosides

The extract was put into 2 separate beakers and dried at 60 ^{o}C. To one beaker: 5ml of dil. H_2SO_4 was added, boiled, filtered and cooled. NaOH solution was added to the filtrate and heated with Fehling's solution (A and B) for 3 mins. Formation of a reddish – brown precipitate indicated the presence of glycosides.

3.3.3 Test for flavonoids

Magnesium turnings were added to alcoholic solution of the extract. Conc. HCl was added drop wise afterwards. Brick-red colouration indicated the presence of flavonoids.

3.3.4 Test for alkaloids

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

3.3.5 Test for Tannins

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

3.4 EVALUATION OF ANTIOXIDANT POTENTIAL

The antioxidant potential of the leafy vegetables and fruits was evaluated using different antioxidant capacity determination assays.

3.4.1 DPPH radical scavenging assay

Radical scavenging activity of the extracts from the vegetable samples against stable DPPH• radical was determined spectrophotometrically. Radical scavenging activity of extracts was measured by slightly modified method of Xiaonan *et al.* (2011). The extract (0.1, 0.3, 1, 3 mg/ml in methanol) was compared with n-propyl gallate (0.01, 0.03, 0.1, 0.3 mg/ml in methanol) as a reference free radical scavenger. The supernatant of the extract (0.1 ml) was added to 1 ml methanolic solution of DPPH (20 mg/l) in a test tube. The reaction mixture was kept at room temperature for 30 minutes. The absorbance of the residual DPPH solution was determined at 515 nm in a UV-Visible spectrophotometer (LKB Biochrom, Cambridge, Cambridge, England, Model 4050). Methanol (0.1 ml) was added to 1 ml DPPH solution and used as control. Methanol was used as the blank. The measurements were done in triplicate. The results were expressed as % Radical Scavenging Activity against concentration and the IC_{50} determined.

3.4.2 Total Phenolic Compounds Assay

The content of total phenolic compounds in the extracts from the samples (0.1, 0.3, 1 and 3 mg/ml) was quantitatively determined by colorimetric assay using Folin-Ciocalteu's reagent (Singleton, 1977) with slight modifications. Tannic acid (0.01, 0.03, 0.1 and 0.3 mg/ml) was used as the reference drug. The extract (1 ml) was added to 1 ml of F-C reagent (diluted five folds in distilled water) in a test tube. The content of the test tube was then mixed and allowed to stand for five minutes at 25^{o}C in an incubator (Gallenkamp model IH, UK). 1 ml of 2 % sodium bicarbonate solution was added to the mixture. The reaction mixture was then incubated at 25^{o}C for 2 hours. The reaction mixture after the incubation period was centrifuged at 3000 rpm for 10 minutes to get a clear supernatant. The absorbance of the supernatant was then measured at 760 nm using

the UV-Visible spectrophotometer (LKB Biochrom, Cambridge, Cambridge, England, Model 4050). Distilled water (1 ml) was added to 1 ml F-C reagent (diluted five folds in distilled water) processed in the same way as done for the test extracts and reference drug. The measurements were done in triplicate. The content of total phenolic compounds was expressed as Tannic Acid Equivalents (TAE) using the GraphPad Prism for Windows version 5.0 (GraphPad Software, San Diego, CA, USA).

3.4.3 Reducing Potential Assay

The reducing potential of the extracts (0.1, 0.3, 1 and 3 mg/ml in methanol) was determined using the method described by Oyaizu (1986), with slight modifications using n-propyl gallate as a reference antioxidant drug. The extract/drug (1 ml) was mixed with 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1 % potassium ferricyanide solution in a test tube. The mixture was incubated at 50^{o}C for 20 minutes. Following this, 1.5 ml of 10 % trichloroacetic acid solution was added to the incubated mixture and centrifuged at 3000 rpm for 10 minutes using the centrifuge (Sanyo MSE, MISTRAL 3000E, UK). 2.5 ml of the supernatant was mixed with 2.5 ml distilled water and 0.5 ml of 0.1 % ferric chloride solution [$FeCl_{3(aq)}$] in a test tube. The absorbance was then measured at 700 nm using the UV-Visible spectrophotometer (LKB Biochrom, Cambridge, Cambridge, England, Model 4050). Distilled water was used in place of the test drug/extract and used as the blank. The absorbance measurements were done in triplicates. Data was presented as concentration-absorbance curves and the IC_{50} (effective concentration that gives 50 % of maximal response) was computed using the GraphPad Prism for Windows version 5.0 (GraphPad Software, San Diego, CA, USA.

3.4.4 Total Antioxidant Capacity (TAC) Assay

The total antioxidant capacity was evaluated using the method described by Prieto *et al*. (1999) with slight modifications. Ascorbic acid was used as the standard antioxidant drug. 3 ml of the 3solution (0.6 M Sulphuric acid, 28 mM Sodium phosphate and 4 Mm Ammonium molybdate) was then added and the resulting mixtures were incubated at 95^{o}C for 90 minutes. After the mixture has cooled to room temperature, the absorbance of each solution was measured in triplicates using the UV-Visible spectrophotometer (LKB Biochrom, Cambridge, Cambridge, England, Model 4050) at 695 nm against a blank. The total antioxidant capacity was expressed as Ascorbic Acid Equivalents (AAE) using the GraphPad Prism for Windows version 5.0 (GraphPad Software, San Diego, CA, USA).

3.4.5 Statistical Analysis

All the experimental data were analyzed statistically by one-way analysis of variance (ANOVA) and "Bonferroni's Multiple Comparison Test" at 95% confidence interval using the software, GraphPad Prism for Windows version 5.0 (GraphPad Software, San Diego, CA, USA) and Excel (Microsoft Corporation, USA). Correlation coefficient (r) was used to determine the relationship between two variables, TAE and AAE. All the points on graphical representation of experimental values were expressed as mean ± s.d. Statistical significance was determined by t test; $P < 0.05$ was considered significant.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

Fruits and Vegetables are in abundance in Ghana, and their daily intake is encouraged all over the world. This study was undertaken to evaluate free-radical quenching properties which includes antioxidant capacity, total phenolic content and determine IC_{50} from the reducing power and DPPH scavenging ability of the six fruits and vegetables: Pawpaw (*Asiminia triloba*), Mango (*mangifera indica*), Avocado (*persea americana*), "Kwawu nsusuaa" (*solanum torvum*), Kontomire (*Xanthosoma colocasia*) and Shallot (*Allium ascalonicum Linn*.) sold on some selected Ghanaian markets with Kumasi metropolis used as the study area.

4.1 EXTRACTION OF CRUDE EXTRACT YIELDS

The percentage yield increased in the order; *Allium ascalonicum Linn* < *Solanum torvum* < *Mangifera indica* < *Persea americana* < *Asiminia triloba* < *Xanthosoma colocasia* ranging between 4.7 % and 11.3 %. The solvent used, its concentration, temperature, chemical properties of the sample and time of extraction are factors that can affect the efficiency of extraction hence the variation in the yield percentage of the samples (Shela et al, 2011). It is reported that there is high extraction yield of phenols when aqueous methanol is used as the extraction solvent since aqueous organic solvents have high recovery of phenols in extracts of vegetable matter due to its polarity as compared with pure solvents (Praveena and Pradeep, 2012; Morrison and Twumasi, 2010; Xiaonan et al, 2011) this is why it was used as the extraction medium in this study. Afolabi et al (2010) suggested that methanolic extracts of studied plants possess significant antioxidant and radical scavenging activities and this is supported by other research works (Badu et al, 2012).

4.2 PHYTOCHEMICAL SCREENING

Phytochemical screening of shallot showed high levels of flavonoids which are in agreement with other research (Suh et al., 1999). Flavonoids were present in all the fruits and vegetables.

Table 4.1 Results of Phytochemical screening of selected samples

Tests	Fruits			Vegetables		
	A. triloba	*P. americana*	*M. indica*	*X. colocasia*	*S. torvum*	*A. ascalonicum Linn*
Alkaloids	+	+	-	+	+	+
Flavonoids	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+
Saponins	+	+	+	-	+	-
Tannins	+	+	+	+	+	+
Terpenoids	-	+	+	-	-	-
Anthraquinones	-	+	-	-	-	-

+ means present - means absent

Phytochemical screening of the fruits and vegetables showed some difference in the constituents. *A. triloba* tested positive for all except terpenoids and anthraquinones. *P. americana* showed positive results for all the phytochemical tests agreeing with previous analysis (Ayoola et. al, 2008). *M. Indica* showed the presence of all the phytoconstituents tested except alkaloids and anthraquinones. *X. colocasia* tested positive for alkaloids, glycosides and flavonoids; it however tested negative for the rest of the analyzed constituents. *S. torvum* showed positive results for all except Terpenoids and

anthraquinones. *A. ascalonicum Linn* tested positive for alkaloids, flavonoids and general glycosides. From the screening tests done it can be deduced that the plant samples may show high total antioxidant activity due to the presence of flavonoids and tannins. Phenolic compound constituents like tannins and flavonoids act highly as antioxidants (protection and regeneration of other dietary antioxidants) and free radical scavengers (Polterait, 1997). Ayoola et al (2010) has stated that flavonoids and tannins are likely to be responsible for the free radical scavenging activities because they are phenolic compounds which in turns are good primary antioxidants or free radical scavengers. Afolabi et al (2010) further attributed phytochemical constituents of some Nigerian indigenous plants as a basis of it being used as a chemoprophylactic agent in the treatment of diseases. *Xanthosoma colocasia* and *Allium ascalonicum Linn* tested negative to saponins and this can be attributed to less phenolic compounds, hence the unstable persistence of the froth from the test.

4.3 DETERMINATION OF ANTIOXIDANT ACTIVITY

Antioxidants are substances which slow down or stop an oxidation reaction. The antioxidant capacities of plants may be influenced by a lot of factors; these could be attributed to the extraction solvent and test systems used. By this analogy, antioxidant activities cannot be fully described by any one single method (Li et al, 2010).

Due to the multifunctional nature of natural antioxidants, it is essential to perform different antioxidant activity in other to realize the various mechanisms of these antioxidant actions (Wong et al, 2006). The evaluation protocols of these activities were done for total phenolic content assay (TPC), total antioxidant capacity assay (TAC), ferric reducing antioxidant potential assay (FRAP) and determination of the scavenging activity using DPPH assay.

4.3.1 Determination of Total Phenolic Content (TPC)

Most antioxidants and medicinal properties of foods are credited to phenolics with flavonoids being the highest contributors (Afolabi et al, 2010). These total phenolics were determined using the principle of the transfer of electrons from phenolic compounds to the Folin-Ciocalteu reagent in alkaline medium; this method has been found to be simple, rapid and reproducible (Singleton, 1965). It is a mixture of tungsten and molybdenum oxides, resulting in blue coloured solutions in the test tubes with absorption at 760 nm. This intensity of the absorption is equal to the sum of the individual contribution by the different classification of phenols in the samples (Singleton et al, 1999). Phenolic compounds in plant extracts contribute significantly to their structure. Phenolics are composed of aromatic ring(s) bearing single or multiple hydroxyl groups and are therefore potentially able to quench free radicals by forming resonance-stabilized phenoxyl radical (Rice-Evans et al, 1997; Bors et al, 2002)

Table 4.2 the total phenolic contents (mg TAE/g) and % yield of 6 plant samples

Botanical name	Common name	Total Phenol Content (TAE mg/DW)	Yield (%, w/w)
Asiminia triloba	Pawpaw	$0.049 \pm 5.99 \times 10^{-5}$ – $0.272 \pm 9.98 \times 10^{-5}$	8.1
Mangifera indica	Mango	$0.014 \pm 4.07 \times 10^{-4}$ – $0.348 \pm 5.34 \times 10^{-3}$	8.5
Persea americana	Avocado	$0.008 \pm 2.23 \times 10^{-5}$ – $0.136 \pm 9.47 \times 10^{-5}$	7.4
Xanthosoma. colocasia	Kontomire	$0.003 \pm 1.87 \times 10^{-7}$ – $0.102 \pm 2.57 \times 10^{-5}$	4.7
Solanum torvum	Kwawu Nsusua	$0.001 \pm 4.28 \times 10^{-6}$ – $0.180 \pm 1.45 \times 10^{-4}$	4.9
Allium ascalonicum L.	Shallot	$0.001 \pm 2.27 \times 10^{-5}$ – $0.124 \pm 7.53 \times 10^{-5}$	11.3

From the results in table 4.2, the total polyphenols content for the various concentrations (0.1 mg/ml to 3 mg/ml) showed an increase in the order *Xanthosoma Colocasia* < *Allium ascalonicum L.* < *Persea americana* < *Solanum torvum* < *Asiminia triloba* < *Mangifera indica.* The total phenolic content in the fruits were higher compared with those of the vegetables. *Mangifera indica* showed the highest total phenol content with *Xanthosoma colocasia* giving the lowest concentration of 0.348 mg TAE/DW and 0.102 mg TAE/DW respectively. *Xanthosoma colocasia* showed the lowest concentration of 0.003 mg TAE/DW and highest of 0.102 mg TAE/DW at experimental concentrations.

0.001 mg TAE/DW was the lowest concentration for *Allium ascalonicum L.* while 0.124 mg TAE/DW has the highest concentration for the vegetable sample. *Persea americana* gave a range of 0.008 mg TAE/DW to 0.136 mg TAE/DW. The lowest concentration obtained for *Solanum torvum, Asiminia triloba* and *Mangifera indica are* 0.049 mg TAE/DW, 0.001 mg TAE/DW, 0.014 mg TAE/DW respectively whiles showing highest total phenol contents in terms of TAE as 0.180 mg TAE/DW, 0.272 mg TAE/DW and 0.348 mg TAE/DW. *Solanum torvum*'s value of 0.180 mg TAE/DW was significantly lower than the results obtained by Li Fu et al (2010) with 0.97 mg GAE/g. Xiaonan et al (2011) showed a slightly different trend in shallot results but falls within the range of the results from Nuutila (2003) and Prakash, Singh, and Upadhyay (2007) and this can be attributed to differences in climate change and cultivars. Manach et al (2004) also established that polyphenols present in foods inhibit oxidative stress due to their free scavenging activities *in vitro* and *in vivo.* From research works and studies, the quantification of phenolic compounds in fruits and vegetables is necessary to increase functional properties of these foods (Virginia et al, 2008). Total phenols have been found out to be highly responsible for the free radical scavenging activities in many plant samples (Olayinka and Anthony, 2010). From literature, it has been realized that various

types of phenols may give rise to various responses with the Folin-Ciocalteu reagent , examples being gallic acid and rutin having similar behaviour, but several of the flavonoids present low absorption leading to an underestimation of various compounds (Medina-Remon et al, 2009; Roura et al, 2006; Chun et al, 2004). Having obtained such results, it should be brought to fore that organic acids and sugars which are non-phenolic reducing compounds could interfere in the determination of the total phenolics by the Folin-Ciocalteu method leading to the overvaluation of the total phenol content (Medina-Remon et al, 2009; Roura et al, 2006; Chun et al, 2004).

4.3.2 Determination of total antioxidant capacity (TAC)

The measure of the ability of substances extracted from food (fruits and vegetables) or herbal matrix to delay oxidative stress in a controlled system is defined as Total Antioxidant capacity (TAC).

Table 4.3: The total antioxidant content (mg AAE/g) and % yield of 6 plant samples

Botanical name	Common name	Total antioxidant capacity (AAE mg/25g)
Asiminia triloba	Pawpaw	$0.044 \pm 4.65 \times 10^{-5}$ – $0.217 \pm 7.75 \times 10^{-5}$
Mangifera indica	Mango	$0.017 \pm 2.83 \times 10^{-4}$ – $0.274 \pm 3.65 \times 10^{-3}$
Persea americana	Avocado	$0.013 \pm 1.73 \times 10^{-5}$ – $0.112 \pm 7.33 \times 10^{-5}$
Xanthosoma colocasia	Kontomire	$0.009 \pm 1.20 \times 10^{-5}$ – $0.085 \pm 2.59 \times 10^{-5}$
Solanum torvum	Kwawu Nsusua	$0.007 \pm 3.27 \times 10^{-6}$ – $0.146 \pm 3.63 \times 10^{-5}$
Allium ascalonicum L.	Shallot	$0.008 \pm 1.86 \times 10^{-5}$ – $0.102 \pm 5.85 \times 10^{-5}$

The total antioxidant capacity was evaluated using the method proposed by Prieto et al, with slight modifications. The modifications were done for temperature and the

concentrations. The temperature stated in the protocol could not be achieved so a more relevant temperature suiting the climate of Ghana was used.

All the extracts showed different range of extent of antioxidant activity and this can be related to the high amounts of flavonoids and phenolic compounds in extracts (Waghulde et al, 2011). All the methanolic extracts gave a positive test for flavonoids with different intensity to the test hence the indication of varying antioxidant capacity since the amount of phenolic compounds in the extracts could be different from one extract to the other.

