SCREENING OF COMMONLY CONSUMED GREEN LEAFY VEGETABLES AND FRUIT SEEDS FOR ANTIOXIDANT ACTIVITY

A PROJECT REPORT

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BONAFIDE CERTIFICATE

Certified that this project report **" SCREENING OF COMMONLY CONSUMED GREEN LEAFY VEGETABLES AND UNUTILIZED FRUIT SEEDS FOR ANTIOXIDANT ACTIVITY "** is the bonafide work of "**Mr. B. SENTHIL KUMAR AND Ms. K. SHANMUGA PRIYA "** who carried out the project work under my supervision.

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ABSTRACT

Four commonly consumed green leafy vegetables (*Amaranthus gangetics, Basella alba, Sesbania grandiflora, Solanum nigrum)* and five fruit seeds (*Carica papaya, Cucumis melo, Citrus lanatus, Benincasa hispida, Cucurbita maxima*) and orange (*Citrus sinensis*) peel were investigated to evaluate their antioxidant activity.preliminary phytochemical analysis of these plant extracts showed the presence of phenols, flavonoids, terpenoids, saponins. Quantitative estimation of flavonoids and total phenolics showed *Citrus sinensis* has highest phenolics which is equal to (32.236 mgTAE/gDW) and *Carica papaya* has highest flavonoids which is equal to (30.25 mgRE/gDW). Antioxidant activity was evaluated by four types of non-enzymatic methods like total antioxidant capacity, DPPH method, FRAP assay and Modified FRAP assay. Among the plant species the ethanol extracts of *Citrus sinensis* has highest total antioxidant capacity of 10.23 (µM/g). In DPPH free radical scavenging assay *Solanum nigrum* has highest free radical scavenging activity of 64-82% in the concentration range of (50-250) µg/ml. ABTS cation radical scavenging assay showed *Basella alba* has highest ABTS cation radical scavenging activity of 37-85% in the concentration range of (50-250) µg/ml. In FRAP assay *sesbania grandiflora* has highest antioxidant activity of absorbance at 700 nm of 2.471 in the concentration range of 100 µg/ml. Modified FRAP assay showed *Citrus sinensis* has highest antioxidant activity of absorbance at 750 nm of 3.057 in the concentration range of 100 µg/ml.

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LIST OF ABBREVIATIONS

µg	microgram
µl	microliter
µM	micromole
ABTS	2, 2,-azinobis (3-ethylbenzoline-6- sulfonic acid)
DNA	Deoxyribose Nucleic Acid
DPPH	1, 1-diphenyl -2-picryl hydrazyl
$FeCl_3$	Ferric Chloride
g	gram
H_2SO_4	Sulphuric acid
TAE	Tannic acid equivalents
DW	Dry weight of extract
RE	Rutin equivalents
mg	milligram
min.	Minute
ml	milliliter
OFR	Oxygen-Free Radicals
˙OH	Hydroxyl radical
ROS	Reactive Oxygen Species
SOD	Superoxide dismutase

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CHAPTER 1
INTRODUCTION

1.1 Significance of antioxidants and their sources

A radical is an atom or group of atoms that have one or more unpaired electrons. Radicals can have positive, negative or neutral charge. They are formed as necessary intermediates in a variety of normal biochemical reactions, but when generated, in excess or not appropriately controlled, radicals can wreak havoc on a broad range of macromolecules. A prominent feature of radicals is that they have extremely high chemical reactivity, which explains not only their normal biological activities, but how they inflict damage on cells.

By definition, a free radical is any atom (e.g. oxygen, nitrogen) with at least one unpaired electron in the outermost shell, and is capable of independent existence. A free radical is easily formed when a covalent bond between entities is broken and one electron remains with each newly formed atom. Free radicals are highly reactive due to the presence of unpaired electron(s). Any free radical involving oxygen can be referred to as reactive oxygen species (ROS). Oxygen centered free radicals contain two unpaired electrons in the outer shell. When free radicals steal an electron from a surrounding compound or molecule, a new free radical is formed in its place. In turn, the newly formed radical then looks to return to its ground state by stealing electrons with antiparallel spins from cellular structures or molecules. Thus, the chain reaction continues and can be "thousand of events long."

There are numerous types of free radicals that can be formed within the body. The most common ROS include: the superoxide anion ($O2^-$), the hydroxyl radical (OH^-), singlet oxygen ($^1O2$), and hydrogen peroxide (H_2O_2). Superoxide anions are formed when oxygen (O_2) acquires an additional electron, leaving the molecule with only one unpaired electron. Within the mitochondria O_2-· is continuously being formed. Hydroxyl radicals are short-lived, but the most damaging radicals within the body. Hydrogen peroxide is produced *in vivo* by many reactions.

The human body has several mechanisms to counteract damage by free radicals and other reactive oxygen species. These act on different oxidants as well as in different cellular compartments.

One important line of defense is a system of enzymes, including glutathione peroxidases, superoxide dismutases and catalase, which decrease concentrations of the most harmful oxidants in the tissues. Several essential minerals including selenium, copper, manganese and zinc are necessary for the formation or activity of these enzymes.

Hence, if the nutritional supply of these minerals is inadequate, enzymatic defenses against free radicals may be impaired.

The second line of defense against free radical damage is the presence of antioxidants. An antioxidant is a molecule stable enough to donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage. Some antioxidants, including glutathione, ubiquinol and uric acid, are produced during normal metabolism in the body. Other lighter antioxidants are found in the diet. Although about 4000 antioxidants have been identified, the best known are vitamin E, vitamin C and the carotenoids. Many other non-nutrient food substances, generally phenolic or poly phenolic compounds, display antioxidant properties and, thus, may be important for health. Antioxidants consist of a group of vitamins, minerals and enzymes that have health enhancing effects for our bodies. Antioxidants work to neutralize free radicals before they do harm to our bodies. Some antioxidants are made in our cells, including enzymes and other molecules. Other essential antioxidants such as Vitamin C, E and selenium must be supplemented in our diets.

Antioxidants are classified into two broad divisions, depending on whether they are soluble in water or in lipids. In general, water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma, while lipid-soluble antioxidants protect cell membranes from lipid peroxidation.

Fresh fruits and vegetables are the best sources of antioxidants as they contain a number of vitamins and minerals. Fruits and vegetables are packed with powerful antioxidants that can lower your risk of heart disease, cancer, diabetes-related damage and even slow down the body's natural aging process. Fruits and vegetables provide the body with an added source of antioxidants that is needed to properly wage war against free radicals. Without the necessary intake of healthy fruits and vegetables, free radicals can spread and eventually lead to stroke, heart attack, arthritis, vision problems, Parkinson's disease, Alzheimer's disease and various types of cancer.

1.2 Oxidative Damage

Free radicals and other reactive oxygen species are derived either from normal essential metabolic processes in the human body or from external sources such s exposure to X-rays, ozone, cigarette smoking, air pollutants and industrial chemicals. Free radicals are atomic or molecular species with unpaired electrons. These unpaired electrons are highly reactive. They are very unstable and react quickly with other compounds, trying to capture the needed electron to gain stability. The attacked molecules now become free radicals, thus initiating the chain reaction. Free radical formation occurs continuously in the cells as a consequence of both enzymatic and non enzymatic reactions.

Reactive oxygen species (ROS) are an entire class of highly reactive molecules derived from oxygen. The formation of oxygen radicals could be the reason for the damaging effects of O_2. A class of enzymes called superoxide dismutases (SOD) is responsible for the catalytic removal of superoxide radical, (Lee *et al*., 2001). An average person

has around 10,000-20,00 free radicals attacking each body cell every day. ROS, including superoxide radicals, hydroxyl radicals, and hydrogen peroxide are often generated as by-products of metabolic processes. *In vivo*, they may also cause great damage to cell membranes and DNA.

1.3 Antioxidants

Antioxidants are capable of stabilizing, or deactivating free radicals before they attack the cells (Mark, 1998). Antioxidants are defined as the substance that when present in low concentrations compared to those of an oxidizable substrate significantly delays or prevents oxidation of that substance (Halliwell and Gutterudge, 1989). Synergism, antagonism, co-antioxidants and oxidant retarders are the other useful concepts related to antioxidants. Synergism can be defined as the phenomenon in which a number of compounds, when present together in the same system, have a more pronounced effect than if they were alone. Antagonism can be defined likewise by substituting “more” with “less”, whereas co antioxidants may be defined by substituting “more” with “same”. The compounds that reduce the rate of oxidation without showing a distinct lag phase of oxidation are retarders of oxidation.