From the table 4.3, the results showed highest antioxidant capacity of 0.274 mg AAE/DW with S.d of 7.7×10^{-5} for *Mangifera indica* and 0.085 mg AAE/DW with SD as 2.59×10^{-5} for *Xanthosoma colocasia* showing the lowest TAC. *Xanthosoma colocasia* however showed the least of the concentrations with 0.085 mg AAE. The results of 0.102 mg AAE/DW for *Allium ascalonicum L.* showed some agreement with previous studies of Prakash et al in 2007. In Leong and Shui (2002), the L-ascorbic acid equivalent antioxidant activity calculated on dry weight was high in *Mangifera indica* and *persea americana* than the results of this study with 6.48 and 4.16 (mg/g DW) respectively. This shows the same trend with *Mangifera indica* having a higher total antioxidant capacity than *Persea americana* with 0.274 and 0.112 AAE mg/DW respectively. With respect to antioxidant capacity, the results of Mia et al (2010) and Ayoola et al (2008) showed high antioxidant capacity for *Mangifera indica, Persea americana* and *Asiminia triloba. Solanum torvum* is a highly regarded vegetable for the prevention of diseases by Ghanaians. The results obtained here shows that perception may have a sound scientific basis.

4.3.3 Correlation between Total Phenol content (TPC) and Total Antioxidant Capacity (TAC)

From the results obtained, it was observed that there is a high and positive correlation between TPC and TAC (Appendix J) with all the extracts ($r = 1$, $P < 0.0001$). The positive correlation could be attributed to the fact that the phyto-constituents of the fruits and vegetables could be responsible for both the total phenol content and total antioxidant capacity. It was realised that the TAE (TPC) was higher than their related AAE (TAC) for all the methanolic extracts, for example given TPC of *Mangifera indica* as 0.348 mg AAE and its TAC given as 0.274 mg TAE. This sequences or relationship between TPC and TAC agrees with the sequence mentioned by Morrison and Twumasi (2010). The amount of total phenol content in a sample relates to the antioxidant activity of the extract (Odukoya et al, 2007; Li Fu et al, 2010). Hence a methanolic extract with high phenol content (TPC) is likely to have high antioxidant activity (TAC).

Table 4.4 AAE and TAE from the selected fruits and vegetables showing correlation between TPC and TAC.

Methanolic Extract	TPC expressed as TAE (mg/GW)	TAC expressed as AAE (mg/GW)
Asiminia triloba	$0.272 \pm 9.98 \times 10^{-5}$	$0.217 \pm 7.75 \times 10^{-5}$
Mangifera indica	$0.348 \pm 5.34 \times 10^{-3}$	$0.274 \pm 3.65 \times 10^{-3}$
Persea americana	$0.136 \pm 9.47 \times 10^{-5}$	$0.112 \pm 7.33 \times 10^{-5}$
Xanthosoma colocasia	$0.102 \pm 2.57 \times 10^{-5}$	$0.085 \pm 2.59 \times 10^{-5}$
Solanum torvum	$0.180 \pm 1.45 \times 10^{-4}$	$0.146 \pm 3.63 \times 10^{-5}$
Allium ascalonicum L.	$0.124 \pm 7.53 \times 10^{-5}$	$0.102 \pm 5.85 \times 10^{-5}$

4.3.4 Ferric Reducing Assay Potential and DPPH Scavenging Assays

The presence of antioxidants in the samples will result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron (Mukherjee et al, 2011). The amount of Fe^{2+} can be monitored by measuring the formation of the Perl's blue colour at 700 nm.

Table 4.5 Ferric Reducing Power Potential (FRAP assay) for 3 selected fruits and vegetables.

FRUITS						
	Mangifera indica		*Persea americana*		*Asiminia triloba*	
Conc (mg/ml)	Absorbance (AV)	mg GAE/GW	Absorbance (AV)	mg GAE/GW	Absorbance (AV)	mg GAE/GW
0.1	0.080067	0.007774	0.051833	0.004077	0.045267	0.003217
0.3	0.194367	0.022766	0.070533	0.006525	0.204467	0.024063
1	0.539233	0.067898	0.169733	0.019515	0.484367	0.060713
3	1.49670	0.193267	0.215067	0.025451	0.982533	0.125944

VEGETABLES						
	Solanum torvum		*Xanthosoma colocasia*		*Allium ascalonicum*	
Conc (mg/ml)	Absorbance (AV)	mg GAE/GW	Absorbance (AV)	mg GAE/GW	Absorbance (AV)	mg GAE/GW
0.1	0.127167	0.013919	0.035400	0.001951	0.079133	0.007673
0.3	0.201367	0.023661	0.094433	0.009624	0.169700	0.019458
1	0.382400	0.047335	0.239500	0.028598	0.207400	0.024381
3	1.139033	0.146406	0.441100	0.055035	0.422833	0.052678

From the results obtained, there was an increase in absorbance from the lowest concentration (0.1 mg/ml) to the highest concentration (3 mg/ml) for all the extracts. The absorbance increased in the order *Persea americana* < *Allium ascalonicum L* < *Xanthosoma colocasia* < *Asiminia triloba* < *Solanum torvum* < *Mangifera indica*, with *Mangifera indica* showing the highest absorbance of 1.4967 and *persea americana* showing the lowest of 0.2148, indicating that *Mangifera indica* could have a potentially high antioxidant activity.

At the lowest concentration of 0.1 mg/ml *solanum torvum* had the highest absorbance of 0.1272 and *xanthosoma colocasia* had the lowest absorbance of 0.0354. At highest concentration of 3 mg/ml *Mangifera indica* had the highest absorbance of 1.4967 and *Persea americana* the lowest with 0.2148. *Solanum torvum* gave a concentration of 0.146 mg GAE/DW at the maximum concentration of 3.0 mg/ml, *Mangifera indica* showed high reducing power ability with 0.193 mg/DW at 3 mg/ml concentration of the extract. 0.146 mg GAE/DW for *Mangifera indica* was higher than earlier reports by Li Fu et al in 2010 in the south region of China, which was 5.24µmol/g. *Persea americana* gave 0.025 mg GAE/DW which represents the least reducing power potential of all the methanolic extracts. From literature and previous studies, an increase in absorbance relatively indicates an increase in reducing ability (Oyalinka and Anthony, 2010).

Smaller IC_{50} value of an extract indicates high reducing power or antioxidant activity (Morrison and Twumasi, 2010).

The reducing power of a compound is a significant indicator of its antioxidant activity (Bhaumik et al, 2008). The amount of polyphenols in a substance determines how it will be able to reduce radicals in a system, hence a positive correlation between TPC and FRAP assay is used to assess the antioxidant activity of that material (Molan et al, 2012).

4.3.5 DPPH scavenging Assay

Plant materials or fruits and vegetables have been reported to have some amount of free radical scavenging activity (Oyalinka and Anthony, 2010). Mukherjee et al (2001) did a study that suggested that the DPPH scavenging activity is an indication that a formulation or extract has a potential antioxidant, hence they suggested that their formulation could contain compounds that are capable of donating hydrogen to a free radical in order to remove odd electron hence it been responsible for the radical's activity. From literature, DPPH is a stable nitrogen-centred free radical, the colour of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron-donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavenger (Dehpour et al, 2009).

The DPPH assay is based on the ability of the antioxidant in these fruits and vegetables to scavenge the DPPH free radicals; n-propyl gallate was used as reference drug based on literature.

Table 4.6 Radical Scavenging Activity (RSA) from the selected fruits and vegetables

			% Scavenging			
Test samples	IC_{50} (mg/ml)	IC_{50} (mg GAE/GW)	0.1 mg/ml	0.3 mg/ml	1 mg/ml	3mg/ml
n-propyl gallate	0.0114	0.0114	43.97	66.56	81.96	85.25
Asiminia triloba	1.238	1.2692	12.33	19.224	75.28	80.75
Mangifera indica	0.9334	1.186	16.99	40.33	74.38	80.44
Persea americana	4.4135	2.5759	5.153	5.53	20.353	34.25
Xanthosoma colocasia	2.4835	1.6753	13.61	18.85	23.39	58.95
Solanum torvum	0.4168	1.0676	28.58	49.71	78.6	78.6
Allium ascalonicum L.	2.2708	1.5978	3.329	7.846	29.328	63.405

The smaller the IC_{50} value, the higher the radical scavenging activity and hence the higher total the antioxidant activity (Morrison and Twumasi, 2010). It can be inferred from table 4.5 that RSA of the n-propyl gallate was more pronounced comparatively to the results obtained for the selected fruits and vegetables. The strongest radical scavenging activity was shown by *solanum torvum* (1.0676 mg GAE) and the lowest IC_{50} shown in *Persea americana* (2.5759 mg GAE). *Allium ascalonicum L*. showed the lowest % scavenging activity showing 3.33% at the lowest concentration of 0.1 mg/ml and the rest followed in the order: *Persea americana* < *Asiminia triloba* < *Xanthosoma colocasia* < *Mangifera indica* < *Solanum torvum.*

At 3.0 mg/ml, the RSA showed *Asiminia triloba* showing a high % scavenging activity of 80.75% with *Persea americana* showing a lowest percentage of 34.25 %. Correlating the data results from the total antioxidant capacity showed irregular trend; *mangifera indica* (0.274 mg AAE) showed high values for antioxidant activity than *solanum torvum* (0.146 mg AAE) but the IC_{50} showed *solanum torvum* (1.0676 mg GAE) having a lower value than *mangifera indica* (1.1860 mg GAE). *Solanum torvum* > *Mangifera indica* > *Asiminia triloba* > *Allium ascalonicum L* > *Xanthosoma colocasia* > *Persea americana* showed the order of descending RSA. From the above data obtained and analysed, the extraction yield was found not to have a positive correlation with the total phenol content and this agrees with previous reports by Chandini et al in 2008.

4.3.6 FT-IR spectral features of crude extracts

The spectral features of the crude samples showed various peaks between the regions of 800 cm^{-1} and 1800 cm^{-1} (fingerprint region) and 2400 – 3600 cm^{-1} (functional group region). The bands in the fingerprint region show the biochemical compositions, especially with moieties of carbohydrates, lipids, protein secondary structures and

polyphenols in plants (Xiaonan et al, 2011). This method of determination of TPC and TAC using FT-IR interspec 200-X is now widely used in food analysis and non-destructive applications (Xiaonan et al, 2011).

FT-IR was used in this study to confirm the antioxidant activity and concentration of the total phenols in the plant samples since it is devoid of degradation of antioxidant components caused by extraction and provides information about the concentration and biological activity of foods and biological variability in antioxidant activity (Xiaonan et al, 2011; Lu and Rasco, 2012).

Table 4.7 Peaks and Band range of 6 fruits and vegetables

SAMPLE	FINGERPRINT REGION	FUNCTIONAL GROUP REGION	INFERENCE
WAVENUMBER RANGE	800 - 1600 cm^{-1}	2400 - 3600 cm^{-1}	
Asiminia triloba	1147-1382, 821, 1513-1600	2941, 3311-3615	C-O stretching vibration ring stretch of phenyl ring, CH deformation, stretching vibrations of OH groups for phenols, CH_2 antisymmetric stretch
Mangifera indica	820, 1055-1510,	2958-3262, 3300-3577,	stretching vibrations of OH groups, CH_2 antisymmetric of methyl groups, vibrational – CH_2OH, in plane CH bending vibration, CH deformation, In plane CH bending vibration from the phenyl ring.
Persea americana	1096-1387, 818-1112,	2924-3262, 3389-3585	stretching vibration of OH groups in the phenols, NH stretching of the protein, CH_2 antisymmetric stretch of methyl groups, CH deformation, C-O stretching vibrations for ring stretch of phenyl groups,
Xanthosoma. Mafafa	816, 1084-1368, 1468-1569,	3300-3596, 2930-3266,	CH deformation, C-OH stretching vibrations, in plane vibration bending from the phenyl
Solanum torvum	816, 1200-1500	3319-3562, 2931,	stretching vibrations of OH groups in phenyl, CH_2 antisymmetric stretch of methyl groups, C-O stretching vibrations ring of phenyls, CH bending vibration.
Allium ascalonicum L.	1511, 1044-1387, 817	2939 , 3339 - 3588	CH deformation, CH_3 assymetric deformation, CH_2 antisymmetric stretching, stretching vibrations of OH groups for phenols

For *Asiminia triloba*, there were clear bands at 1513 cm^{-1} which indicates an in-plane CH bending vibrations from phenyl rings (Schulz and Baranska, 2007). Between 1147-1382 cm^{-1} showed a weak C-O stretching vibration ring stretch of phenyl ring. Peaks at 1147 and 821 cm^{-1} indicated C-OH stretch vibration of hydrogen bonding and CH deformation (Schulz and Baranska, 2007) respectively. There was a broad peak between bands of 1513-1600 cm^{-1} showing a ring base (Doubeskha et al, 1997). From the functional group region peaks from 3311 to 3615 cm^{-1} stretching vibrations of OH groups for phenols and peroxides. The spectrum again showed a peak at 2941 cm^{-1} indicating CH_2 antisymmetric stretch of methyl groups mainly of lipids (Lu and Rasco, 2010).

Allium ascalonicum Linn showed clear peaks at 1511, 1113, 1044 and 1200 -1387 cm^{-1} which is in agreement with previous results by Mordechai et al (2001) and Andrus (2006) from the fingerprint region. Peak at 817 cm^{-1} and 1400 indicates CH deformation (Schulz and Baranska, 2007) and CH_3 assymetric deformation respectively. Broad and weak peaks appeared between 1511 and 1600 cm^{-1} which is a ring base spectra. From the functional group region, there were peaks at 2939 cm^{-1} for CH_2 antisymmetric stretching of methyl groups mainly of lipids (Lu and Rasco, 2010) and between 3339 - 3588 indicating stretching vibrations of OH groups for phenols.

Xanthosoma colocasia showed similar peaks at the regions of 816, 1104, and from 1084-1368 cm^{-1} indicating CH deformation, C-OH stretching vibrations for C-OH groups and C-O stretching vibrations for ring stretch of phenyl following in that order. The band between 1468 and 1569 cm^{-1} indicates a ring base with a weak peak at 1525 cm^{-1} signalling an in plane vibration bending from the phenyl.

Mangifera indica which showed highest total phenol content and hence high antioxidant activity gave strong signals with sharp peaks at 2958 cm^{-1}, 3262 cm^{-1} and from 3300 to

3577 cm^{-1} in the functional group region of the spectrum. This indicates stretching vibrations of OH groups (3300-3577 cm^{-1}), CH_2 antisymmetric of methyl groups. In the fingerprint region, there were bands at 1055 cm^{-1} (vibrational $–CH_2OH$), 1493 cm^{-1} (in plane CH bending vibration), 820 cm^{-1} (CH deformation; Shulz and Baranska, 2007) and 1510 cm^{-1} (In plane CH bending vibration from the phenyl ring). The sharp bands from 3300 to 3577 cm^{-1} could explain the high phenols contained in the crude extract hence its high antioxidant activity.

Persea americana gave bands at 3262 cm^{-1}, 2924 cm^{-1} and 3389-3585 cm^{-1} all in the functional group region and was similar to the rest of the samples with slight shift in the position of the bands. 3389-3585 cm^{-1} accounted for the stretching vibration of OH groups in the phenols. 3262 cm^{-1} was for the NH stretching of the protein content in the sample whiles 2924 cm^{-1} gave the indication of a CH_2 antisymmetric stretch of methyl groups mainly of lipids (Lu and Rasco, 2010). For the fingerprint region, bands at 818 cm^{-1} for CH deformation (Shulz and Baranska, 2007) was obtained. Again, there was broad and weak peak from 1096 to 1387 cm^{-1} indicating C-O stretching vibrations for ring stretch of phenyl groups. 1112 cm^{-1} was for realized for C-OH stretching vibrations of hydrogen bonding for C-OH groups.

Solanum torvum spectrum gave bands from 3319-3562 cm^{-1} for the stretching vibrations of OH groups in phenyl, 2931 cm^{-1} for CH_2 antisymmetric stretch of methyl groups mainly of lipids (Lu and Rasco, 2010); all in the functional group region. In the fingerprint region, sharp peaks at 816 cm^{-1}, 1500 cm^{-1} and a broad band from 1200 to 1376 cm^{-1} were shown. This inference the presence of phenyls due to the C-O stretching vibrations ring of phenyls (1200-1376 cm^{-1}) and the in plane CH bending vibration from phenyl rings (Schulz and Baranska, 2007).

Based on the obtained and cited data it can be concluded that fruits are a rich source of diverse antioxidants, therefore the efforts in the promotion of variety fruits, especially exotic fruits, for health benefits (Barreto et al., 2009) have to be done.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

Xanthosoma colocasia gave high extraction yield of 11.3 % and *A. ascalonicum Linn* gave the lowest of yield 4.7 %. All the fruit and vegetable extracts showed variations of total antioxidant capacity, total phenol content, radical scavenging activity and the reducing power compared with the reference standards used. For total phenolic content, *Mangifera indica* had the highest total phenol content with *Xanthosoma colocasia* giving the lowest concentration of 0.102 mg TAE/DW and 0.348 mg TAE/DW respectively. *Mangifera indica* again showed high total antioxidant capacity than all the selected fruit and vegetable extracts with 0.274 mg AAE/DW. There was perfect linear correlation between the TAC and TPC and this was evident in the correlation graphs with r = 1 and P < 0.0001. The absorbance increased in the order *Persea americana* < *Allium ascalonicum L* < *Xanthosoma colocasia* < *Asiminia triloba* < *Solanum torvum* < *Mangifera indica,* with *Mangifera indica* showing the highest absorbance of 1.4967 and *persea americana* showing the lowest of 0.2148

The strongest radical scavenging activity was demonstrated by *solanum torvum* (1.0676 mg GAE) and the lowest shown in *Persea americana* (2.5759 mg GAE). *Allium ascalonicum L*. showed the lowest % scavenging activity with 3.33% at the lowest concentration of 0.1 mg/ml hence this indicates that *Mangifera indica* could have a potentially high antioxidant activity.

5.2 RECOMMENDATIONS

1. In future works, more findings into other indigenous fruits and vegetables can be looked into, with also findings of some of the foreign fruits that has currently flooded our market and hence comparing of the findings could be done and the appropriate recommendation made.
2. From the concluded findings I recommend that more fruits and vegetables be taken in by people either as main course meals or supplementary to the main meals.

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APPENDICES

APEENDIX A: TABLE OF RESULTS FOR DPPH SCAVENGING ASSAY

n-propyl gallate						
Conc (mg/ml)	% Scavenging	1	2	3	AVG ABS	-LOG X
0.01	43.97	0.1934	0.1937	0.1935	0.1935	2
0.03	66.56	0.1156	0.1154	0.1156	0.1155	1.52
0.1	81.96	0.0625	0.0621	0.0623	0.0623	1
0.3	85.25	0.052	0.0513	0.0506	0.0513	0.52

Mangifera indica						
Conc (mg/ml)	% Scavenging	1	2	3	AVG ABS	-LOG X
0.1	16.99	0.2874	0.2866	0.2861	0.2867	1
0.3	40.33	0.2063	0.2061	0.2058	0.2061	0.52
1	74.38	0.0884	0.0881	0.089	0.0885	0
3	80.44	0.0677	0.0674	0.0664	0.0672	-0.48

Asiminai triloba						
Conc (mg/ml)	% Scavenging	1	2	3	AVG ABS	-LOG X
0.1	12.33	0.3026	0.3027	0.3031	0.3028	1
0.3	19.224	0.2811	0.2794	0.2792	0.2799	0.52
1	75.28	0.0861	0.0853	0.0853	0.0856	0
3	80.75	0.0662	0.0665	0.0667	0.0665	-0.48

Allium ascalonicum Linn						
Conc (mg/ml)	% Scavenging	1	2	3	AVG ABS	-LOG X
0.1	3.329	0.3339	0.3342	0.3336	0.3339	1
0.3	7.846	0.319	0.318	0.3179	0.3183	0.52
1	29.328	0.2448	0.2438	0.2437	0.2441	0
3	63.405	0.1267	0.1263	0.1261	0.1264	-0.48

Persea americana						
Conc (mg/ml)	% Scavenging	1	2	3	AVG ABS	-LOG X
0.1	5.153	0.3285	0.327	0.3274	0.3276	1
0.3	5.53	0.3271	0.3263	0.3254	0.3263	0.52
1	20.353	0.2753	0.2749	0.275	0.2751	0
3	34.25	0.2273	0.2269	0.2272	0.2271	-0.48

Xanthosoma colocasia						
Conc (mg/ml)	% Scavenging	1	2	3	AVG ABS	- LOG X
0.1	13.61	0.2974	0.2994	0.2985	0.2984333	1
0.3	18.85	0.2805	0.2799	0.2795	0.2799667	0.52
1	23.39	0.2638	0.2661	0.2638	0.2645667	0
3	58.95	0.1418	0.1421	0.1415	0.1418	-0.48

Solanum torvum						
Conc (mg/ml)	% Scavenging	1	2	3	AVG ABS	- LOG X
0.1	28.58	0.2467	0.2462	0.2462	0.2464	1
0.3	49.71	0.1737	0.1738	0.1741	0.1739	0.52
1	78.6	0.0728	0.0728	0.0734	0.073	0
3	78.6	0.074	0.0737	0.0741	0.0739	-0.48

INHIBITORY CONCENTRATION (IC50)		
Botanical name	IC_{50} (mg/ml)	IC_{50} (GAE mg/25g)
Mangifera indica	0.9339	1.1860
Asiminia triloba	1.2380	1.2692
Allium ascalonicum Linn	2.2708	1.5978
persea americana	4.4135	2.5759
xanthosoma colocasia	2.4835	1.6753
solanum torvum	0.4618	1.0676

APPENDIX B: FERRIC REDUCING POWER POTENTIAL (FRAP)

Mangifera indica

Conc (mg/ml)	1	2	3	-LOG X	AVG ABS
0.1	0.0797	0.0801	0.0804	1	0.0801
0.3	0.1941	0.1941	0.1949	0.52	0.1944
1	0.5389	0.5389	0.5399	0	0.5392
3	1.4967	1.4967	1.4967	-0.48	1.4967

Xanthosoma colocasia

Conc (mg/ml)	1	2	3	-LOG X	AVG ABS
0.1	0.0353	0.0353	0.0356	1	0.0354
0.3	0.0946	0.0945	0.0942	0.52	0.0944
1	0.2395	0.2399	0.2391	0	0.2395
3	0.4413	0.441	0.441	-0.48	0.4411

Solanum torvum

Conc (mg/ml)	1	2	3	-LOG X	AVG ABS
0.1	0.1272	0.1273	0.127	1	0.1272
0.3	0.2013	0.2014	0.2014	0.52	0.2014
1	0.3818	0.3832	0.3822	0	0.3824
3	1.1395	1.1388	1.1388	-0.48	1.1390

Persea americana

Conc (mg/ml)	1	2	3	-LOG X	AVG ABS
0.1	0.0524	0.0521	0.0510	1	0.0518
0.3	0.0706	0.0706	0.0704	0.52	0.0705
1	0.17	0.1694	0.1698	0	0.1697
3	0.2153	0.2151	0.2148	-0.48	0.2151

Asiminia Triloba

Conc (mg/ml)	1	2	3	-LOG X	AVG ABS
0.1	0.0452	0.0454	0.0452	1	0.0453
0.3	0.2035	0.2045	0.2054	0.52	0.2045
1	0.4844	0.4844	0.4843	0	0.4844
3	0.9829	0.9818	0.9829	-0.48	0.9825

Allium ascalonicum Linn

Conc (mg/ml)	1	2	3	-LOG X	AVG ABS
0.1	0.079	0.0791	0.0793	1	0.0791
0.3	0.1696	0.1702	0.1693	0.52	0.1697
1	0.2073	0.2069	0.208	0	0.2074
3	0.4225	0.423	0.423	-0.48	0.4228

APPENDIX C: TOTAL ANTIOXIDANT CAPACITY (TAC)

Ascorbic Acid				
Conc (mg/ml)	1	2	3	AVERAGE
0.01	0.0375	0.0372	0.0375	0.0374
0.03	0.1558	0.1559	0.1560	0.1559
0.1	0.4889	0.4889	0.4891	0.4890
0.3	0.9288	0.9453	0.9270	0.9337

Mangifera indica				
	Absorbance			
Conc (mg/ml)	1	2	3	AVERAGE
0.1	0.3887	0.3887	0.3881	0.3885
0.3	0.4845	0.4868	0.4862	0.4858
1	0.9586	0.9552	0.9562	0.9567
3	1.1266	1.1266	1.1281	1.1736

Xanthosoma colocasia				
	Absorbance			
Conc (mg/ml)	1	2	3	AVERAGE
0.1	0.0587	0.0585	0.0588	0.0587
0.3	0.0968	0.0961	0.0963	0.0964
1	0.2090	0.2089	0.2102	0.2094
3	0.3975	0.3964	0.3990	0.3976

Solanum torvum				
	Absorbance			
Conc (mg/ml)	1	2	3	AVERAGE
0.1	0.3517	0.3513	0.3510	0.3513
0.3	0.3595	0.358	0.3562	0.3579
1	0.5142	0.5137	0.5134	0.5138
3	0.9552	0.9567	0.9552	0.9557

Persea americana				
	Absorbance			
Conc (mg/ml)	1	2	3	AVERAGE
0.1	0.1371	0.1377	0.1360	0.1369
0.3	0.1858	0.1888	0.1884	0.1877
1	0.3212	0.3210	0.3211	0.3211
3	0.7128	0.7079	0.7088	0.7098

	Allium ascalonicum Linn			
	Absorbance			
Conc (mg/ml)	1	2	3	AVERAGE
0.1	0.2294	0.2344	0.2379	0.2339
0.3	0.3910	0.3931	0.3931	0.3924
1	0.6368	0.6404	0.6378	0.6383
3	1.6323	1.6345	1.6368	1.6345

Asiminia triloba				
	Absorbance			
Conc (mg/ml)	1	2	3	AVERAGE
0.1	0.5215	0.5215	0.5215	0.5215
0.3	0.5488	0.5520	0.5520	0.5509
1	1.0038	1.0049	1.0049	1.0045
3	1.9310	1.9310	1.9310	1.9310

APPENDIX D: ASCORBIC ACID EQUIVALENCE (AAE mg/DW)

Mangifera indica					
	Ascorbic acid equivalence (AAE mg/25g)				
Conc (mg/ml)	1	2	3	AVERAGE	S.D
0.1	0.0173	0.01674	0.017528	0.017173	$2.83\ 10^{-4}$
0.3	0.0365	0.036389	0.036348	0.036362	1.67×10^{-5}
1	0.1200	0.119885	0.119885	0.119885	0
3	0.275443	0.268674	0.278823	0.274313	$3.65\ 10^{-3}$

Xanthosoma colocasia					
	Ascorbic acid equivalence (AAE mg/25g)				
Conc (mg/ml)	1	2	3	AVERAGE	S.D
0.1	0.008751	0.008752	0.008781	0.008761	1.21×10^{-5}
0.3	0.012246	0.01227	0.012222	0.012246	1.69×10^{-5}
1	0.03456	0.034569	0.034569	0.034566	3.67×10^{-6}
3	0.084861	0.084869	0.084928	0.084886	2.59×10^{-5}

Solanum torvum					
	Ascorbic acid equivalence (AAE mg/25g)				
Conc (mg/ml)	1	2	3	AVERAGE	S.D
0.1	0.007314	0.007322	0.007314	0.007317	3.26×10^{-6}
0.3	0.018251	0.018146	0.018292	0.018230	5.33×10^{-5}
1	0.056241	0.056265	0.056241	0.056249	9.79×10^{-6}
3	0.145726	0.145815	0.145815	0.145785	3.63×10^{-5}

Persea americana					
	Ascorbic acid equivalence (AAE mg/25g)				
Conc (mg/ml)	1	2	3	AVERAGE	S.D
0.1	0.012611	0.012636	0.012587	0.012611	1.73×10^{-5}
0.3	0.01665	0.016594	0.016683	0.016642	3.18×10^{-5}
1	0.079961	0.079961	0.079961	0.079961	0
3	0.111815	0.11188	0.111677	0.111791	7.33×10^{-5}

Allium ascalonicum Linn					
	Ascorbic acid equivalence (AAE mg/25g)				
Conc (mg/ml)	1	2	3	AVERAGE	S.D
0.1	0.006745	0.006696	0.006704	0.006715	1.86×10^{-5}
0.3	0.01674	0.016821	0.016715	0.016759	3.92×10^{-5}
1	0.044449	0.044507	0.044417	0.044458	3.23×10^{-5}
3	0.102259	0.102153	0.102316	0.102243	5.85×10^{-5}

Asiminia triloba					
	Ascorbic acid equivalence (AAE mg/25g)				
Conc (mg/ml)	1	2	3	AVERAGE	S.D
0.1	0.043914	0.043832	0.043962	0.043903	4.65×10^{-5}
0.3	0.059564	0.059581	0.059524	0.059556	2.07×10^{-5}
1	0.170827	0.170827	0.170819	0.170824	3.27×10^{-6}
3	0.216837	0.216894	0.217049	0.216927	7.76×10^{-5}

APPENDIX E: TOTAL PHENOLIC CONTENT (TPC)

Tannic Acid				
Absorbance				
Conc (mg/ml)	1	2	3	AVERAGE
0.01	0.1481	0.1481	0.1481	0.1481
0.02	0.2342	0.2341	0.2341	0.2341
0.1	1.1331	1.1331	1.1331	1.1331
0.3	2.9123	2.9123	2.9124	2.9123

Allium ascalonicum Linn				
	Absorbance			
Conc (mg/ml)	1	2	3	AVERAGE
0.1	0.088	0.0874	0.0875	0.0876
0.3	0.212	0.2107	0.2102	0.2110
1	0.5527	0.5516	0.5516	0.552
3	1.2621	1.2641	1.2640	1.2634

Persea americana				
	Absorbance			
Conc (mg/ml)	1	2	3	AVERAGE
0.1	0.1605	0.1603	0.1599	0.1602
0.3	0.2092	0.2103	0.2102	0.2099
1	0.9890	0.9890	0.9890R	0.9890
3	1.3818	1.3793	1.3818	1.3810

Asiminia triloba				
	Absorbance			
Conc (mg/ml)	1	2	3	AVERAGE
0.1	0.5444	0.5458	0.5460	0.5454
0.3	0.7382	0.7375	0.7382	0.7380
1	2.1072	2.1072	2.1071	2.1072
3	2.6741	2.676	2.6700	2.6734

Solanum torvum				
	Absorbance			
Conc (mg/ml)	1	2	3	AVERAGE
0.1	0.0951	0.0950	0.0948	0.0950
0.3	0.2283	0.2301	0.2303	0.2296
1	0.6974	0.6971	0.6969	0.6971
3	1.7994	1.7994	1.7960	1.7983

Xanthosoma colocasia				
	Absorbance			
Conc (mg/ml)	1	2	3	AVERAGE
0.1	0.1127	0.1129	0.1132	0.1129
0.3	0.1560	0.1554	0.1558	0.1557
1	0.4304	0.4304	0.4301	0.4303
3	1.0494	1.0500	1.0500	1.0498

Mangifera indica				
	Absorbance			
Conc (mg/ml)	1	2	3	AVERAGE
0.1	0.2110	0.2207	0.2203	0.2173
0.3	0.4528	0.4523	0.4518	0.4523
1	1.4803	1.4803	1.4803	1.4803
3	3.3113	3.4362	3.4362	3.3946

APPENDIX F: TANNIC ACID EQUIVALENCE (TAC mg/DW)

Allium ascalonicum Linn					
	Tannic Acid Equivalence (TAE mg/GW)				
Conc (mg/ml)	1	2	3	AVERAGE	S.D
0.1	0.000577	0.000514	0.000535	0.000542	2.27×10^{-5}
0.3	0.013485	0.013590	0.013453	0.013509	5.07×10^{-5}
1	0.049290	0.049343	0.049228	0.049287	4.07×10^{-5}
3	0.123924	0.123788	0.123998	0.123903	7.53×10^{-5}

Persea americana					
Tannic Acid Equivalence (TAE mg/GW)					
Conc (mg/ml)	1	2	3	AVERAGE	S.D
0.1	0.008154	0.008185	0.008122	0.008154	2.23×10^{-5}
0.3	0.013369	0.013296	0.013411	0.013359	4.13×10^{-5}
1	0.095129	0.095129	0.095129	0.095129	1.20×10^{-17}
3	0.136265	0.136349	0.136087	0.136234	9.47×10^{-5}

Asiminia triloba					
Tannic Acid Equivalence (TAE mg/GW)					
Conc (mg/ml)	1	2	3	AVERAGE	S.D
0.1	0.048577	0.048472	0.048640	0.048563	5.99×10^{-5}
0.3	0.068789	0.06881	0.068736	0.068778	2.68×10^{-5}
1	0.212473	0.212473	0.212463	0.212470	4.28×10^{-6}
3	0.271891	0.271964	0.272163	0.272006	9.98×10^{-5}

Solanum torvum					
Tannic Acid Equivalence (TAE mg/GW)					
Conc (mg/ml)	1	2	3	AVERAGE	S.D
0.1	0.001312	0.001322	0.001312	0.001315	4.28×10^{-6}
0.3	0.015437	0.015300	0.015489	0.015409	6.89×10^{-5}
1	0.064497	0.064528	0.064496	0.064507	1.29×10^{-5}
3	0.180173	0.180173	0.179816	0.180054	1.46×10^{-4}

Xanthosoma colocasia					
Tannic Acid Equivalence (TAE mg/GW)					
Conc (mg/ml)	1	2	3	AVERAGE	S.D
0.1	0.003190	0.003169	0.003222	0.003194	1.87×10^{-5}
0.3	0.007692	0.007713	0.00765	0.007685	2.27×10^{-5}
1	0.036509	0.036509	0.036509	0.036509	0
3	0.101467	0.101530	0.10153	0.101509	2.57×10^{-5}

Mangifera indica					
Tannic Acid Equivalence (TAE mg/GW)					
Conc (mg/ml)	1	2	3	AVERAGE	S.D
0.1	0.013485	0.014503	0.014461	0.014149	4.07×10^{-5}
0.3	0.038860	0.038755	0.038807	0.038807	3.71×10^{-5}
1	0.146686	0.146686	0.146686	0.146686	0
3	0.338832	0.351939	0.351939	0.34757	5.35×10^{-3}