Antioxidants are divided into two classes: preventive antioxidants and chain breaking antioxidants. Preventive antioxidants inhibit oxidation by reducing the rate of chain initiation. Preventive antioxidants convert the hyperoxides to molecular products that are not potential sources of free radicals (Burton *et al*., 1985). Most biological preventive antioxidants are also peroxide decomposers. Antioxidants can also be manufactured synthetically. These belong to the class of synthetic antioxidants. The main disadvantage with these antioxidants is their side effect when taken *in vivo* (Chen *et al*., 1992). Most of the natural antioxidants are found to have higher antioxidant activity when compared with that of the synthetic ones. Previous epidemiological studies have consistently shown that consumption of fruits and vegetables has been associated with reduced risk of chronic diseases, such as cardiovascular diseases and cancer (Sargeant et al., 2001) and neurodegenerative disorders, including Parkinson’s and Alzhemier’s diseases (Gella and Durany, 2009) as well as inflammation and problems caused by cel and cutaneous aging. Diabetic disease is increasing rapidly and consumes vast resources in all countries. The oxidative products, mainly the superoxide anion radical(O_2^-), from diabetic monocytes during oxidative stress lead researchers to give more attention to the protective functions of naturally occurring antioxidants.

1.4 Phytochemicals

Phytochemicals are non- nutritive plant chemicals that contain protective disease preventive compounds. They have been long known to protect plants, and now known to protect humans against disease. They are involved in many processes including ones that help to prevent cancer cell replication and decrease cholesterol levels. Specific phytochemicals are found in different colours of fruits and green leafy vegetables.

1.5 Sources of antioxidants

Plants produce a very impressive array of antioxidant compounds that includes carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols and tocotrienols to prevent oxidation of the susceptible substrate (Hollman, 2001). Common antioxidants include Vitamin A, Vitamin C, Vitamin E, and certain compounds called carotenoids (like lutein and beta-carotene) (Hayek, 2000). These plant-based dietary antioxidants are believed to have an important role in the maintenance of human health because our endogenous antioxidants provide insufficient protection against the constant and unavoidable challenge of reactive oxygen species (Holt et al., 2009).

Green leafy vegetables occupy an important place among the food crops as these provide adequate amounts of many vitamins and minerals for humans. They are rich source of carotene, ascorbic acid, riboflavin, folic acid and minerals like calcium, iron and phosphorous. In nature, there are many underutilized greens of promising nutritive value, which can nourish the ever increasing human population (Gupta and Prakash, 2009). Many of them are resilient, adaptive and tolerant to adverse climatic conditions.

The nutritional value of green leafy vegetables and the lack of awareness of the antioxidant potential of some unexploited green leafy varieties has provoked us to screen the antioxidant activity of four under exploited green leafy vegetables such as ***Amaranthus gangetics, Basella alba, Sesbania grandiflora, Solanum nigrum*** and six unutilized fruit seeds such as ***Cucurbita maxima, Benincasa hispida, Cucumis melo, Carica papaya, Citrullus lanatus*** and peels of ***Citrus sinensis.***

OBJECTIVES

The main objectives of our study are:

- To screen and compare the in vitro antioxidant activities of the ten plant varieties by different free radical scavenging assays.
- To analyse the phytochenicals such flavonoids, tannins, saponons, carbohydrates, phenols present in the ten plant varieties qualitatively.
- To estimate total phenol content and flavonoid content in the selected plant varieties.
- To analyse the fatty acids present in the selected plant variety qualitatively.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Free radicals

Free radicals are the chemical species possessing an unpaired electron, which is formed by homolytic cleavage of a covalent bond of a molecule by the loss of a single electron from a normal molecule or by the addition of a single electron to a normal molecule. Free Radicals are a group of active molecules with unpaired electrons that react with biological membrane, resulting in cell damage. Antioxidants are capable of taking up these free radicals. In doing so, they protect healthy cells from damage and abnormal growth. A number of antioxidant activity assays have been published, and its antioxidant activity is often expressed as Trolox Equivalent.

Most of the molecular oxygen consumed by aerobic cells during metabolism is reduced to water by using cytochrome oxidases in mitochondria. However, when oxygen is partially reduced, it becomes activated and reaches readily with a variety of biomolecules. This partial reduction occurs in one step, by addition of one, two and four electrons to oxygen, which leads to successive formation of reactive oxygen metabolites (ROMs). These ROMs include superoxide anion, hydroperoxy radical, peroxide ion, hydrogen peroxide and hydroxyl radical (Green and Hill, 1984).

The oxygen and hydrogen peroxide so formed may lead to the formation of the most reactive OH. This hydroxyl radical oxidizes lipids giving rise to lipid peroxidation. Hydrogen peroxide is known to cause DNA breaks in intact cells and purification DNA. Malondialdehyde (MDA) is the major reactive aldehyde resulting from the peroxidation of biological membranes of polyunsaturated fatty acid (PUFA) (Vaca *et al.,* 1998). MDA is the secondary product of LPO and used as an indicator of tissue damage (Ohkawa *et al.,* 1979). MDA can modify xanthine oxidoreductase activity through interaction with xanthine oxide and for xanthine dehydrogenase (XDH) (Cighetti *et al.,* 2001). Lipid hydroperoxides may directly induce DNA chain breaking (Cochrane, 1991), and lipid peroxyl and alkoxyl radicals may cause base oxidation in DNA (Park, 1992).

The reactive oxygen metabolites produced during cell metabolism are believed to be involved in various disease processes including cancer. Their main targets are membrane lipids and enzymatic proteins, where they cause cell injury and death (Nadkarni *et al.,* 1991). The damage induced by activated oxygen species leads to fluid loss, protein modification, ion leaks and eventually cell lysis (Paller et *al.,* 1991). However, these problems are overruled by administering the antioxidant compounds.

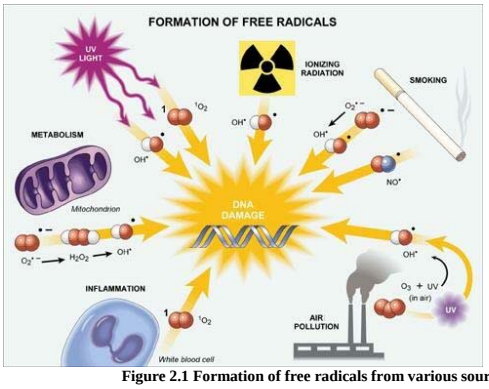

Figure 2.1 Formation of free radicals from various sources

2.2 Role in oxidative stress

Free radicals can also cause extensive damage to the cells and tissues. The major mechanism of damage is due to the induction of oxidative stress. Oxidative stress is caused by the imbalance between the production of ROS and a biological system's inability to readily detoxify the reactive species or easily repair the resulting damage. All forms of life maintain a reducing environment within their cells. The enzymes through a constant input of metabolic energy preserve this reducing environment. Disturbances in this normal redox state can cause toxic effects through the production of peroxides and free radicals that damage all the components of the cell, including proteins, lipids and DNA.

In chemical terms, oxidative stress is a large increase in the cellular reduction potential or a large decrease in the reducing capacity of the cellular redox couples, such as glutathione (Schafer *et al.*, 2001). The effects of oxidative stress depend on the size of these changes, with a cell being able to overcome small perturbations and regain its original state. However, more severe oxidative stress can cause cell death and even moderate oxidation can trigger apoptosis, while more intense stress can cause necrosis (Lennon *et al*., 1991).

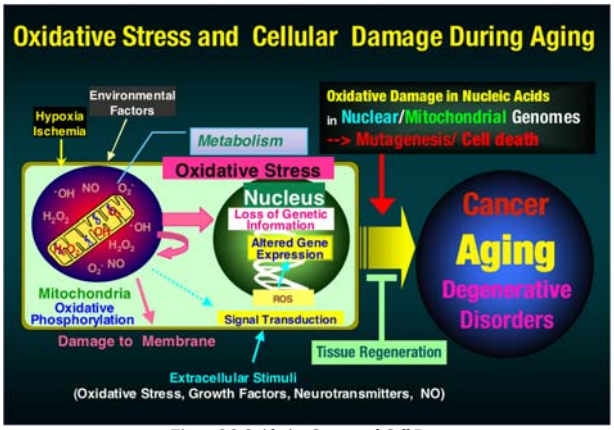

Figure 2.2 Oxidative Stress and Cell Damage

2.3 Bioactive constituents in plant seeds

Seeds are the primary stage of plant life cycle having a strong defence mechanism possibly due to presence of phytoconstituents contributing to antioxidant and/or antimicrobial activity. Many fruits have inedible seeds and are not part of human diet. However, such seeds are a part of ayurvedic preparations against many diseases.