APPENDIX G: CORRELATION BETWEEN TPC AND TAC

Allium ascalonicum Linn	
TAC (AAE mg/GW)	TPC (TAE mg/GW)
0.006715	0.000542
0.016759	0.0135093
0.044458	0.049287
0.102243	0.1239033

Persea americana	
TAC (AAE mg/GW)	TPC (TAE mg/GW)
0.012613	0.008154
0.016642	0.013359
0.079961	0.095129
0.111791	0.136234

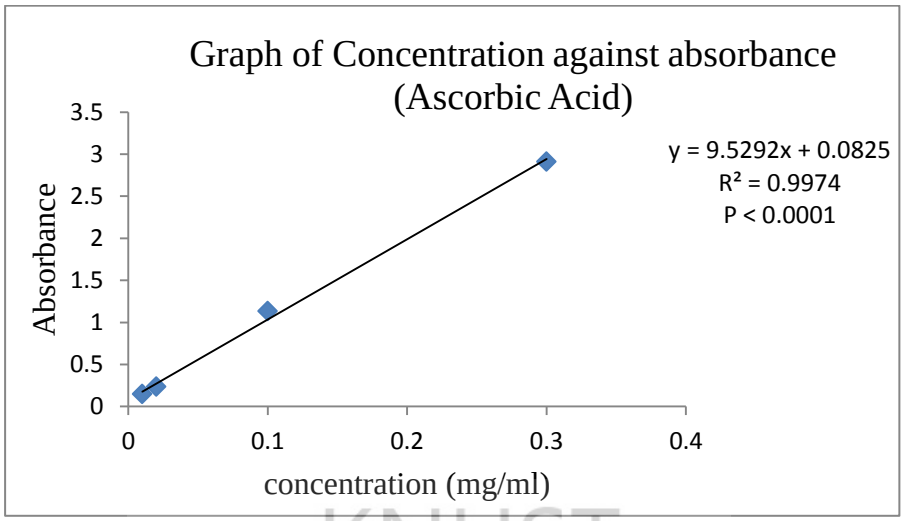
Mangifera indica	
TAC (AAE mg/GW)	TPC (TAE mg/GW)
0.017173	0.014149
0.036362	0.038808
0.119885	0.146686
0.274313	0.347571

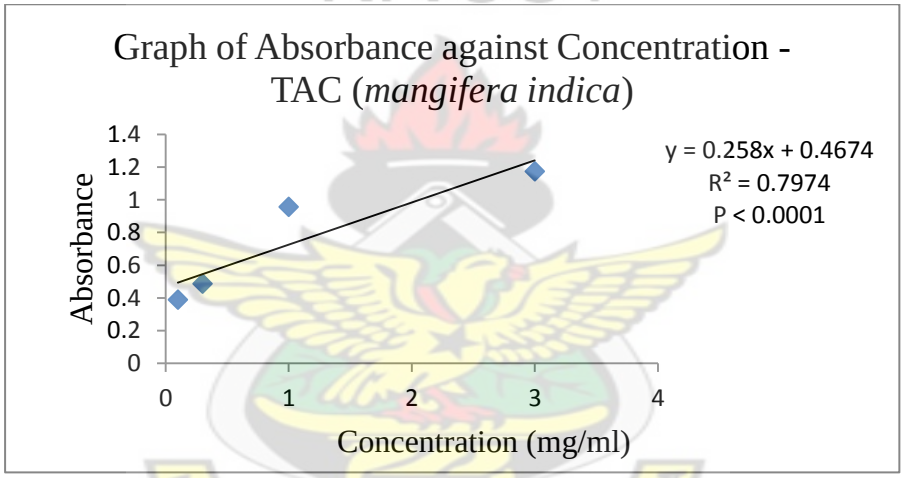
Asiminia triloba	
TAC (AAE mg/GW)	TPC (TAE mg/GW)
0.043903	0.048563
0.059556	0.068778
0.170824	0.21247
0.216927	0.272006

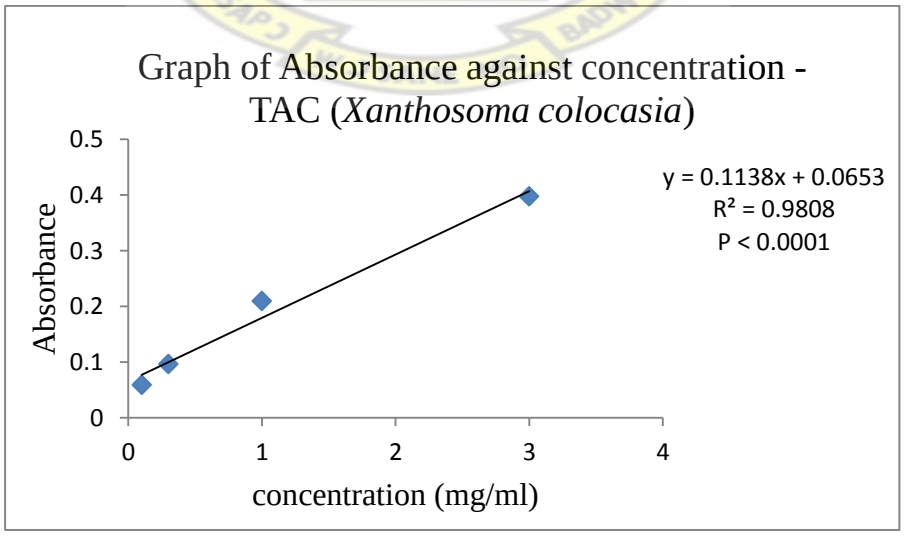
Xanthosoma colocasia	
TAC (AAE mg/GW)	TPC (TAE mg/GW)
0.008761	0.003194
0.012246	0.007682
0.034566	0.036509
0.084886	0.101509

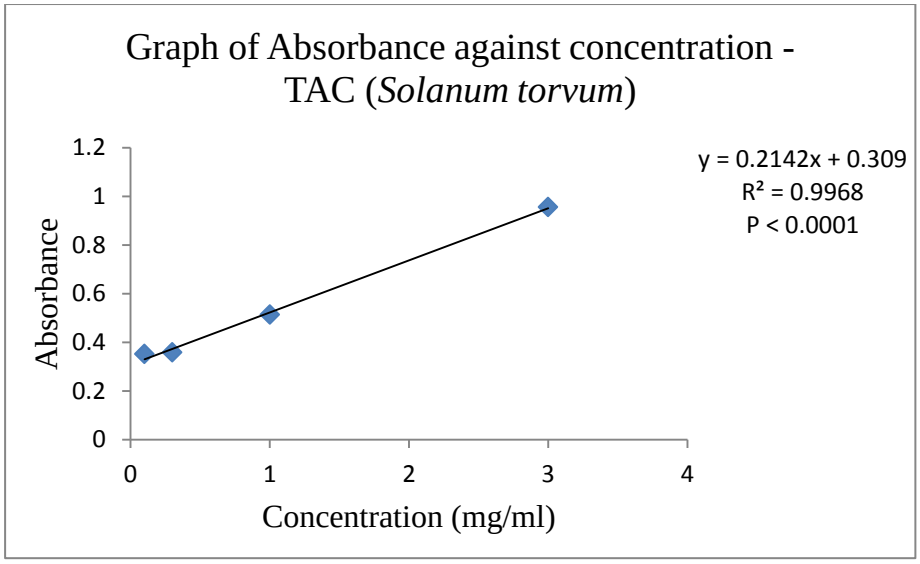
Solanum torvum	
TAC (AAE mg/GW)	TPC (TAE mg/GW)
0.007317	0.001315
0.01823	0.015409
0.056249	0.064507
0.145785	0.180054

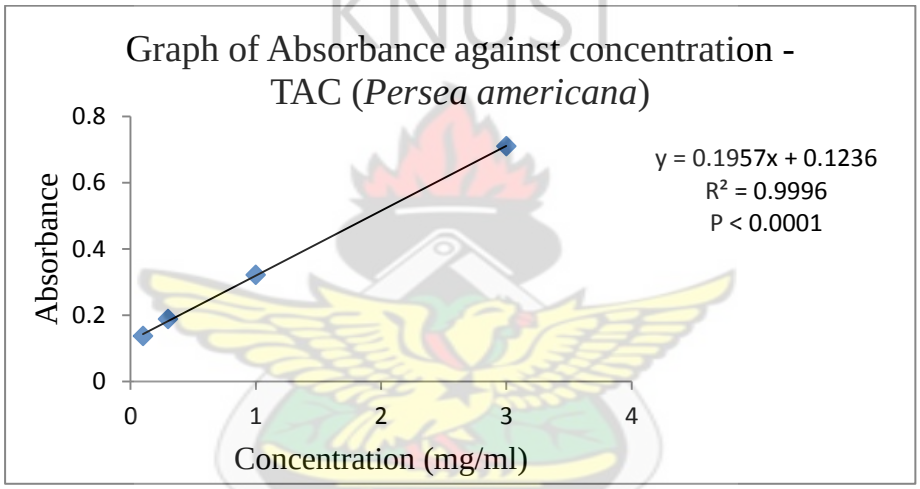
APPENDIX H: GRAPH OF RESULTS OF TOTAL ANTIOXIDANT CAPACITY

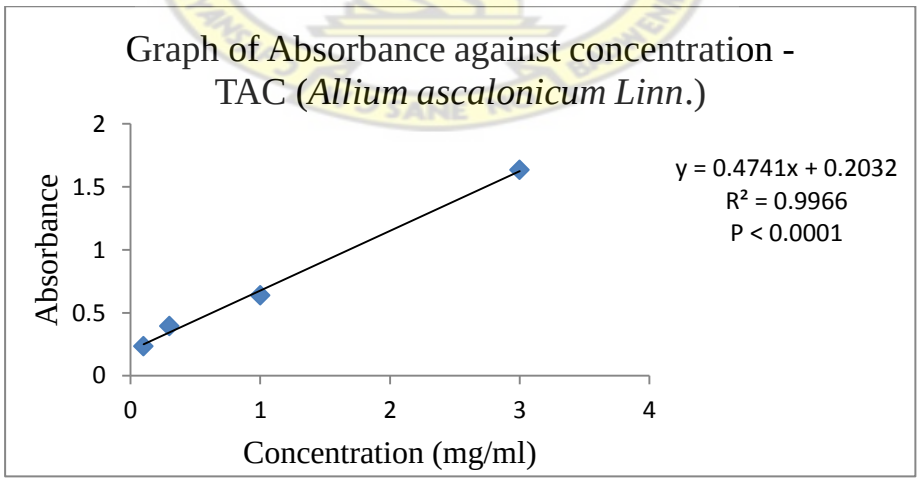


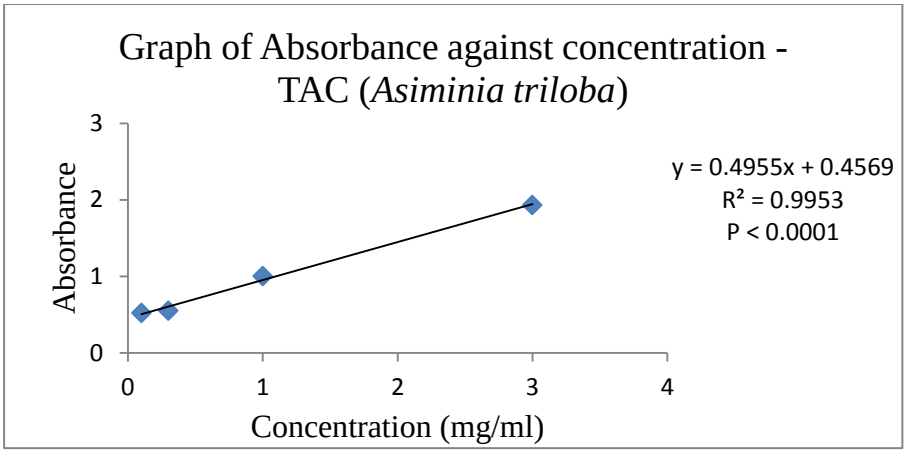


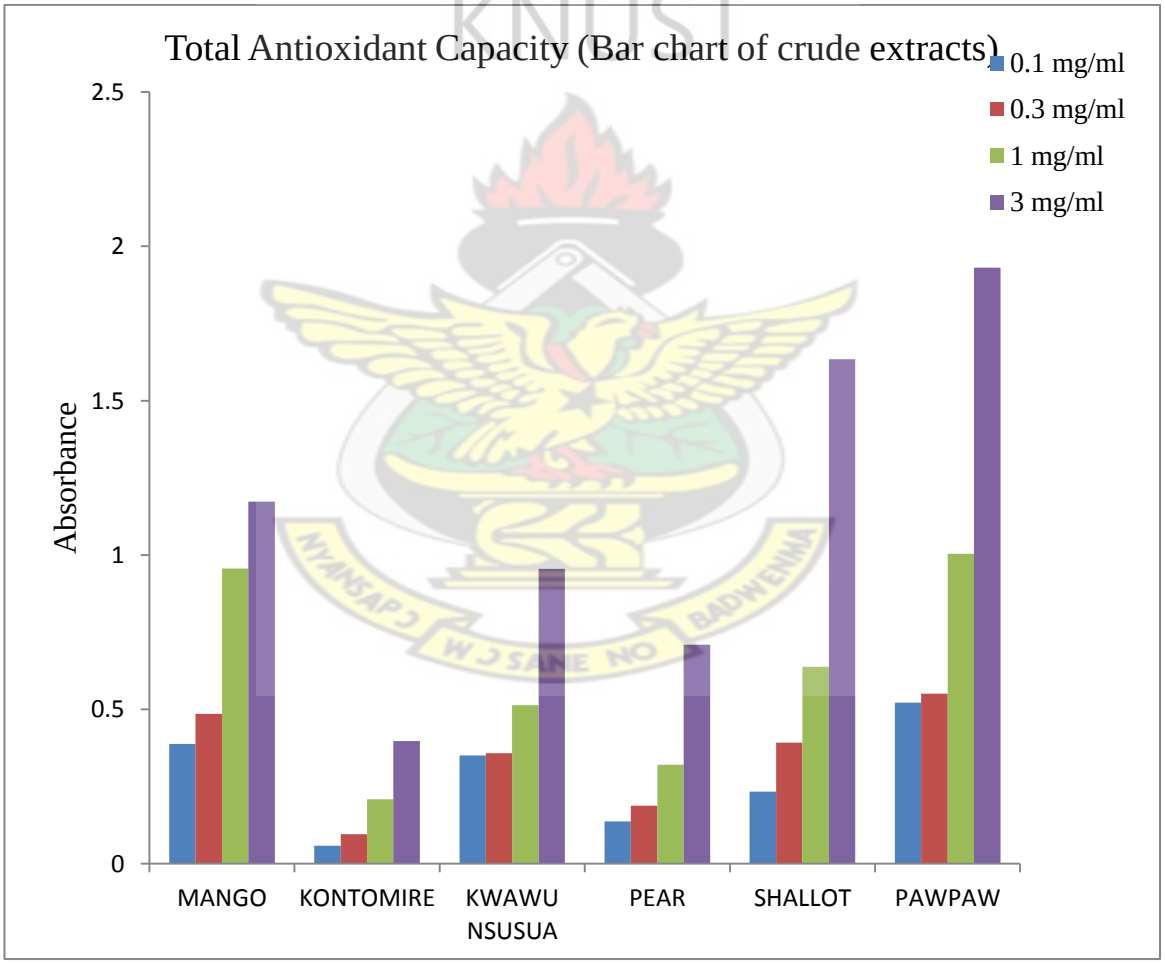



Graph of Absorbance against concentration - TAC (*Solanum torvum*)
y = 0.2142x + 0.309
R² = 0.9968
P < 0.0001
Absorbance
Concentration (mg/ml)
0
0.2
0.4
0.6
0.8
1
1.2
0
1
2
3
4

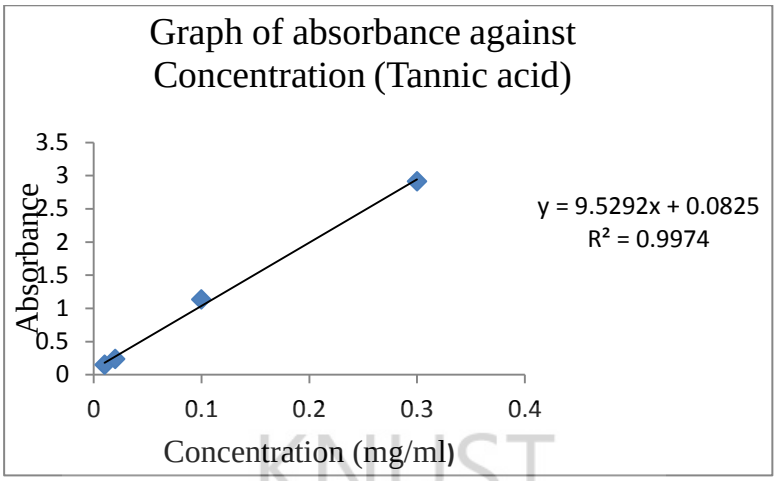

Graph of Absorbance against concentration - TAC (*Persea americana*)
y = 0.1957x + 0.1236
R² = 0.9996
P < 0.0001
Absorbance
Concentration (mg/ml)
0
0.2
0.4
0.6
0.8
0
1
2
3
4

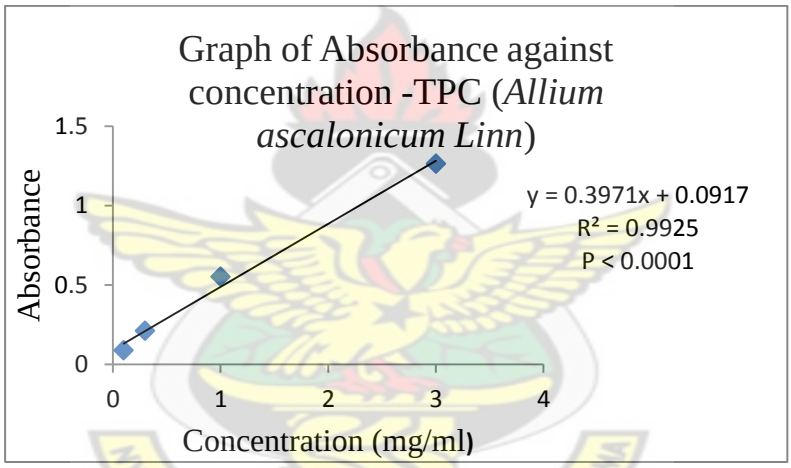

Graph of Absorbance against concentration - TAC (*Allium ascalonicum Linn.*)
y = 0.4741x + 0.2032
R² = 0.9966
P < 0.0001
Absorbance
Concentration (mg/ml)
0
0.5
1
1.5
2
0
1
2
3
4

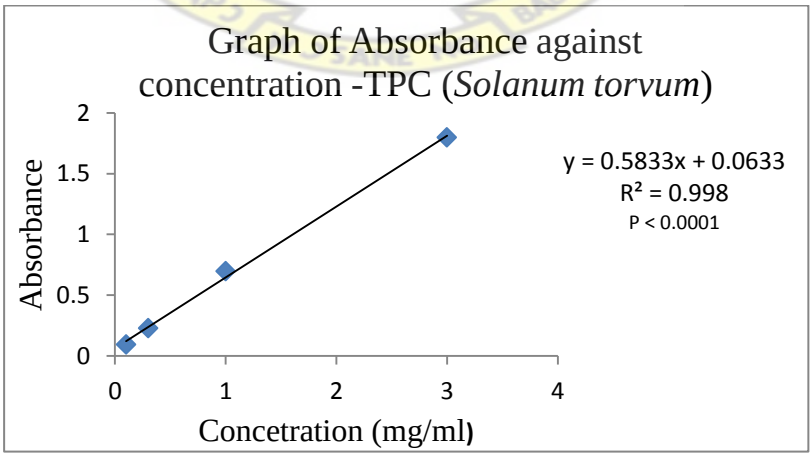

Graph of Absorbance against concentration - TAC (*Asiminia triloba*)
Absorbance
Concentration (mg/ml)
y = 0.4955x + 0.4569
R² = 0.9953
P < 0.0001
0
1
2
3
4

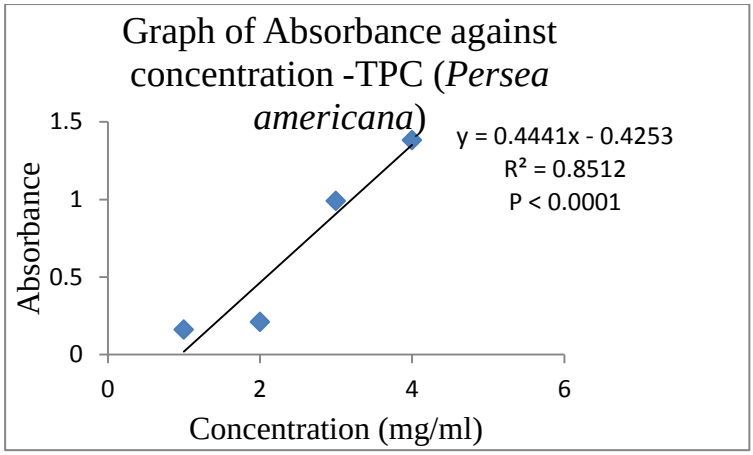

Total Antioxidant Capacity (Bar chart of crude extracts)
Absorbance
0.1 mg/ml
0.3 mg/ml
1 mg/ml
3 mg/ml
MANGO
KONTOMIRE
KWAWU NSUSUA
PEAR
SHALLOT
PAWPAW

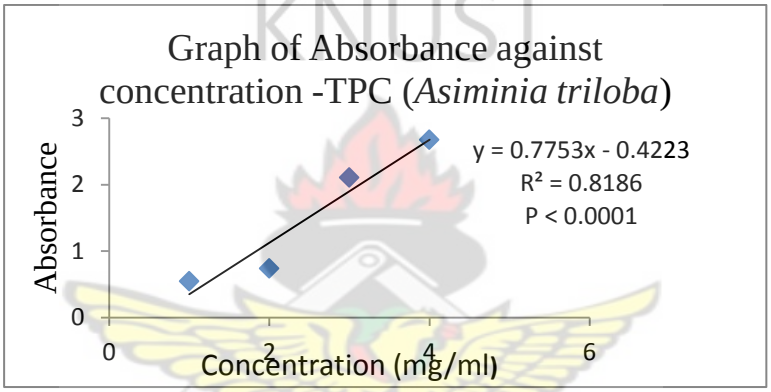
APPENDIX I: GRAPHS OF RESEULTS FOR TOTAL PHENOL CONTENT (TPC)

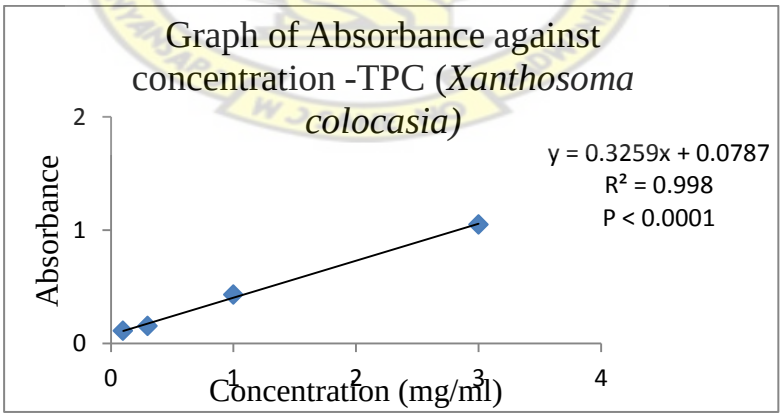


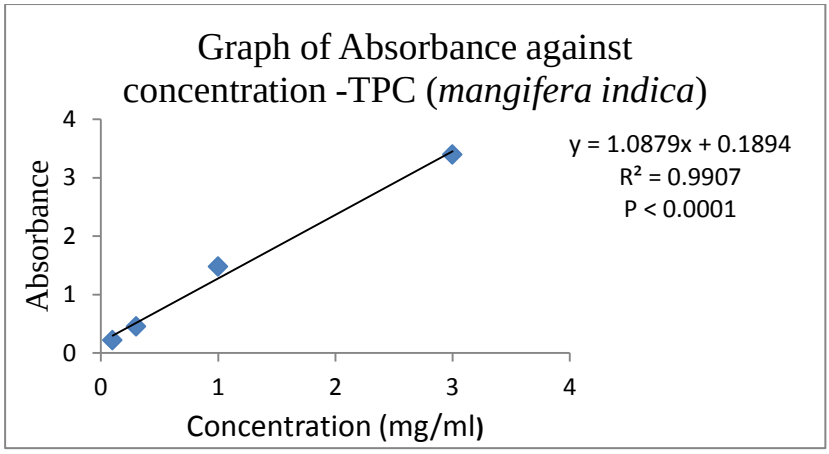




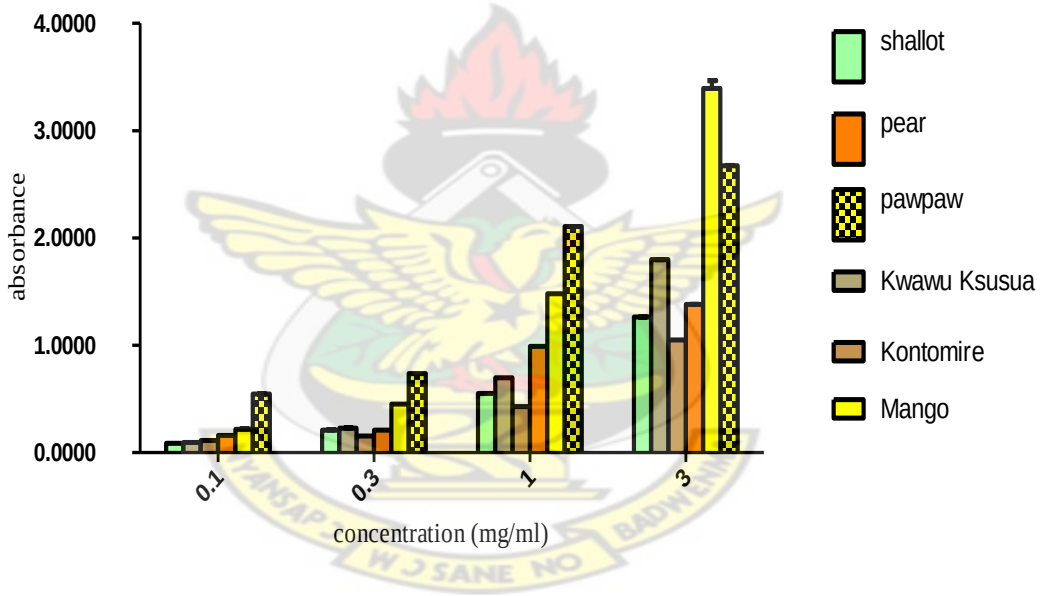

Graph of Absorbance against concentration -TPC (*Persea americana*)
y = 0.4441x - 0.4253
R² = 0.8512
P < 0.0001
Absorbance
Concentration (mg/ml)
0
0.5
1
1.5
0
2
4
6


Graph of Absorbance against concentration -TPC (*Asiminia triloba*)
y = 0.7753x - 0.4223
R² = 0.8186
P < 0.0001
Absorbance
Concentration (mg/ml)
0
1
2
3
0
2
4
6

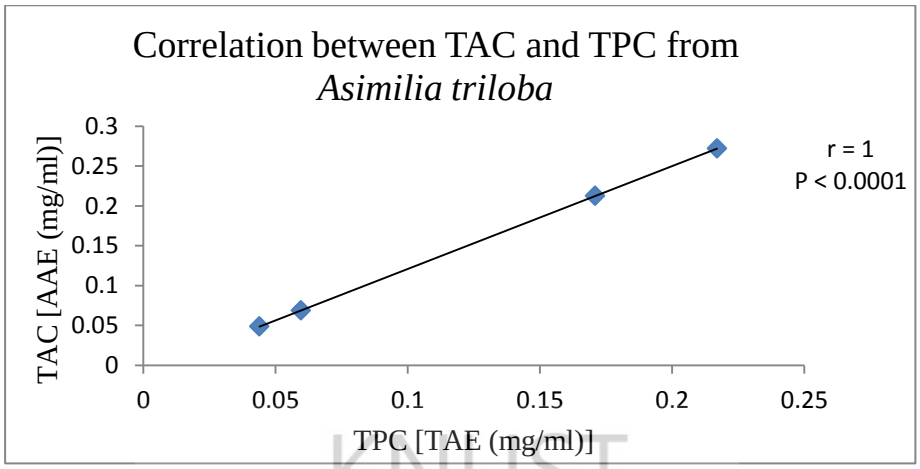

Graph of Absorbance against concentration -TPC (*Xanthosoma colocasia)*
y = 0.3259x + 0.0787
R² = 0.998
P < 0.0001
Absorbance
Concentration (mg/ml)
0
1
2
0
1
2
3
4

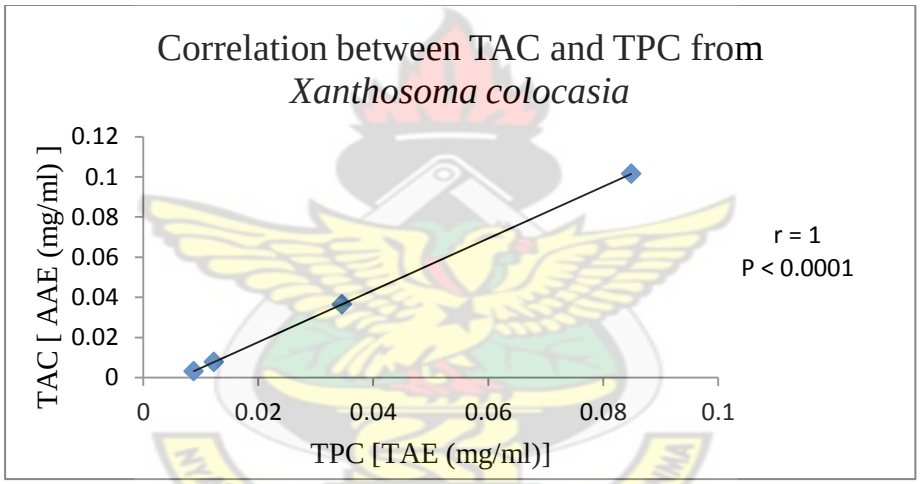


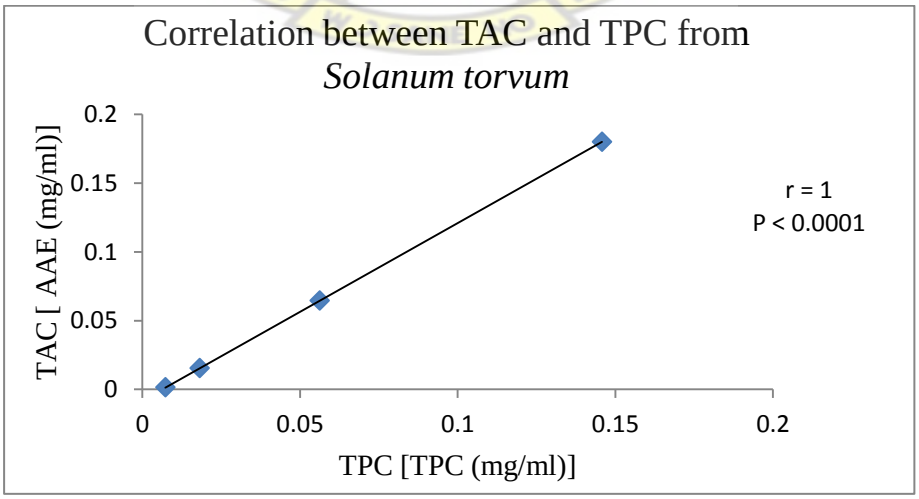
Total phenolic capacity graph of absorbance against concentration