Numerous reports are there in literature concerning presence of bioactive substances in plant seeds. Seeds from the genus *Lupinus* are known to accumulate large quantities of poisonous quinolizidine alkaloids. Lignans in seeds either help defend against various pathogens or act as antioxidants. Sesamin, from the sesame seed, has in vitro antioxidant properties that stabilize sesame oil against turning rancid during commercial storage. Red sorghum produces proanthocyanidin antifeedant compounds, condensed tannins, which deter birds from feeding on the seed. Coumarins in seeds coats appear to possess antimicrobial, antifeedant, and germination inhibitor properties.

Methanol extract of *Garcinia kola* seeds were reported for their bactericidal action against *B. anthracis* and *E. coli*. *Prunus armeniaca* kernels were reported to possess antioxidant and antimicrobial activities.A particularly destructive aspect of oxidative stress is the production of ROS, which include free radicals and peroxides. Some of the less reactive of these species can be converted by oxidoreduction reactions with transition metals or other redox

cycling compounds including quinones into more aggressive radical species that can cause extensive cellular damage (Valko *et al*., 2005). Under the severe levels of oxidative stress that cause necrosis, the damage causes ATP depletion, preventing controlled apoptotic death and causing the cell to simply fall apart (Lelli *et al*., 1998; Lee and Shacter, 1999).

S.NO	Plant Name	Bioactive Component Present
1	*Coffea Arabica*	Diterpenoids
2	*Leucas aspera*	Phenyl propanoids
3	*Mallotus anomalus*	Benzyl benzoate
4	*Stemona parviflora*	Parvistemoninine
5	*Alysicarpus vaginalis*	Flavonoids
6	*Liriodendron tulipifera.*	Saponins
7	*Brassica Nigra*	Glucosinolates
8	*Prunus spp.*	Cyanogenic glycosides
9	*Digitalis purpura*	Cardiac glycosides
10	*Fagus grandifolia*	Tannins

Table 2.1 Plants and their Bioactive components

2.4 Antioxidants

Oxygen is essential for aerobic life process. However, cells under aerobic conditions are threatened with the reactive oxygen metabolites and are efficiently taken care by the powerful antioxidant system. Aerobic life is characterized as continuous production of oxidants balanced by equivalent synthesis of antioxidants (Rice Evans and Diplock, 1993).

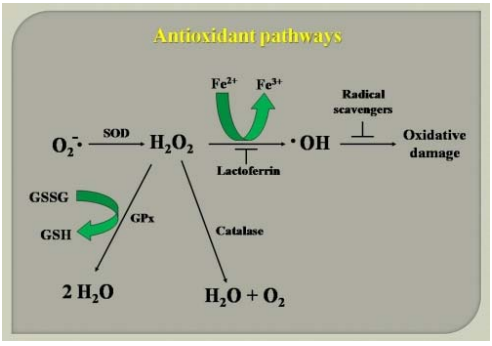

Figure 2.3 Antioxidant Pathway

A shift of the balance on the oxidant side may trigger a cascade of reaction leading to the formation of highly reactive cytotoxic compounds such as Reactive Oxygen Metabolites, ROMs (Halliwell and Gutteridge, 1989). The improper balance between ROMs production and antioxidant defense results in oxidative stress, which deregulates the cellular functions leading to various pathological conditions including cancer (Bandyopathyay *et al.,* 1999). In order to counteract the lethal effects of oxidative damage of DNA, normal living cells have developed multiple antioxidative defenses (Demple and Harrison, 1994).

2.5 Antioxidants as Scavengers

Antioxidants have also been suggested to have a well-defined role as preservatives. These have been defined by the US Food and Drug Administration (FDA) as substance used to preserve food by retarding deterioration, rancidity or discoloration caused by oxidation (Dziezak, 1986). Lipid peroxidation is an important deteriorative reaction of foods during processing and storage. Toxic substance formed by lipid peroxidation may lead to adverse effects such as carcinogenesis, cell DNA mutagenesis and aging.

Antioxidants therefore, according to their mode of action, have also been classified as the compounds that terminate the free radical chain in lipid peroxidation by donating electrons or hydrogen to fat containing a free radical and to the formation of a complex between the chain and a free radical. Antioxidants stop the reactions by contributing hydrogen from the phenolic hydroxyl hydrogen from the phenolic hydroxyl groups, themselves forming stable free radicals that do not initiate or propagate further oxidation of lipids (free radical terminators). Some of the important

synthetic antioxidants of this class are butylated hydroxyanisole (BHA), butylated hydroxy toluene, terbutyl hydroquinone (TBHQ), propyl gallate (PG) and tocopherols.

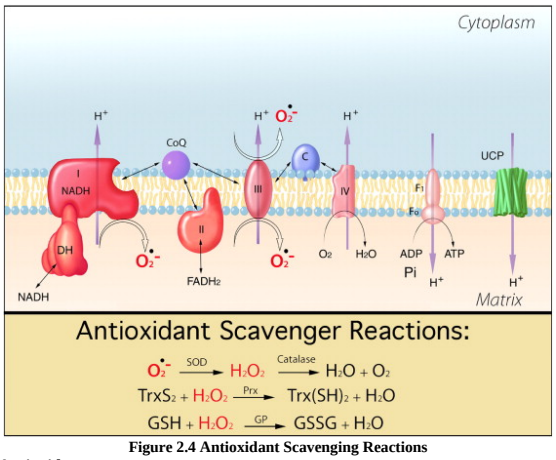

Figure 2.4 Antioxidant Scavenging Reactions

2.6 Types of Antioxidants

Antioxidants are of different types such as natural or enzymic antioxidants, non-enzymic antioxidants, scavenging or chain breaking antioxidants, pharmacologic antioxidants and others.

2.6.1 Non-Enzymatic Antioxidants

Total reduced glutathione (GSH)

Reduced glutathione, most commonly called glutathione or GSH, is a relatively small molecule ubiquitous in living systems (Kidd, 2000). Occurring naturally in all human cells, GSH is a water-phase orthomolecule. Its intracellular depletion ultimately results in cell death and its clinical relevance has been researched for decades. Technically N-L-gamma-glutamyl-cysteinyl glycine or L-glutathione, the molecule has a sulfhydryl (SH) group on the cysteinyl portion, which accounts for its strong electron-donating character. As electrons are lost the molecule becomes oxidized, and two such molecules become linked (dimerized) by a disulfide bridge to form glutathione disulfide or oxidized glutathione (GSSG). GSH is an extremely important cell protectant. It directly quenches reactive hydroxyl

free radicals, other oxygen-centered free radicals, and radical centres on DNA and other biomolecules. It contains an unusual peptide linkage between the amine group of cysteine and the carboxyl group of the glutamate side chain. Glutathione, an antioxidant, protects cells from toxins such as free radicals.

Antioxidative vitamins

The major protective functions of the vitamins are the scavenging of ROMs (Torn *et al.,* 1995). Antioxidative vitamin A, E and C have a number of biological activities such as immune stimulation, alteration of metabolic activations of carcinogens, mopping up free radicals, etc., other natural body antioxidant compounds include cysteine, glutathione and transferrin. Although free radicals are implicated in many diseases, it is not possible that free radicals are not the cause, but consequence of the disease at least in some situations.

Vitamin C

Vitamin **C** or L-ascorbic acid or L-ascorbate is an essential nutrient for humans and certain other animal species. In living organisms ascorbate acts as an antioxidant by protecting the body against oxidative stress. The vast majority of animals and plants are able to synthesize their own vitamin C, through a sequence of four enzyme-driven steps, which convert glucose to vitamin C. The glucose needed to produce ascorbate in the liver (in mammals and perching birds) is extracted from glycogen; ascorbate synthesis is a glycogenolysis-dependent process. Ascorbic acid is well known for its antioxidant activity, acting as a reducing agent to reverse oxidation in liquids. Individuals experiencing oxidative stress have ascorbate blood levels lower than 45 µmol/L, compared to healthy individual who range between 61.4-80 µmol/L.

Ascorbic Acid (Vitamin C)

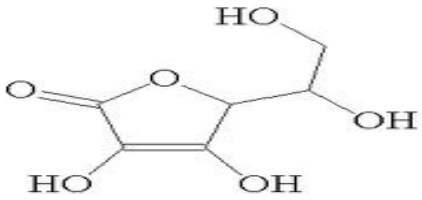

Figure 2.5 Vitamin C

Vitamin E

Vitamin E refers to a group of eight fat-soluble compounds that include both tocopherols and tocotrienols. There are many different forms of vitamin E, of which γ-tocopherol is the most common. α-Tocopherol is an important lipid-soluble antioxidant. It performs its functions as antioxidant in what is known by the glutathione peroxidase pathway and it protects cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction. This would remove the free radical intermediates and prevent the oxidation reaction from continuing. The oxidized α-tocopheroxyl radicals produced in this process may be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, retinol or ubiquinol**.**

Tocopherol (Vitamin E)

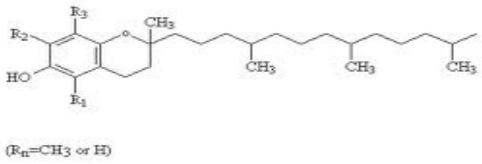

Figure 2.6 Vitamin E

Carotenoids

Carotenoids are tetraterpenoid organic pigments that are naturally occurring in the chloroplasts and chromoplasts of plants and some other photosynthetic organisms like algae, some bacteria, and some types of fungus. Carotenoids in general absorb blue light. In photosynthetic organisms, specifically flora, carotenoids play a vital role in the photosynthetic reaction centre. They either participate in the energy-transfer process, or protect the reaction center from auto-oxidation. In non-photosynthesizing organisms, specifically humans, carotenoids have been linked to oxidation-preventing mechanisms. It can dissipate the energy of singlet oxygen, thus preventing this active molecule from generating free radicals. Its other antioxidant properties include the scavenging of free radicals.

Lycopenes

Lycopene is a bright red carotene and carotenoid pigment and phytochemical found in tomatoes and other red fruits and vegetables. Although lycopene is chemically a carotene, it has no vitamin A activity. Lycopene may be the most

powerful carotenoid quencher of singlet oxygen, being 100 times more efficient in test tube studies of singlet-oxygen quenching action.

Edaravone

Edaravone (Radicut) is a neuroprotective agent used for the purpose of aiding neurological recovery following acute brain ischemia and subsequent cerebral infarction. It acts as a potent antioxidant and strongly scavenges free radicals, protecting against oxidative stress and neuronal apoptosis.

Hydroxytyrosol

Hydroxytyrosol (3,4-dihydroxyphenylethanol; DOPET) is a phytochemical with antioxidant properties. After gallic acid, hydroxytyrosol is believed to be one of the most powerful antioxidants. Its oxygen radical absorbance capacity is 40,000 umolTE/g, which is ten times higher than that of green tea, and two times higher than that of CoQ10.

L-Carnitine

Carnitine is a quaternary ammonium compound biosynthesized from the amino acids lysine and methionine. In living cells, it is required for the transport of fatty acids from the cytosol into the mitochondria during the breakdown of lipids (fats) for the generation of metabolic energy. It is often sold as a nutritional supplement. Carnitine was originally found as a growth factor for mealworms and labeled vitamin Bt. Carnitine exists in two stereoisomers: Its biologically active form is L-carnitine, whereas its enantiomer, D-carnitine, is biologically inactive.

Ladostigil

Ladostigil (TV-3,326) is a novel neuroprotective agent being investigated for the treatment of neurodegenerative disorders like Alzheimer's disease, Lewy body disease, and Parkinson's disease. It acts as a reversible acetylcholinesterase and butyrylcholinesterase inhibitor, and an irreversible monoamine oxidase B inhibitor, and combines the mechanisms of action of older drugs like rivastigmine and rasagiline into a single molecule. In addition to its neuroprotective properties, ladostigil enhances the expression of neurotrophic factors like GDNF and BDNF, and may be capable of reversing some of the damage seen in neurodegenerative diseases via the induction of neurogenesis. Ladostigil also has antidepressant effects, and may be useful for treating comorbid depression and anxiety often seen in such diseases as well.

Melatonin

Melatonin, also known chemically as N-acetyl-5-methoxytryptamine, is a naturally occurring compound found in animals, plants, and microbes. In animals, circulating levels of the hormone melatonin vary in a daily cycle, thereby allowing the entrainment of the circadian rhythms of several biological function.

N-Acetylserotonin

N-Acetylserotonin (NAS), also known as normelatonin, is a naturally-occurring chemical intermediate in the endogenous production of melatonin from serotonin. It is produced from serotonin by the enzyme aralkylamine N-

acetyltransferase (AANAT) and is converted to melatonin by acetylserotonin O-methyltransferase (ASMT). Like melatonin, NAS is an agonist at the melatonin receptors MT1, MT2, and MT3, and may be considered to be a neurotransmitter. In addition, NAS is distributed in some areas of the brain where serotonin and melatonin are not, suggesting that it may have unique central duties of its own instead of merely functioning as a precursor in the synthesis of melatonin.

Oleocanthal

Oleocanthal is a natural organic compound isolated from extra virgin olive oil. It is responsible for the slightly peppery "bite" of extra virgin olive oil. Oleocanthal is an tyrosol ester and its chemical structure is related to oleuropein that is also found in olive oil.

Oleuropein

Oleuropein is a chemical compound found in olive leaf from the olive tree (and leaves of privet) together with other closely related compounds such as 10-hydroxyoleuropein, ligstroside, and 10-ydroxyligstroside. All these compounds are tyrosol esters of elenolic acid that are further hydroxylated and glycosylated. Oleuropein and its metabolite hydroxytyrosol have powerful antioxidant activity both in vivo and in vitro and give extra-virgin olive oil its bitter, pungent taste. Oleuropein preparations have been claimed to strengthen the immune system. A study in rats suggests oleuropein enhances thermogenesis by increasing the thermogenin content in brown adipose tissue and the secretion of noradrenaline and adrenaline.

Rasagiline

Rasagiline (Azilect) is an irreversible inhibitor of monoamine oxidase used as a monotherapy in early Parkinson's disease or as an adjunct therapy in more advanced cases. It is selective for MAO type B over type A by a factor of fourteen.

Resveratrol

Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a phytoalexin produced naturally by several plants when under attack by pathogens such as bacteria or fungi.

Selegiline

Selegiline (l-deprenyl, Eldepryl) is a drug used for the treatment of early-stage Parkinson's disease, depression and senile dementia. In normal clinical doses it is a selective irreversible MAO-B inhibitor, however in larger doses it loses its specificity and also inhibits MAO-A. Dietary restrictions are common for MAOI treatments, but special dietary restrictions for lower doses have been found to be unnecessary, and dietary restrictions appear to be unnecessary at standard doses when selegiline is taken as Emsam, the transdermal patch form, as no adverse events due to diet have ever been reported with Emsam. The drug was researched by Jozsef Knoll et al. (Hungary). Selegiline

belongs to a class of drugs called phenethylamines. Selegiline consists of a l-desoxyephedrine (levomethamphetamine) skeleton with a propargyl group attached to the nitrogen atom.

Selenium

Selenium is a chemical element with the atomic number 34, represented by the chemical symbol Se, an atomic mass of 78.96. It is a nonmetal, chemically related to sulfur and tellurium, and rarely occurs in its elemental state in nature.

Vulgaxanthin

Vulgaxanthins are a group of betaxanthins, or the predominant yellow plant pigments found in red beets, among other plants like Mirabilis jalapa and swiss chard. They are antioxidant pigments, types I, II, III, IV, and V. Like all betaxanthins, it cannot be hydrolyzed by aglycone without degradation. Water activity also affects stability of this antioxidant.

Tartaric acid

Tartaric acid is a white crystalline diprotic organic acid. It occurs naturally in many plants, particularly grapes, bananas, and tamarinds, and is one of the main acids found in wine. It is added to other foods to give a sour taste, and is used as an antioxidant. Salts of tartaric acid are known as tartrates. It is a dihydroxy derivative of succinic acid.