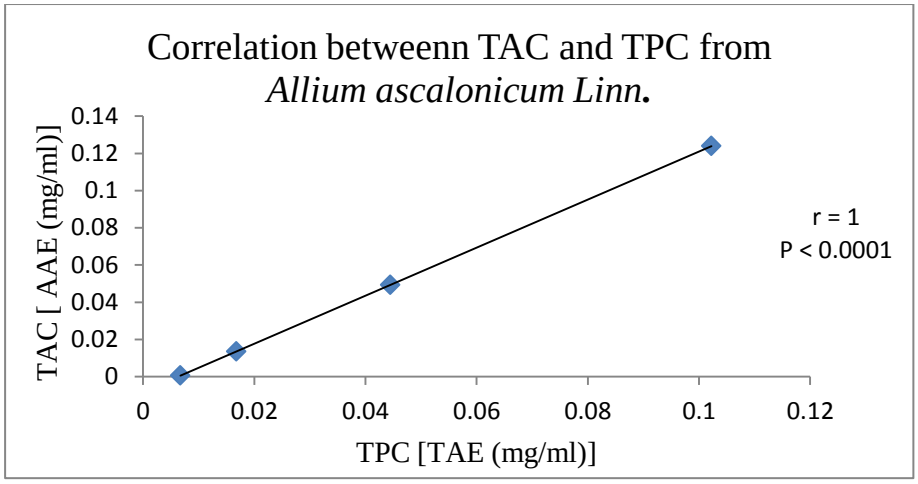


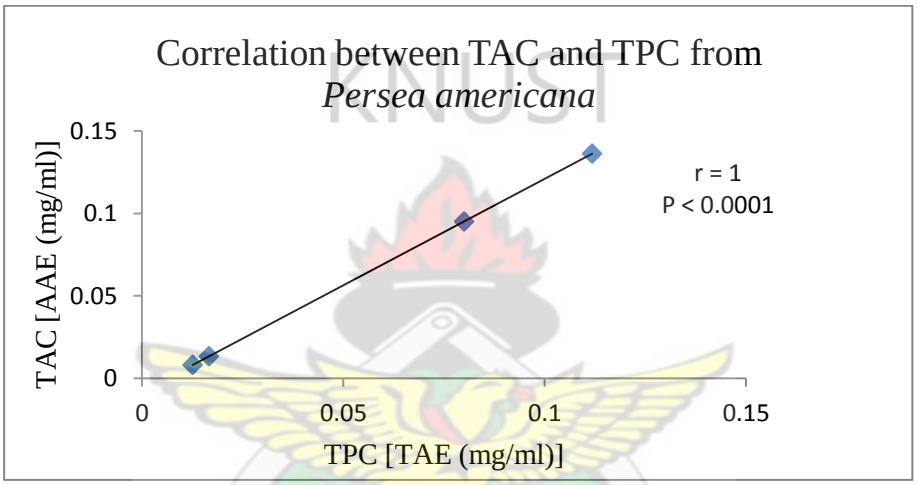
APPENDIX J: GRAPH OF RESULTS FOR CORRELATION BETWEEN TPC AND TAC

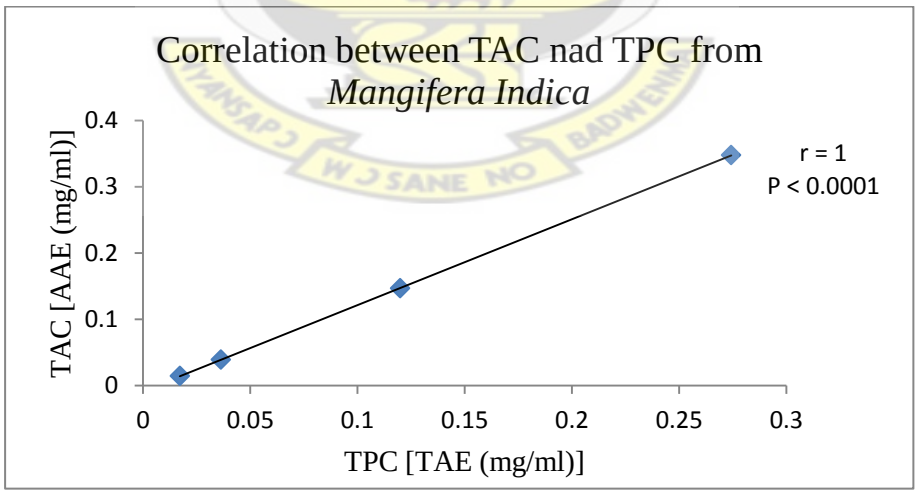




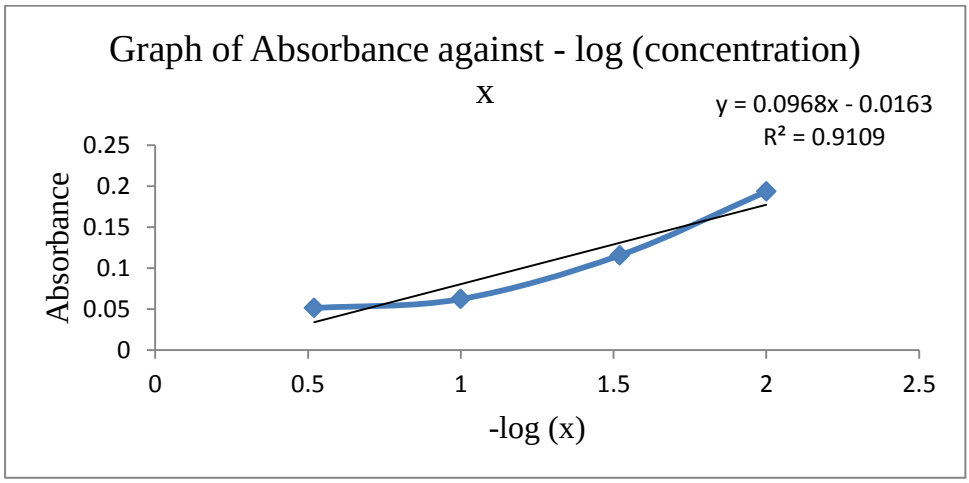


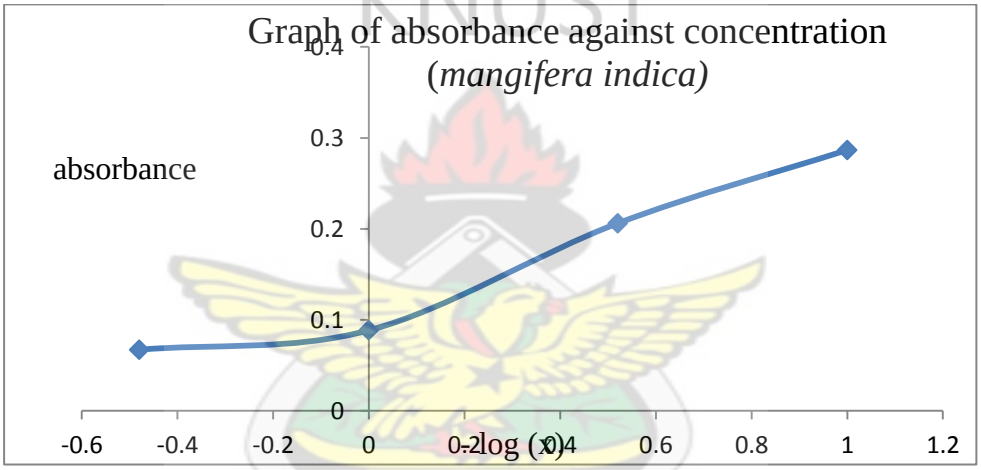

Correlation betweenn TAC and TPC from
Allium ascalonicum Linn.
TAC [AAE (mg/ml)]
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0
0.02
0.04
0.06
0.08
0.1
0.12
TPC [TAE (mg/ml)]
r = 1
P < 0.0001

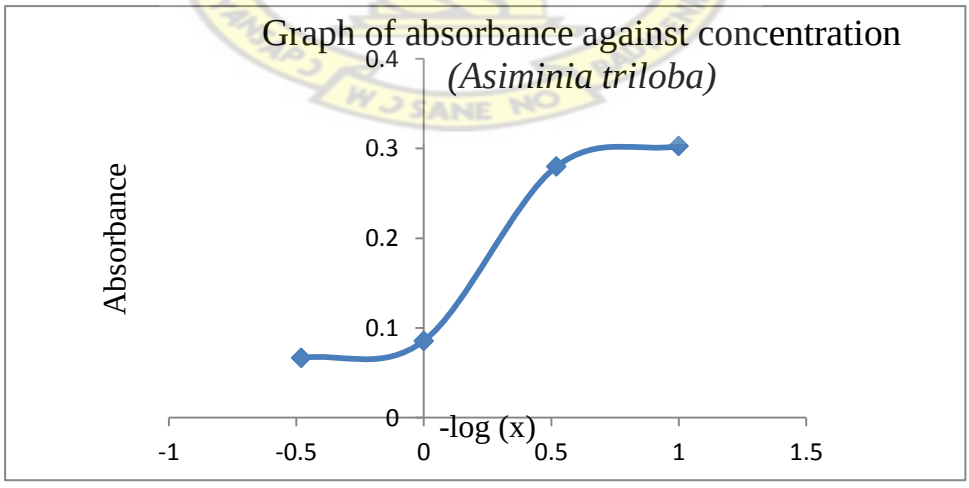

Correlation between TAC and TPC from
Persea americana
TAC [AAE (mg/ml)]
0.15
0.1
0.05
0
0
0.05
0.1
0.15
TPC [TAE (mg/ml)]
r = 1
P < 0.0001

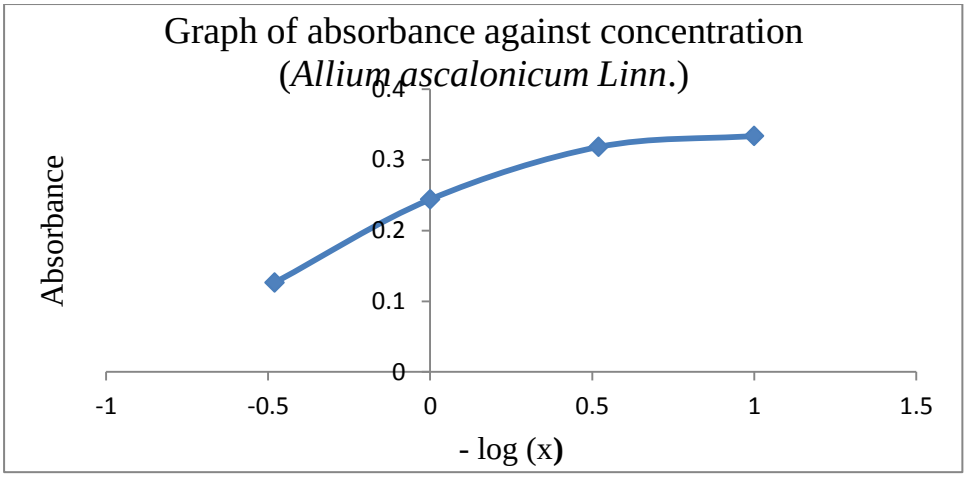

Correlation between TAC nad TPC from
Mangifera Indica
TAC [AAE (mg/ml)]
0.4
0.3
0.2
0.1
0
0
0.05
0.1
0.15
0.2
0.25
0.3
TPC [TAE (mg/ml)]
r = 1
P < 0.0001

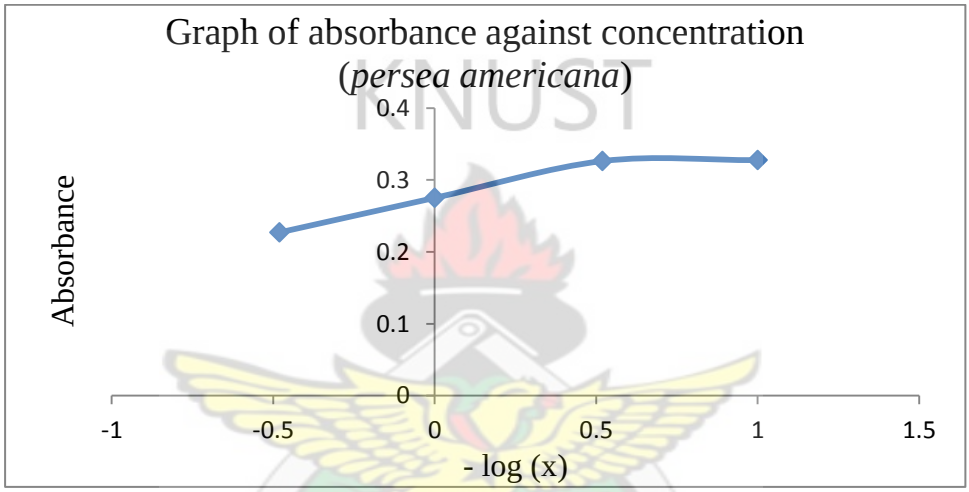
APPENDIX K: GRAPHS OF RESULTS FOR DPPH SCAVENGING ASSAY.

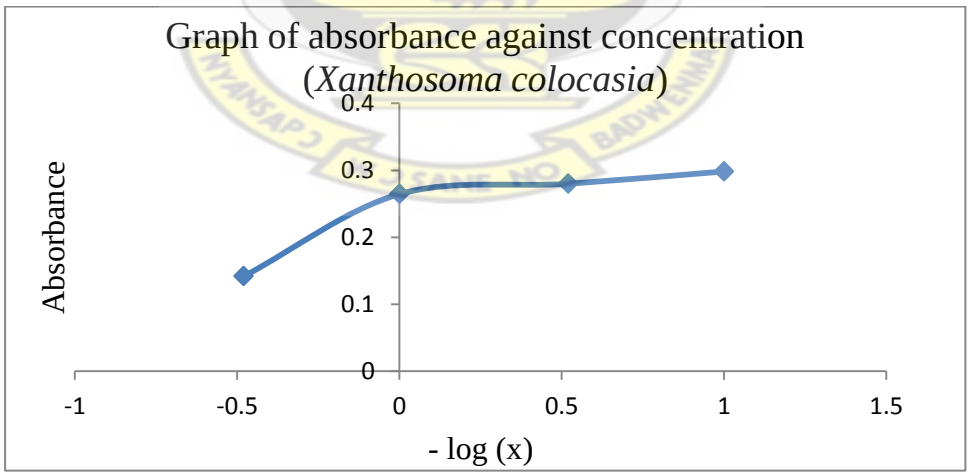


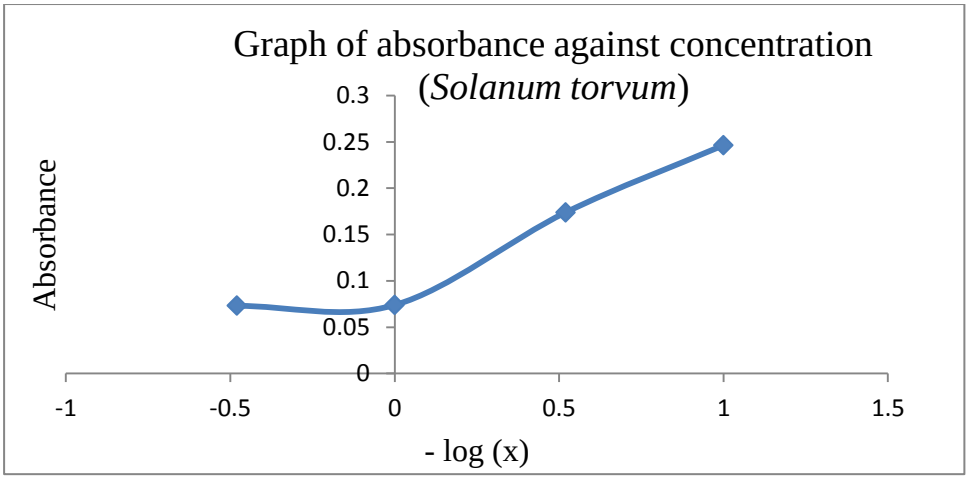




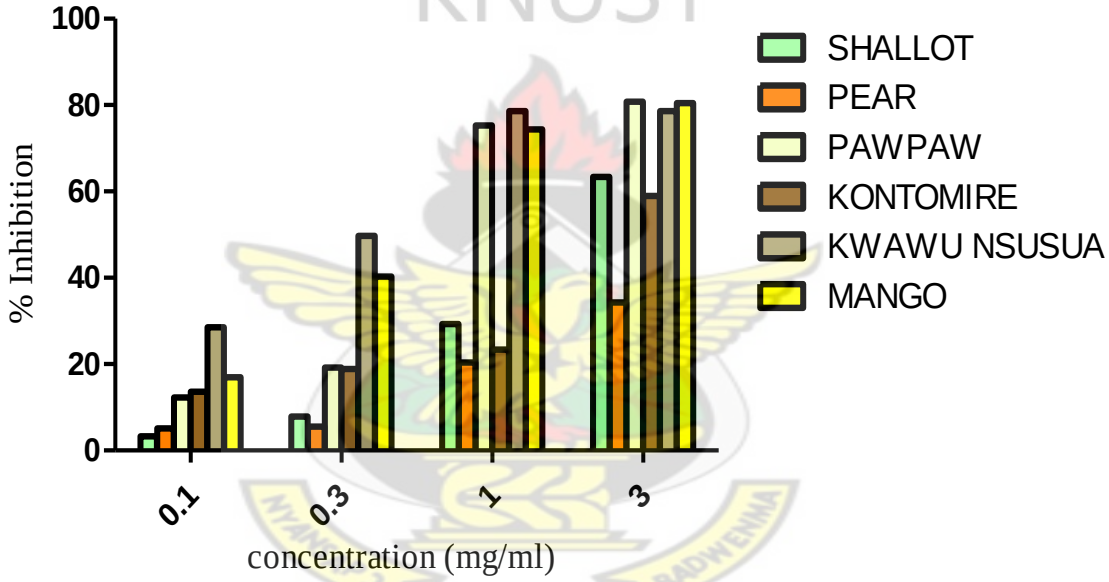

Graph of absorbance against concentration
(*Allium ascalonicum Linn*.)
Absorbance
0.4
0.3
0.2
0.1
0
-1
-0.5
0
0.5
1
1.5
- log (x)


Graph of absorbance against concentration
(*persea americana*)
Absorbance
0.4
0.3
0.2
0.1
0
-1
-0.5
0
0.5
1
1.5
- log (x)