Green tea

Green tea is tea made solely with the leaves of Camellia sinensis that have undergone minimal oxidation during processing. Green tea originates from China and has become associated with many cultures in Asia from Japan and South Korea to the Middle East. Recently, it has become more widespread in the West, where black tea is traditionally consumed. Many varieties of green tea have been created in countries where it is grown. These varieties can differ substantially due to variable growing conditions, processing, and harvesting time.

Iodide

An iodide ion is the ion I-1. Compounds with iodine in formal oxidation state -1 are called iodides. Iodides include ionic compounds such as sodium iodide and organic compounds such as methyl iodide. In everyday life, iodide is most commonly encountered as a component of iodized salt, which many governments mandate. Worldwide, iodine deficiency affects two billion people and is the leading preventable cause of mental retardation.

Polyphenol

A polyphenol is a chemical compound belonging to a group of chemical substances found in plants, characterized by the presence of more than one phenol unit or building block per molecule. Polyphenols are generally divided into hydrolyzable tannins (gallic acid esters of glucose and other sugars) and phenylpropanoids, such as lignins, flavonoids, and condensed tannins.

Proanthocyanidin

Proanthocyanidin (PA or PAC), also known as procyanidin, oligomeric proanthocyanidin (OPC), leukocyanidin, leucoanthocyanin and condensed tannins, is a class of flavanols. Proanthocyanidins are essentially polymer chains of flavonoids such as catechins. One was discovered in 1948 by Jacques Masquelier and called Vitamin P, although this name did not gain official category status and has since fallen out of usage. It was Masquelier who first developed techniques for the extraction of proanthocyanidins from certain plant species.

2.7 Enzymatic Antioxidants

The first line of defense against O_2 and H_2O_2 mediated injury are antioxidant enzymes like superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT). The term antioxidant has been defined as any substance that delays or inhibits oxidative damage to a target molecule (Halliwell and Gutteridge, 1989). Antioxidant enzymes, together with the substances that are capable of either reducing ROMs or preventing their formation, form a powerful reducing buffer which affects the ability of the cell to counteract the actions of oxygen metabolites and which maintain the lowest possible levels of ROMs inside the cells (Sies, 1997).

2.7.1 Superoxide dismutase (SOD)

Superoxide dismutases (SODs) are a class of closely related enzymes that catalyse the breakdown of the superoxide anion into oxygen and hydrogen peroxide (Zelco *et al*., 2002). SOD enzymes are present in almost all aerobic cells and in extracellular fluids. Superoxide dismutase enzymes contain metal ion cofactors that, depending on the isozyme, can be copper, zinc, manganese or iron. In humans, the copper/zinc SOD is present in the cytosol, while manganese SOD is present in the mitochondrion (Bannister *et al*., 1987). There also exists a third form of SOD in extracellular fluids, which contains copper and zinc in its active sites (Nozik-Grayck *et al.*, 2005). The mitochondrial isozyme seems to be the most biologically important of these three.

2.7.2 Glutathione peroxidase (GPx)

Glutathione peroxidase is an enzyme containing four selenium-cofactors that catalyses the breakdown of hydrogen peroxide and organic hydroperoxides. There are at least four different glutathione peroxidase isozymes in animals (Brigelius-Flohe, 1999). Glutathione peroxidase 1 is the most abundant and is a very efficient scavenger of hydrogen peroxide, while glutathione peroxidase 4 is most active with lipid hydroperoxides.

2.7.3 Catalase (CAT)

Catalases are enzymes that catalyse the conversion of hydrogen peroxide to water and oxygen, using either an iron or manganese cofactor (Chelikani *et al.,* 2004). This protein is localized to peroxisomes in most eukaryotic cells. Catalase is an unusual enzyme since, although hydrogen peroxide is its only substrate, it follows a ping-pong mechanism. Here, its cofactor is oxidised by one molecule of hydrogen peroxide and then regenerated by transferring the bound oxygen to a second molecule of substrate (Hiner *et al.*, 2002). Despite its apparent importance in hydrogen

peroxide removal, humans with genetic deficiency of catalase —"acatalasemia"— or mice genetically engineered to lack catalase completely, suffer few ill effects (Mueller *et al.,* 1997).

2.7.4 Glutathione-S- transferase (GST)

GSTs catalyze the conjugation of reduced glutathione via the sulfhydryl group, to electrophilic centres on a wide variety of substrates. This activity is useful in the detoxification of endogenous compounds such as peroxidised lipids as well as the metabolism of xenobiotics.

2.7.5 Glutathione reductase (GR)

Glutathione reductase, also known as GR, is an enzyme that reduces glutathione disulfide (GSSG) to the sulfhydryl form GSH, which is an important cellular antioxidant. For every mole of oxidized glutathione (GSSG), one mole of NADPH is required to reduce GSSG to GSH. The enzyme forms a FAD bound homodimer.

2.7.6 Glucose-6-phosphate dehydrogenase (G6PD)

Glucose-6-phosphate dehydrogenase (G6PD) is a cytosolic enzyme in the pentose phosphate pathway (see image), a metabolic pathway that supplies reducing energy to cells (such as erythrocytes) by maintaining the level of the co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH). The NADPH in turn maintains the level of glutathione in these cells that helps protect the red blood cells against oxidative damage.

2.7.7 Polyphenol oxidase (PPO)

Polyphenol oxidase (PPO) enzymes catalyze the *o*-hydroxylation of monophenols (phenol molecules in which the benzene ring contains a single hydroxyl substituent) to *o*-diphenols (phenol molecules containing two hydroxyl substituents). They can also further catalyze the oxidation of *o*-diphenols to produce *o*-quinones. It is the rapid polymerization of *o*-quinones to produce black, brown or red pigments (polyphenols) that is the cause of fruit browning. The amino acid tyrosine contains a single phenolic ring that may be oxidized by the action of PPOs to form *o*-quinone. Hence, PPOs may also be referred to as tyrosinases. Polyphenol oxidase is found in fruits and is the enzyme responsible for them turning brown. Enzymatic browning is not unique to apples. PPO — a mixture of monophenol oxidase and catechol oxidase enzymes are present in nearly all plant tissues, and can also be found in bacteria, animals, and fungi. In fact, browning by PPO is not always an undesirable reaction; the familiar brown color of tea, coffee and cocoa is developed by PPO enzymatic browning during product processing.

2.8 Antioxidants and peroxidation

Antioxidants are seemingly magical nutrients that can repair cell damage that happens in all our bodies over time. Although antioxidants are produced naturally, our body needs a supply of antioxidants from dietary sources.

The process of peroxidation due to free radicals continues in a chain reaction and cells are damaged. Peroxidation is important because it helps the body destroy cells that have outlived their usefulness and kills germs and parasites. However, peroxidation, when left unchecked, also destroys or damages healthy cells.

Antioxidants help prevent widespread cellular destruction by donating components to stabilize free radicals. More important, antioxidants return to the surface of the cell to stabilize, rather than damage, other cellular components.

When there are not enough antioxidants to hold peroxidation in check, free radicals begin damaging healthy cells, which can lead to problems. For example, free radical damage to immune cells can lead to an increased risk of infections.

2.9 Antioxidant Activity

Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, and reperfusion injury of many tissues. Oxidation process is one of the most important routes for producing free radicals in food, drugs and even living systems. Strong restrictions have been placed on the application of synthetic antioxidants, and there is a trend to substitute them with naturally occurring antioxidants. Moreover, synthetic antioxidants such as butylated hydroxyl anisole (BHA) and gallic acid esters also show low solubility and moderate antioxidant activity. Recently there has been anupsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical induced tissue injury.

Antioxidant activity of plants might be due to their phenolic compounds.Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of oxidative enzymes and anti-inflammatory action. There have been reports of relationship of total flavonoid and phenol contents with antioxidant activity. In the longer term, plant species (or their active constituents) identified as having high levels of antioxidant activity *in vitro* may be of value in the design of further studies to unravel novel treatment strategies for disorders associated with free radicals induced tissue damage. It may be that such simple and cost-effective measures as improving our diet through supplementation with an umber of key antioxidants (antioxidant therapy) can dramatically improve the health of the general population and the individual person.

Antioxidants, however,when administered in higher amounts exert detrimental effects on health. The dose and the route of administration of antioxidants are important factors to be considered before taking in any exogenous supplements of antioxidants. Many research groups are focusing on the appraisal of antioxidant properties of various parts of plants. Metabolic engineering of plants can improve the yield of antioxidants. In order to identify the compatible targets for metabolic engineering,screening of a large number of plant species for their antioxidant activity needs to be done. More research on natural antioxidants can certainly help in increasing our average life expectancy in the coming decades.

Capacity of polyphenolic antioxidants to scavenge free radicals has been evidenced by a large number of tests measuring the antioxidant activity *in vitro*. Antioxidant tests can be classified into two groups: those assays used to evaluate lipid peroxidation, and those assays used to measure free radical scavenging ability. In view of the diversity of the methods, there is a great need to standardize them for measurement of antioxidant activity (Moreno, 2002).

2.10 Phenolic antioxidants

Electron-donating groups at the ortho positions of a phenol characterize a number of efficient antioxidants. A further feature of the structure of phenols is their acidity, or ability to ionize; the phenolate anion is even more readily oxidized than the protonated form. Naturally occurring poplyphenols have repeatedly been shown to scavenge peroxyl radicals. Many phenolic compounds, in addition to being potent quenchers of free radical reactions, also react quite rapidly with singlet oxygen. Some flavonoids, too, which are also well-known radical scavengers, appear to be reactive with singlet oxygen. The most chemically reactive quencher types are flavonols such as quercetin and fisetin. The flavonoid quenches singlet oxygen without undergoing much change in concentration. Flavonoids represent a large and diverse group of phenolic compounds derived from higher plants. Derived from the type structure, flavone, these heterocyclic compounds display a wide range of substitution patterns and oxidation states including flavonols, flavanols, flavanones, and flavans or catechins. These compounds appear to have the capacity of radical scavenging and metal ion complexation. Quercetin is known to form

stable complexes with Cu(II). Flavonoids having greater numbers of hydroxyl groups are more effective antioxidants. Isoflavonoids are to be noted for their distribution being restricted to a single plant family, the Leguminosae. Among free phenolic acids, compounds derived from the C6-C3 phnylpropanoid unit are especially abundant in seeds and bark.

In addition, gallic acid and related phenols in red wines have been suggested to be responsible for the “French paradox”, that is the fact that residents of France have lower rates of cardiovascular disease than those of other countries, despite consuming a diet high in fats. Gallic acid derivatives are often powerful antioxidants.

2.11 Extraction

Extraction as a pharmaceutically used term can be defined as the technique used for the separation of therapeutically desired active constituent(s) and elimination of unwanted insoluble material by treatment with selective solvents. Extraction mainly involves the release of complex plant constituents and solubilization of secondary metabolites from the matrix, followed by separation of soluble target compounds from the crude extract through selective use of solvents.

The basic parameters influencing the quality of an extract are the plant part used as tarting material, the solvent used for extraction, the extraction technology used with the type of equipment employed, and crude drug :

extract ratio. Other important parameters affecting the yield of the extraction procedure are the moisture content of the plant material and temperature.

Traditional extraction processes involve extraction with water or organic solvents. Water is almost universally the solvent used to extract activity. Initial screenings of plants for possible antimicrobial activities typically begin by using crude aqueous or alcohol extractions. Starches, polypeptides, and lectins are better extracted in water. Coumarins and fatty acids are better extracted in ether, whereas methanol is reported to be good for extracting lactones and phenones.

The choice of extraction procedure depends on the nature of the plant material and the components to be isolated. Various procedures that can be used for the extraction of medicinal plants include- maceration, infusion, percolation, decoction, Soxhlet extraction, counter current extraction, sonication, supercritical fluid extraction, steam distillation, etc. The head space trapping technique, microwave assisted extraction (MAE), solid phase microextraction, and molecular distillation are some of the newer methods of extraction. Ultrasound may enhance the extraction process for some plant materials.

2.12 Antioxidant and its natural sources

Antioxidant compounds in food play an important role as a health-protecting factor. Most of the antioxidant compounds in a typical diet are derived from plant sourcesand belong to various classes of compounds with a wide variety of physical and chemical properties. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease (Miller, 1997).

Antioxidant is a group of molecule that is capable of slowing or preventing the oxidation of other molecules, thus damaging our body. They are substances or nutrients that are found within our foods. Oxidation reactions produce free radical, which in turn start a chain reaction that causes damage to the cells. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. Antioxidant is important because they act as 'free radical scavengers' and prevent and also repair damage done by these free radicals. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases.

Antioxidant could be found abundant in plants and other living things. Most common antioxidants are vitamin A, vitamin C, vitamin E and carotenoids which can be found in plants. On the other hand, selenium is a type of

antioxidant that is found in fish, shellfish, red meat, chicken and eggs. Our body also produces antioxidant in the form of enzymes, such as, sup eroxide dismutase (SOD), catalase and glutathione peroxidase.

Low levels of antioxidants, or inhibition of the antioxidant enzymes, causes oxidative stress and may damage or kill cells. The use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases. Antioxidants are also widely used as ingredients in dietary supplements. Although initial studies suggested that antioxidant supplements might promote health, later large clinical trials did not detect any benefit and suggested instead that excess supplementation may be harmful. In addition, these compounds have many industrial uses, such as preservatives in food and cosmetics and preventing the degradation of rubber and gasoline.

2.13 Evaluation and Comparison of Antioxidant potential in fruit seeds and green leafy vegetable extracts

Research on antioxidant activity has been carried out in many of the commonly available edible plants and almost all fruits and vegetables have shown significant antioxidant activity. The nutritional value and the lack of awareness of the antioxidant potential of commonly available varieties have provoked us to evaluate and compare the antioxidant potential.

2.13.1 Plants

The green leafy vegetables and unutilized fruit seeds taken for the studies are ***Amaranthus gangetics, Basella alba, Sesbania grandiflora, Solanum nigrum***, ***Cucurbita maxima, Benincasa hispida, Cucumis melo, Carica papaya, Citrullus lanatus, Citrus sinensis.***

Sesbania grandiflora

It is a soft-wooded tree belonging to the family Leguminoseae. Leaves are considered to be excellent sources of vitamin C and calcium.The antioxidant activity of ethanol and aqueous extracts of leaves of *Sesbania grandiflora* were determined by DPPH radical scavenging method and various other methods (Shyamala gowri and Vasantha, 2010)

Cucurbita maxima

It is studied for seed oil content and fatty acid composition. Oil content ranged from 10.9 to 30.9%. Total unsaturated fatty acid content ranged from 73.1 to 80.5%.These oils have 4 main fatty acids: Linoleic acid, C18:2 (49-69%); oleic acid, C18:1 (9-25%); stearic acid,C18:O (7-11%) and palmitic acid, C16:O (10-19%).The study showed potential for pumpkin seed oil to have high oxidative stability that would be suitable for food and industrial applications, as well as high unsaturation that could potentially improve the nutrition of human diets (Gajewski marek *et al.,* 2008)

Benincasa hispida

Winter melon (*Benincasa hispida*) is a vegetable crop, popular, especially among Asian communities both for nutritional and medicinal attributes. The oils were extracted and were analyzed for physicochemical parameters, fatty acids and sterols profiles. According to the GLC analysis linoleic acid (C18:2) was established to be the principal fatty acid (63.10-70.64%).Analysis of oil sterol fractions, using GC and GC-MS, revealed the presence of β-sitosterol (54.62–60.50%), campesterol (15.10–18.50%), stigmasterol (11.00–14.30% and Δ5-avenasterol (6.40–8.14 %) as the four main components.The seeds, which are under-utilized and often discarded as an agrowate, from winter melon should be explored for extraction of high-linoleic oil with additional tocopherols and phytosterol benefits (Raghu *et al.,* 2011).

Citrullus lanatus

The lipids content of *Citrullus lanatus* seeds were determined. Also the iodine value, saponfication value,acid value, free fatty acid, peroxide value, specific gravity and refractive index of the extracted oil were analysed.The lipid content was found to be 50.23%. The iodine value was 119.82. Saponfication value of *C. lanatus* was 189.35. The acid value was 5.120. The peroxide value was 7.51. Specific gravity and refractive indexes were found to be ranged between 0.91 – 0.93 (Naresh singh gill *et al.,* 2010)

Carica papaya

The antioxidant activity of the seeds of papaya was evaluated by 2,2 – diphenyl -1- picrylhydrazhyl (DPPH) method. This study was carried out to determine the total phenolic content (TPC). From the results, it showed that the ethanol extract had higher total phenolic content than the water extract. In *Carica papaya* value for ethanol extract (353.2 mg GAE/g) compared to water extract is (102.4 mg GAE/g). Ethanol extract showed the higher radical scavenging activity. The radical scavenging activities of *carica papaya* in ethanol extract was (83%) compared water extract was (82.7%) (Srikanth *et al.,* 2010).