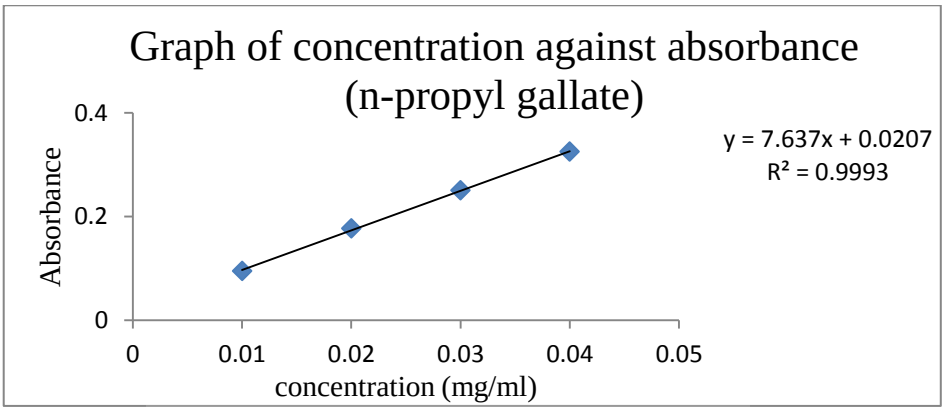

Graph of absorbance against concentration
(*Xanthosoma colocasia*)
Absorbance
0.4
0.3
0.2
0.1
0
-1
-0.5
0
0.5
1
1.5
- log (x)

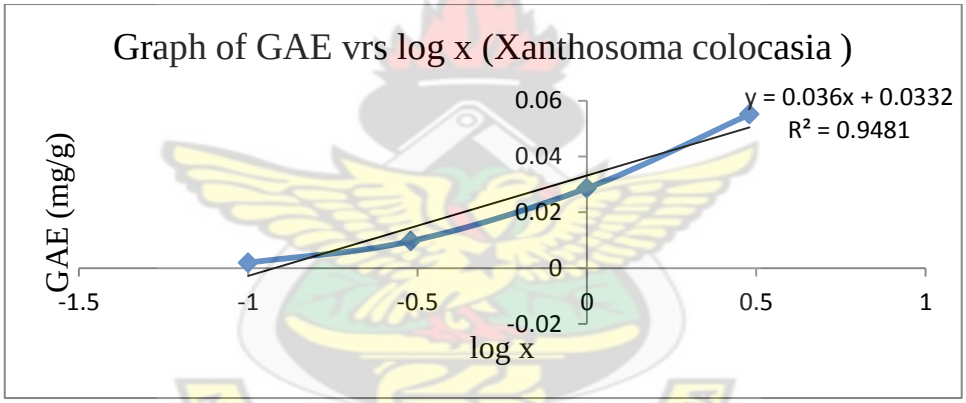


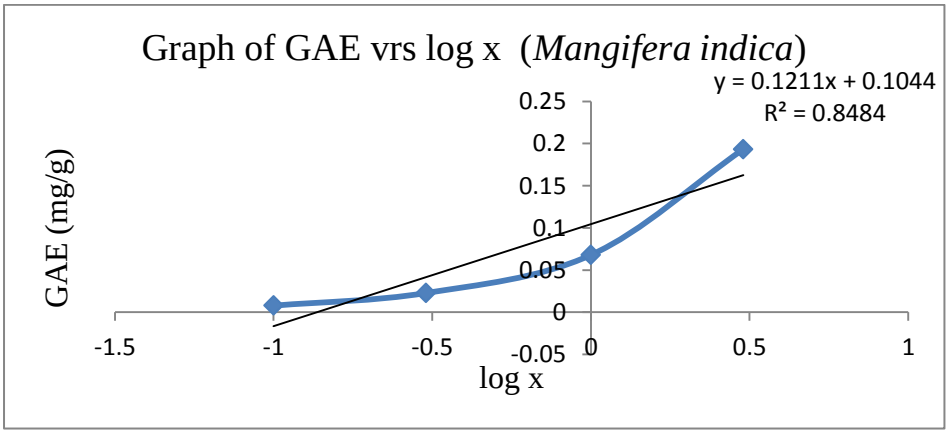
Graph of DPPH % Inhibition against Concentration

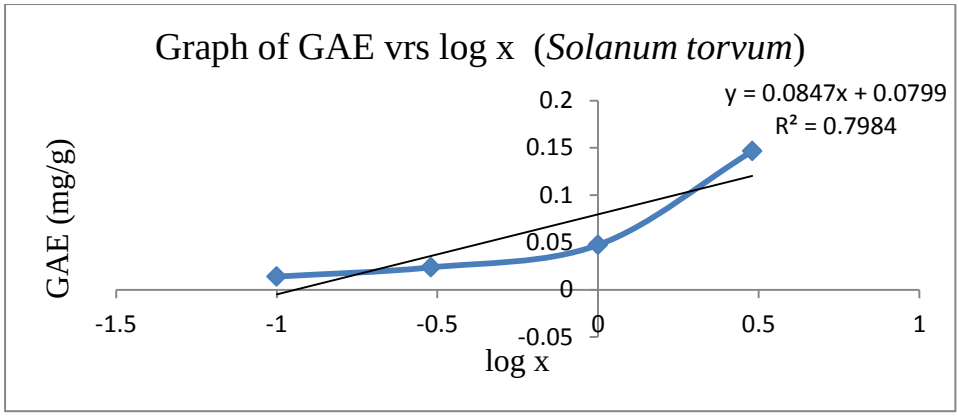


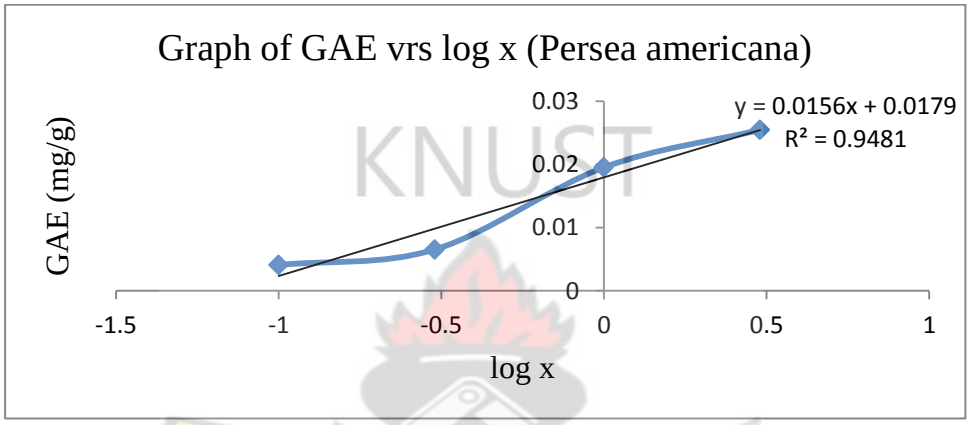
APPENDIX L: GRAPH RESULTS FOR FERRIC REDUCING POWER POTENTIAL

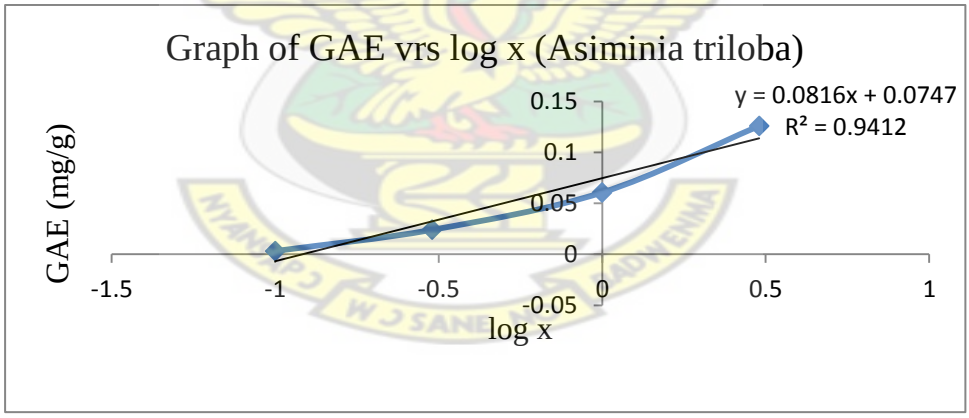


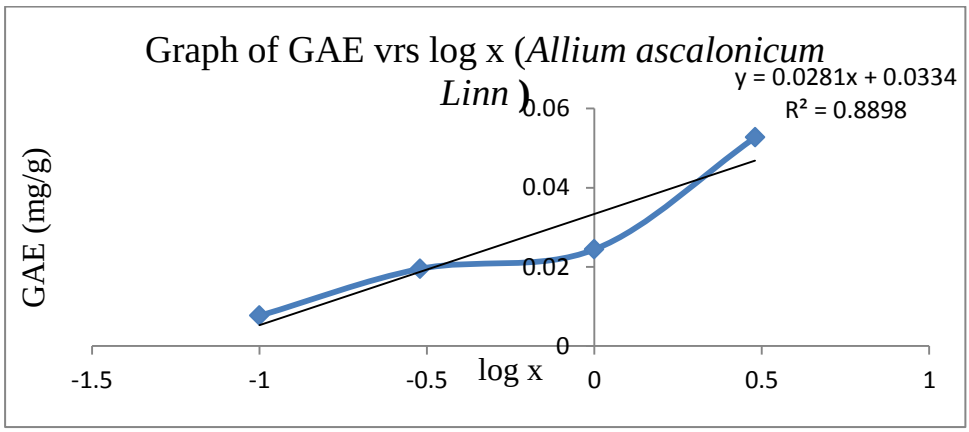





Graph of GAE vrs log x (*Solanum torvum*)
y = 0.0847x + 0.0799
R² = 0.7984
GAE (mg/g)
0.2
0.15
0.1
0.05
0
-0.05
-1.5
-1
-0.5
0
0.5
1
log x


Graph of GAE vrs log x (Persea americana)
y = 0.0156x + 0.0179
R² = 0.9481
GAE (mg/g)
0.03
0.02
0.01
0
-1.5
-1
-0.5
0
0.5
1
log x


Graph of GAE vrs log x (Asiminia triloba)
y = 0.0816x + 0.0747
R² = 0.9412
GAE (mg/g)
0.15
0.1
0.05
0
-0.05
-1.5
-1
-0.5
0
0.5
1
log x


Graph of GAE vrs log x (*Allium ascalonicum Linn*)
y = 0.0281x + 0.0334
R² = 0.8898
GAE (mg/g)
0.06
0.04
0.02
0
-1.5
-1
-0.5
log x
0
0.5
1

APPENDIX M: TABLES OF RESULTS FOR GALLIC ACID EQUIVALENCE FOR FRAP ASSAY

GALLIC ACID EQUIVALENCE (GAE mg/GW)					
Conc (mg/ml)	1	2	3	Average	S.D
0.01	0.0944	0.0944	0.0948	0.0946	0.000188562
0.02	0.1772	0.1772	0.177	0.177	9.42809E-05
0.03	0.2493	0.2493	0.2518	0.2501	0.001178511
0.04	0.3247	0.3247	0.3248	0.3248	4.71405E-05

Mangifera indica					
Gallic acid equivalence (GAE mg/GW)					
Conc (mg/ml)	1	2	3	Average	**S.D**
0.1	0.007726	0.007778	0.007817206	0.007773559	3.25163E-05
0.3	0.022705	0.022784	0.022810003	0.022766356	3.85481E-05
1	0.067854	0.067854	0.06798481	0.067897516	5.34564E-05
3	0.19327	0.19327	0.193269608	0.193269608	0

Xanthosoma colocasia					
Gallic acid equivalence (GAE mg/GW)					
Conc (mg/ml)	1	2	3	Average	S.D
0.1	0.001912	0.001912	0.001951028	0.00192484	1.6037E-05
0.3	0.009677	0.009663	0.009624198	0.009654751	1.92741E-05
1	0.02865	0.028702	0.028597616	0.028649993	3.70361E-05
3	0.055074	0.055035	0.055034699	0.055047793	1.60368E-05

Solanum torvum					
Gallic acid equivalence (GAE mg/GW)					
Conc (mg/ml)	1	2	3	Average	S.D
0.1	0.013945	0.013958	0.013919078	0.013940901	1.41431E-05
0.3	0.023648	0.023661	0.023661123	0.023656758	5.3456E-06
1	0.047283	0.047466	0.047335341	0.047361529	6.67672E-05
3	0.146497	0.146406	0.146405656	0.146436209	3.74196E-05

Persea americana					
Gallic acid equivalence (GAE mg/GW)					
Conc (mg/ml)	1	2	3	Average	S.D
0.1	0.004151	0.004112	0.003967527	0.004076644	6.82489E-05
0.3	0.006534	0.006534	0.006507791	0.00652525	1.06913E-05
1	0.01955	0.019471	0.019523373	0.019514643	2.82867E-05
3	0.025481	0.025455	0.025415739	0.025450656	2.33009E-05

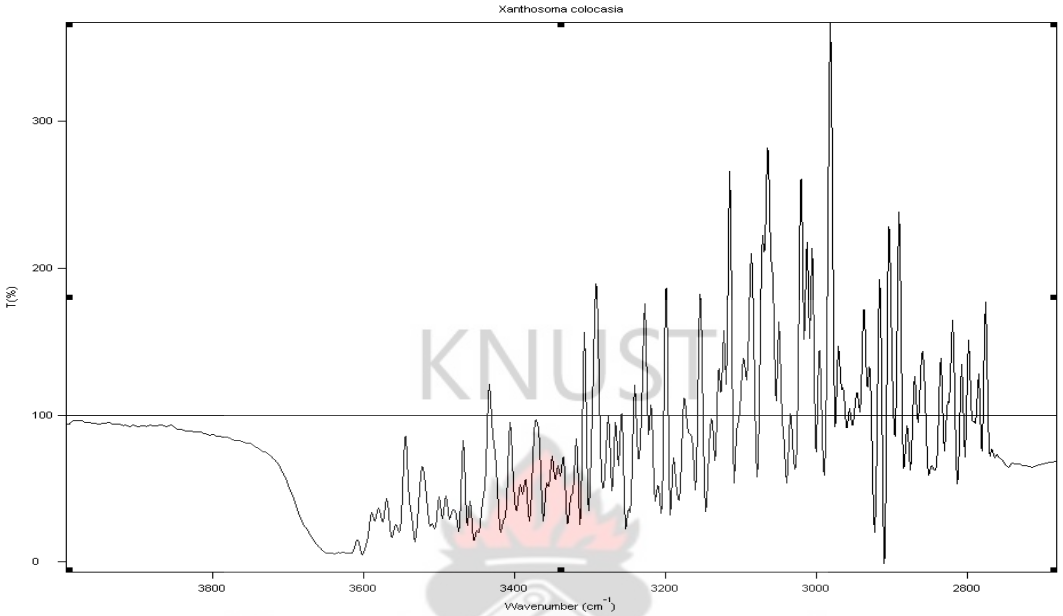
Asiminia triloba					
Gallic acid equivalence (GAE mg/GW)					
Conc (mg/ml)	1	2	3	Average	S.D
0.1	0.003208	0.003234	0.003208066	0.003216795	1.06913E-05
0.3	0.023936	0.024067	0.024184889	0.024062677	8.80008E-05
1	0.060718	0.060718	0.060704465	0.060713194	5.3456E-06
3	0.125992	0.125848	0.125991881	0.125943869	5.8802E-05

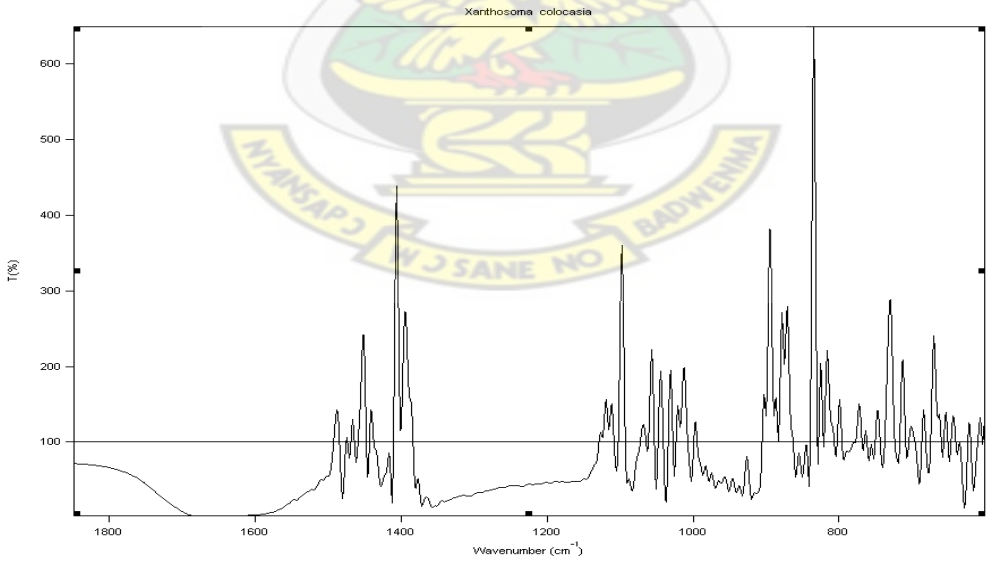
Allium ascalonicum Linn					
Gallic acid equivalence (GAE mg/GW)					
Conc (mg/ml)	1	2	3	Average	S.D
0.1	0.007634	0.007647	0.00767317	0.007651347	1.41433E-05
0.3	0.019497	0.019576	0.019457902	0.019510278	4.24298E-05
1	0.024434	0.024381	0.024381301	0.02439876	2.13828E-05
3	0.052612	0.052678	0.052677753	0.052655929	2.67284E-05