Cucumis melo

The In Vitro antioxidant study was performed by 2,2 – diphenyl -1- picrylhydrazhyl (DPPH) method.The methanolic seed extract was found to have scavenging activity of 75.59% for 300ug/ml. Presence of phytochemicals like iterpenoids,alkaloids,tannins,flavonoids,carbhohydrates contribute to the observed antioxidant activity (Kaur Manpreet and Arora, 2011).

CHAPTER 3

MATERIALS AND METHODS

3.1 Chemicals used

1,1-Diphenyl-2-Picryl Hydrazyl (DPPH), methanol, ethanol, ferric chloride, phosphomolybdic acid, ammonia, chloroform, sulfuric acid, sodium hydroxide, hydrochloric acid, metallic zinc and magnesium, acetic acid, lead acetate, ammonium chloride, iodine, potassium iodide, pyridine, sodium nitroprusside, sodium bicarbonate, mercury, nitric acid, cupric acetate, potassium sodium tartarate, olive oil, ammonium molybdate, disodium hydrogen phosphate, sodium di hydrogen phosphate, potassium ferric cyanide, trichloro acetic acid (TCA), SDS, tannic acid, rutin, sodium nitrite, aluminium chloride from Himedia, Mumbai (India). All reagents used were of the analytical grade.

3.2 Plant Materials

The plant materials were collected from local market in Coimbatore (Tamil Nadu, India) during the months of December 2011. The name of the plants and plant parts used for the study are tabulated in Table 3.1

Table 3.1 Name of the plant and plant parts used

S.No	BOTANICAL NAME	VERNACULAR NAME	PLANT PART USED
1.	*Amaranthus gangetics*	Mulai keerai	Leaves
2.	*Basella alba*	Pasalai keerai	Leaves
3.	*Sloanum nigrum*	Manathakali keerai	Leaves
4.	*Sesbania grandiflora*	Agathi keerai	Leaves
5.	*Carica papaya*	Papaya	Seeds
6.	*Cucumis melo*	Muskmelon	Seeds
7.	*Citrullus lanatus*	Watermelon	Seeds
8.	*Benincasa hispida*	Ashgourd	Seeds
9.	*Cucurbita maxima*	Pumpkin	Seeds
10.	*Citrus sinensis*	Orange	Peels

3.3. Preparation of plant extracts

The leaves and seeds were shade-dried for around 5 days. The dried leaves and seeds were then crushed to make a coarse powder. Then two kinds of extraction process carried out.

3.3.1. Aqueous extraction

5g of dried powder was extracted separately with 100 ml water. The extracts were boiled for 1 hour in boiling mantle. Then filtered using whatman filter paper, onto the petri plates. Then the filtered extracts were evaporated at 40^0C for 2 days in hot air oven. Then scraped it by adding 3 ml of water and stored at 4^0c for further phytochemical and pharmacological screening.

3.3.2 Solvent extraction

5g of dried powder was extracted separately with 50 ml ethanol. The extracts were placed in shaker for 2 days in conical flasks. Then filtered using whatman filter paper, onto the petri plates. Then the filtered extracts were evaporated at room temperature for 2 days. Then scraped it by adding 3 ml of methanol and stored at 4^0C for further phytochemical and pharmacological screening.

3.4 Preliminary Phytochemical Analysis

Qualitative tests for the presence of plant secondary metabolites such as carbohydrates, alkaloids, tannins, flavonoids, saponins and glycosides and terpenoids, were carried out on the leaf powder using standard procedures (Chitravadivu *et al*., 2009) (Mariita *et al*., 2010).

3.4.1 Identification of Phenols by Ferric chloride test

2 ml of ferric chloride solution was added to 2 ml of the aqueous extract and formation of deep bluish green solution indicates the presence of phenols.

3.4.2 Identification of Phenols by Phosphomolybdic test

Phosphomolybdic acid reagent was added to the ethanolic extract and liquor ammonia. Appearance of blue colour indicates the presence of phenols.

3.4.3 Identification of Sterols and Steroids Salkowski test

The ethanolic extract was dissolved in chloroform and mixed with an equal volume of concentrated sulphuric acid. Appearance of red colour in the chloroform layer and green fluorescence in the acid layer indicates the presence of cholesterol.

3.4.4 Identification of Flavonoids by Decolourization test

The water extract of the sample was reduced to dryness in a boiling water bath. Then the residue was diluted with Naoh followed by addition of diluted HCL. A yellow solution with NaOH which turns colourless with dilute HCL confirms the presence of flavonoids.

3.4.5 Identification of Flavonoids by Ammonia test

The filter paper strips was dipped in the water and ethanolic extract and exposed to ammonia vapours. The change of the colour of the filter paper to yellow indicates the presence of flavonoids. 10 ml of the concentrated sulphuric acid was added to the above yellow coloured filter paper. Disappearance of the yellow colour indicates the presence of flavonoids.

3.4.6 Identification of Flavonoids by Lead acetate test

0.5% acetic acid was added to the equal volume of ethanolic extract and then filtered. 1 ml of 1% lead acetate was added to the filtrate. Flocculant white precipitate indicates the presence of flavonoids.

3.4.7 Identification of Flavonoids by Aluminium chloride test

2 drops of 1% aluminium chloride was added to 1 ml of the aqueous extract. Yellow coloration indicates the presence of flavonoids.

3.4.8 Identification of Tannins by Braemer's test

0.5g of ethanolic extract was dissolved in 10 ml of water and it was boiled and then filtered. Few drops of 10% ferric chloride were added to the filtrate. A dark green, blue or brown colour indicates the presence of tannin.

3.4.9 Identification of Alkaloids by Dragendorff's test

To 1 ml of the extract was added 1 ml of Dragendroff's reagent. Preparation of Dragendroff's reagent is given in Appendix 1. Appearance of orange precipitate indicates the presence of alkaloids.

3.4.10 Identification of Alkaloids by Wagner's test

The acid extract was treated with few ml of Wagner.s reagent. Reagent preparation is given in Appendix 2. Reddish brown precipitate indicates the presence of alkaloids.

3.4.11 Identification of Glycosides by Keller – Killani test

The ethanolic extract was dissolved in glacial acetic acid containing a trace of ferric chloride. Then dame amount of $FeCl_3$ dissolved in concentrated sulphuric acid was added along the sides of the test tube to settle at the bottom. Apearance of reddish brown colour changing to bluish green colour at the junction of two reagents within 2-5 minutes spreading slowly into the acetic acid layer confirms the presence of glycosides.

3.4.12 Identification of Glycosides by Legal's test

A few ml of ethanolic extract was dissolved in few drops of pyridine. Then a drop of 2% w/v sodium nitroprusside solution and a drop of 20% NaOH solution were added. Appearance of a pink or deep red colour indicates the presence of glycosides.

3.4.13 Identification of Saponins by Froth test

1g of the sample was dissolved 10 ml of sterile distilled water and boiled for 5 minutes. Then 2.5 ml of the filtrate added to 10 ml of sterile distilled water in a test tube and mixed vigorously for about 30 seconds, allowed to stand for half an hour and honeycomb froth indicates the presence of saponins. Then the froth was vigorously mixed with 3 drops of olive oil. Formation of emulsion indicates the presence of saponins.

3.4.14 Identification of Saponins by Sodium bicarbonate test

Few drops of sodium bicarbonate were added to few ml of the ethanolic extract and mixed well. The formation of honey comb indicates the presence of saponins.

3.4.15 Identification of Terpenoids by Terpenoids test

2 ml of chloroform was added to 0.5g of the sample. Then add 5 mla of concentrated sulphuric acid was added along the sides of the test tube. A reddish brown coleration in the interphase indicates the presence of terpenoid.

3.4.16 Identification of Proteins by Millon's test

5-6 drops of Millon's reagent was added to 2 ml of the filtrate of water extract. Preparation of reagent solution is explained in Appendix 3. Formation of red precipitate indicates the presence of proteins and free aminoacids.