Crude extract	**IC_{50} (GAE mg/g)**
Mangifera indica	412.0198
Xanthosoma colocasia	1487.107
Solanum torvum	589.375
Persea americana	3203.98
Asiminia triloba	611.829
Allium ascalonicum Linn	1784.521

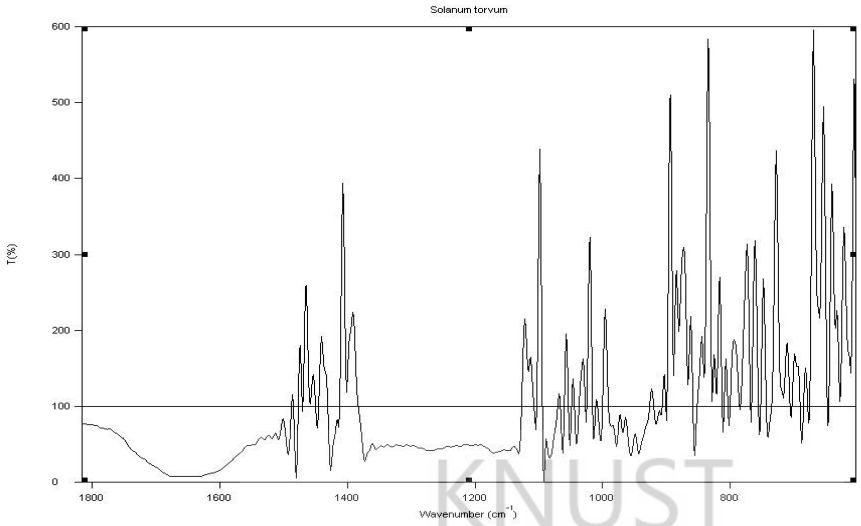
APPENDIX N: IR SPECTRUM OF FRUITS AND VEGETABLES

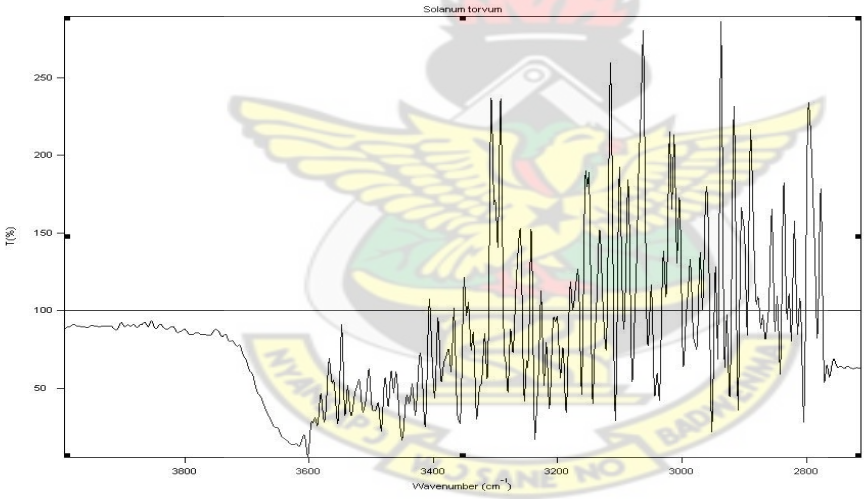
Xanthosoma colocasia

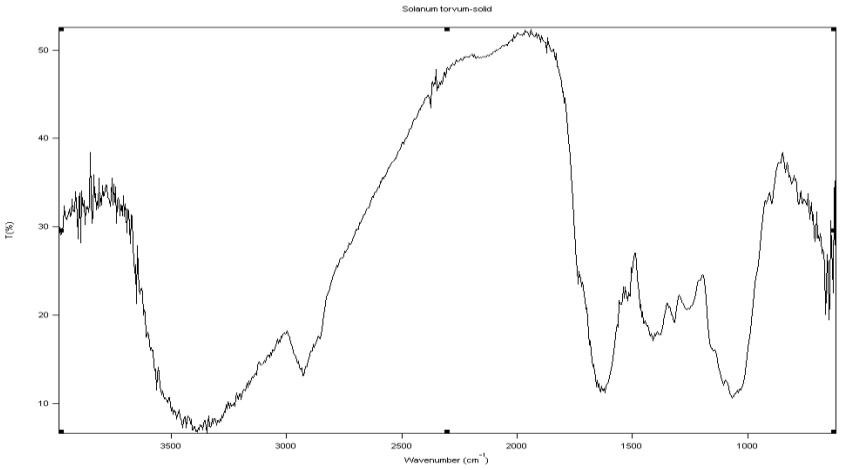




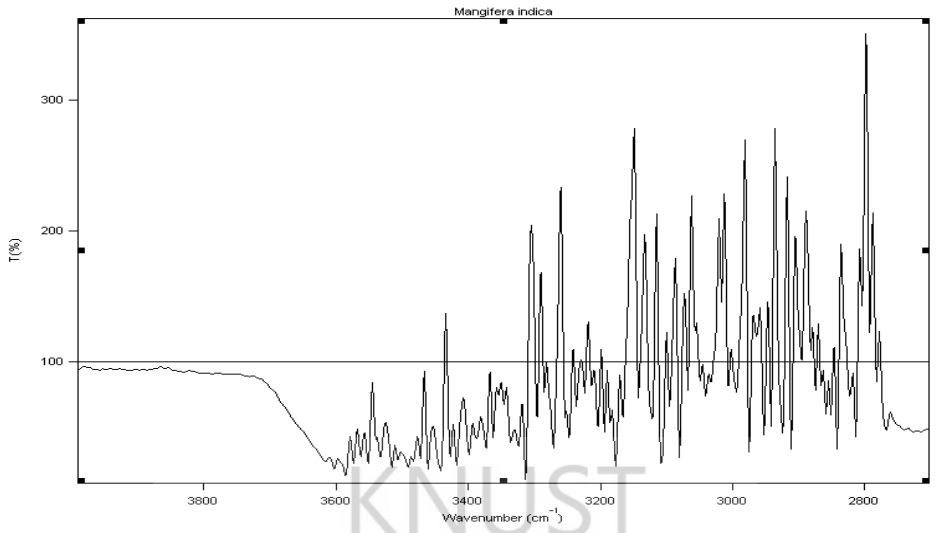
Solanum torvum

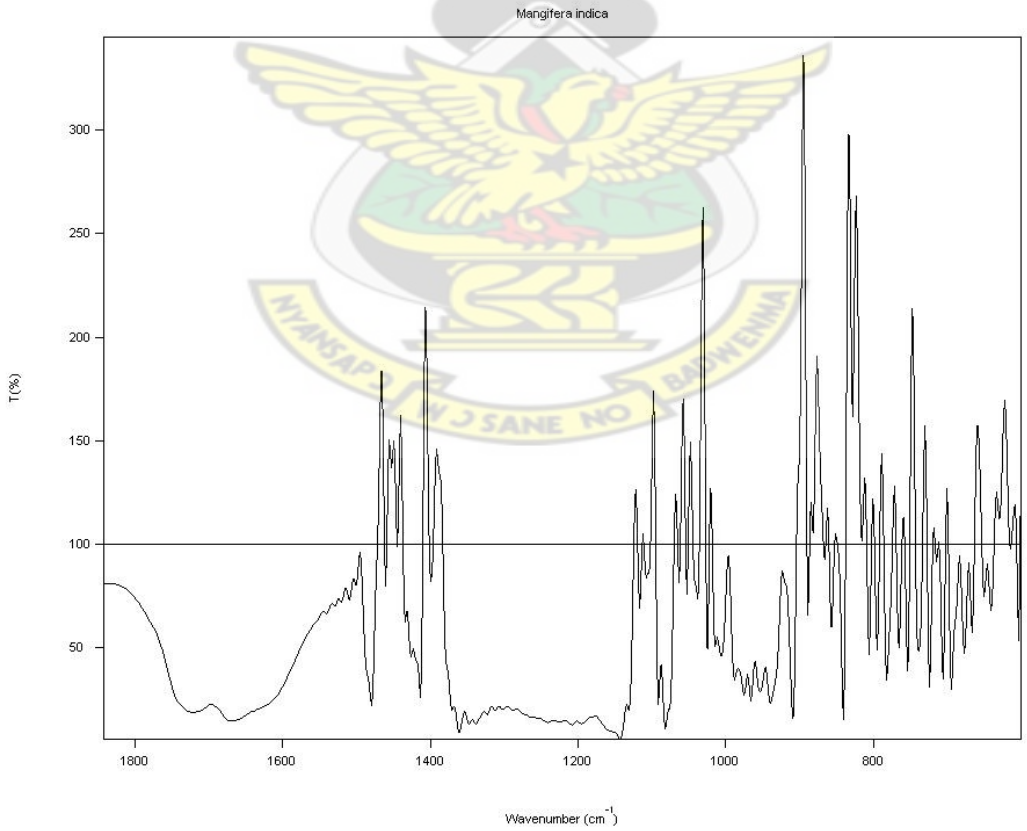




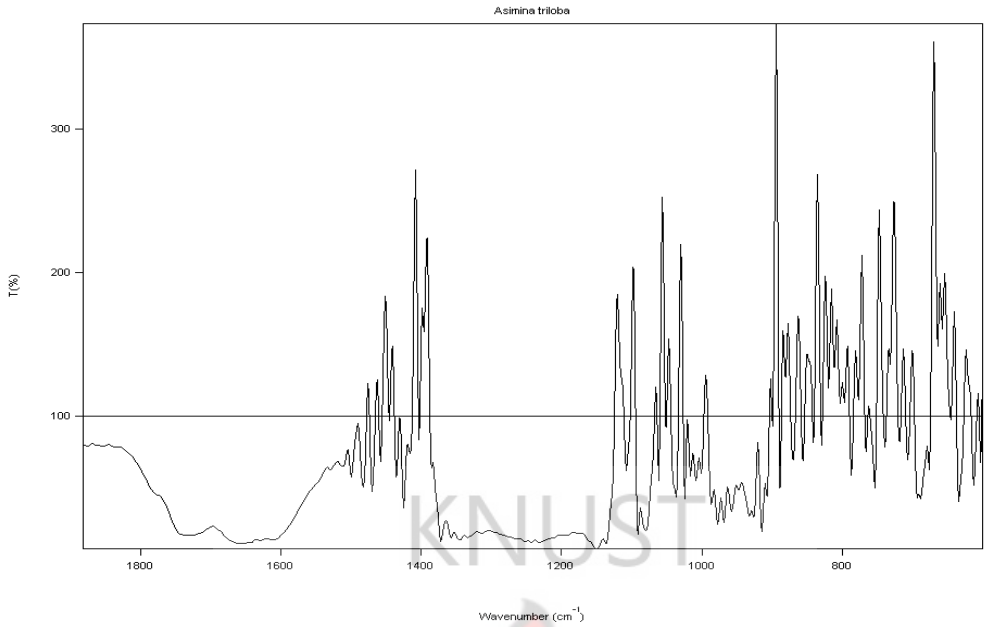


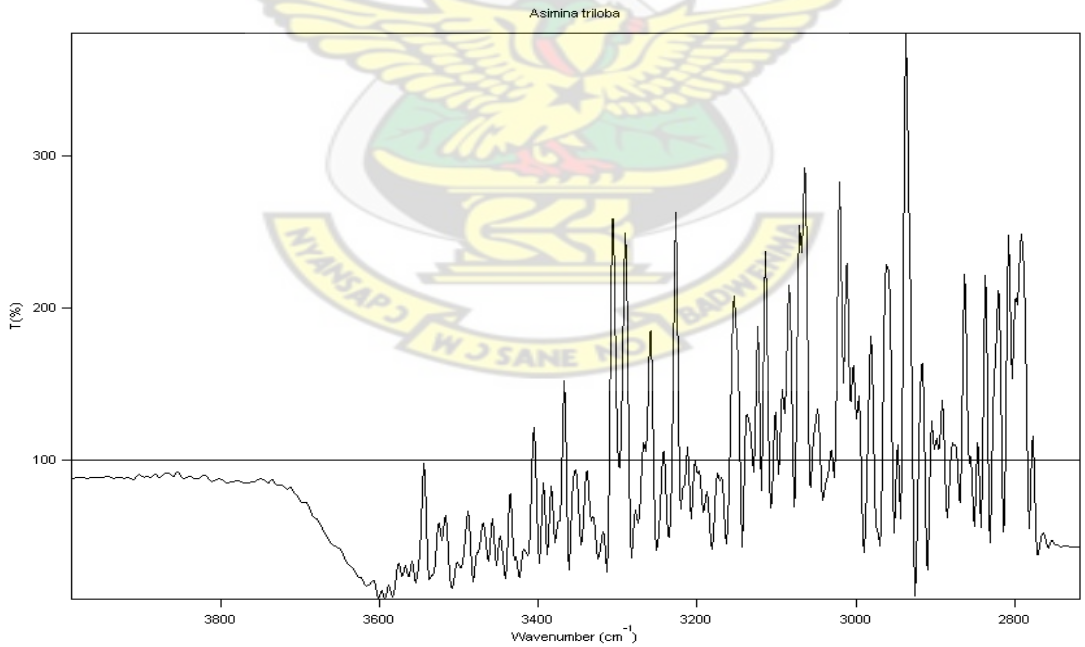
Mangifera indica



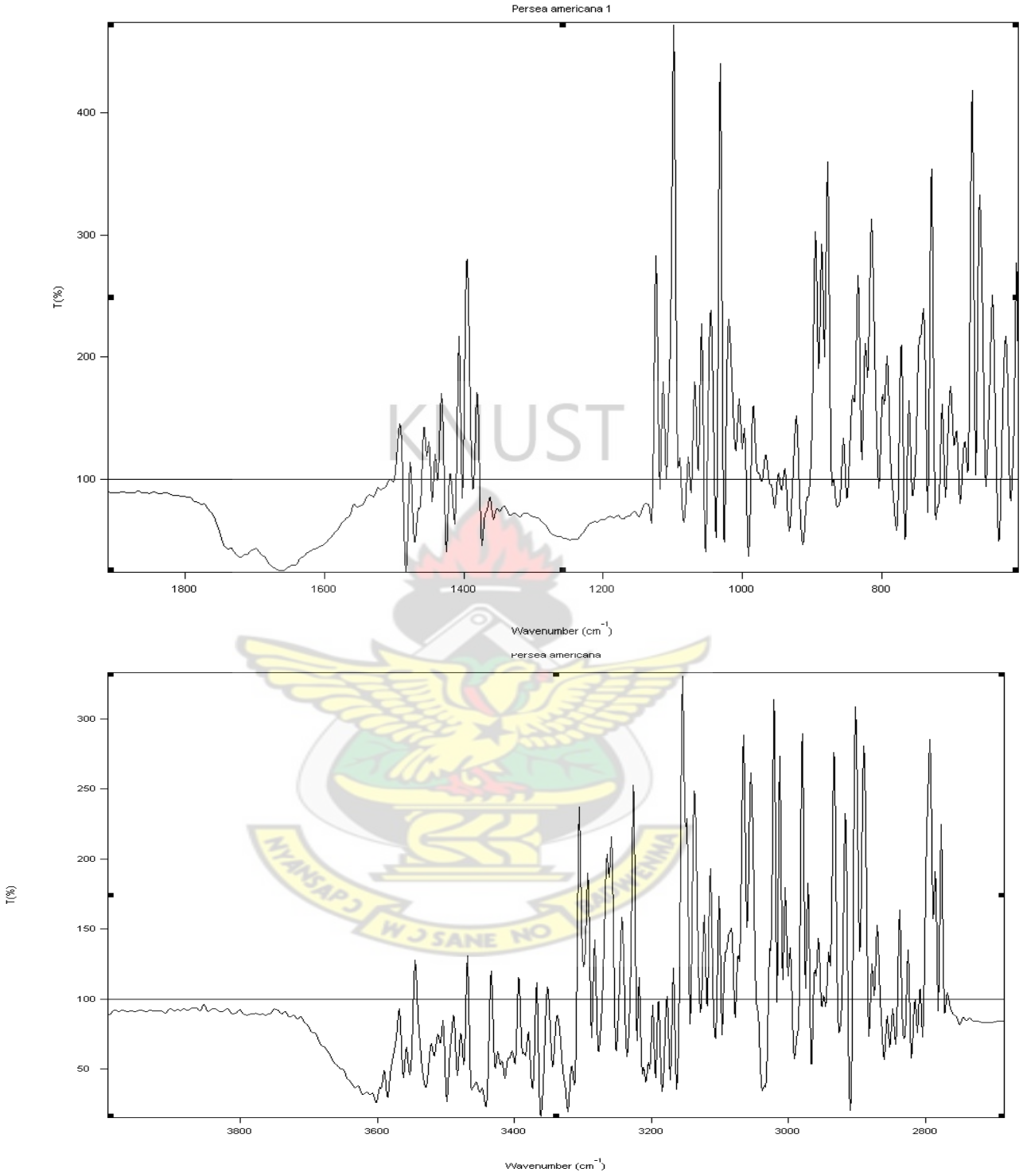


Asimina triloba





Persea Americana



Allium Ascolinicum Linn