3.4.17 Identification of Proteins by Bradford's test

Few drops of Bradford's reagent were added to 1 ml of the extract (Coomassie Brilliant Blue G 250) and formation of blue colour product indicates the presence of proteins. The procedure of Bradford's reagent preparation is given in Appendix 4.

3.4.18 Identification of Carbohydrates by Fehling's test

1 ml of Fehling's reagent (copper sulphate in alkaline conditions) was added to the filtrate of the extract in distilled water and heat in steam bath. The reagent preparation is given in Appendix 5. Brick red precipitate indicates the presence of carbohydrates.

3.4.19 Identification of Carbohydrates by Starch test

Few drops of iodine solution was added to 1 ml of the extract. Formation of blue colour indicates the presence of starch.

3.4.20 Identification of Carbohydrates by Cellulose test

1 ml of the extract was added to 2-3 drops of iodine solution followed by 2 drops of sulphuric acid. Apearance of dark/ deep brown/ chery red colour indicates the presence of cellulose.

3.5 Estimation of Total Phenols by Folin-Ciocalteau method (Singleton and rossi, 1965)

3.5.1 Principle

The hydroxyl (-OH) groups of phenolic compounds reduced the phosphomolybdic acid to molybdenum blue in the presence of an alkaline medium (present in Folin's reagent). The blue coloured complex was then spectrophotometrically measured at 650 nm.

3.5.2 Reagents

Stock solution for standard:

Dissolved 100mg of tannic acid in 100 ml of distilled water (1 ml=1mg).

Working standard solution:

10 ml of the stock solution was made upto 100 ml with distilled water (1 ml=100µg).

80% Ethanol

Dissloved 80 ml of absolute ethanol and made upto 100 ml with distilled water.

Folin-Ciocalteau reagent

Folin's reagent was mixed with distilled water in the ratio of 1:1. This reagent should be prepared freshly.

20% Sodium carbonate

Dissolved 20g of sodium carbonate in 100 ml distilled water.

3.5.3 Sample preparation

1. Weighed 1g of the sample and grind with 10 ml of 80% aqueous ethanol in a mortar and pestle.
2. Centrifuged at 6000 rpm for 10 minutes.
3. Obtained supernatant in a separate tube.
4. Re-extracted pellet with 5 ml ethanol.
5. Centrifuged again at 6000 rpm for 10 minutes.
6. Pooled the supernatant s and allowed to boiled to evaporate ethanol.
7. Dissolved residue in 10 ml of distilled water.

3.5.4 Procedure

1. Pipetted out 0.1,0.2,0.3,0.4 and 0.5 ml (concentration varying from 10 to 50µg) of the working standard solution into a series of test tubes.
2. Pipetted out 0.1 ml of the sample into a test tube.
3. To all the tubes, including the blank, distilled water was added to make up to 4 ml.
4. To all the tubes, added 0.5 ml of Folin's reagent.
5. The tubes were incubated at room temperature for 3 minutes.
6. Then added 2 ml of 20% sodium carbonate in all the test tubes.
7. Kept at boiling water bath for 1 minute.
8. The absorbance was read spectrophotometrically at 650 nm.

9. A graph was drawn by plotting the concentration of total phenolics along the X-axis and the optical density reading along Y-axis.
10. From the graph, the unknown sample concentration was calculated and the results were expressed in mg of tannic acid equivalents/g dry weight of extract.

3.5.5 Calculation

Optical density reading (y) corresponds to (a) µg of total phenolics.

i.e. 0.1 ml of unknown sample contains (a) µg of total phenolics.

Therefore, 100 ml of unknown sample contains (a*100)/(0.1*1000) mgTAE/gDW.

.

Reagents	Blank	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Plant extracts
Volume of Working standard (ml)	-	0.1	0.2	0.3	0.4	0.5	0.1
Concentration of working standard (µg/ml)	-	10	20	30	40	50	-
Volume of distilled water (ml)	3.0	2.9	2.8	2.7	2.6	2.5	2.9
Volume of Folin's phenol reagent (ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Incubation for 3 minutes at room temperature							
Volume of 20% sodium carbonate (ml)	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Incubation for 1 minute in boiling water bath							
OD value at 510 nm							

Table 3.2 Estimation of total phenols

3.6. Estimation of Flavonoids by Aluminium chloride method (Zhishen, Mengcheng, and Jianming, 1999)

3.6.1 Principle

Flavonoids present in the extract formed a charge transfer complex with several heavy metals to give a characteristic colour. In this reaction, the high electro positive nature of aluminium (AL^{3+}, Aluminium chloride) attracts the atomic nuclei of the aromatic rings in the flavonoids through the µ-electrons (Flavonoids) and creates a charge – transfer

resonance hybrid. This hybrid is highly stable in the aqueous medium, which then interacted with the sodium nitrite in an alkaline medium to form a pink coloured complex that is spectrophotometrically measured at 510 nm.

3.6.2 Reagents

Stock standard rutin solution:

Dissolved 100mg of rutin in 100 ml of 80% ethanol (1 ml=1mg).

Working standard solution:

Dissolved 10 ml of the stock solution in 100 ml of 80% ethanol (1 ml=1mg).

5% Sodium nitrite

Dissolved 5g of sodium nitrite in 100 ml of distilled water.

10% Aluminium chloride

Dissolved 10g of aluminium chloride in 100 ml of distilled water.

1M Sodium hydroxide

Dissolved 4g of sodium hydroxide in 100 ml distilled water.

3.6.3 Sample preparation

1. Weighed 1 g of the sample and grind with 10 ml of 80% aqueous methanol in a mortar and pestle.
2. Filtered ground sample with the help of Whatman filter paper.
3. Obtain clear sample solution to be used for experimental purpose,

3.6.4 Procedure

1. Pipetted out 0.5,1.0,1.5,2.0 and 2.5 ml (concentration varying from 50 to 250µg) of the standard solution into a series of test tubes.
2. Pipetted out 0.1 ml of the sample into a test tube.
3. To all the tubes, including the blank, distilled water was added to make up to 2.5 ml.
4. To all the tubes, added 75 µl of 5% sodium nitrite and incubated at room temperature for 5 minutes.
5. Added 150 µl of 10% aluminium chloride and incubated at room temperature for 6 minutes.
6. Then added 0.5 ml of 1M sodium hydroxide, mixed well and the pink colour formed was spectrophotometrically measured at 510 nm.
7. A graph was drawn by plotting the concentration of rutin along the X-axis and the optical density reading along Y-axis.

8. From the graph, the unknown sample concentration was calculated and the results were expressed as mg of rutin equivalents/g dry weight of extract.

3.6.5 Calculation

Optical density reading (y) corresponds to (a) µg of rutin.

i.e. 0.1 ml of unknown sample contain (a) µg of rutin.

Therefore, 100 ml of unknown sample contains (a*100)/(0.1*1000) mgRE/g DW.

Reagents	Blank	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Plant extracts
Volume of Working standard (ml)	-	0.5	1.0	1.5	2.0	2.5	0.1
Concentration of working standard (µg/ml)	-	50	100	150	200	250	-
Volume of distilled water (ml)	2.5	2.0	1.5	1.0	0.5	-	2.4
Volume of 5% sodium nitrite (µl)	75	75	75	75	75	75	75
Incubation for 5 minutes at room temperature							
Volume of 10% aluminium chloride (µl)	150	150	150	150	150	150	150
Incubation for 6 minutes in room temperature							
Volume of sodium hydroxide (ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
OD value at 510 nm							

Table 3.3 Estimation of total flavonoids

3.7 Estimation of Total Antioxidant Capacity

3.7.1 Principle

Phosphomolybdenum assay used to determine the total antioxidant capacity was based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/ Mo (V) complex at acidic pH.

3.7.2 Materials

- Standard ascorbic acid – stock: 10 mg in 1ml distilled water.

- Working standard: 100µl of stock diluted to 900µl with water.
- Sulphuric acid (0.6M): 5.88 ml of H_2So_4 mixed in 94.12 ml of water.
- Ammonium molybdate (0.4mM): 0.49g dissolved in 100 ml water.
- Disodium hydrogen phosphate (28mM): 0.397g dissolved in 100 ml water.

3.7.3 Procedure

The method described by Prieto et al was used to determine the total antioxidant capacity of the extract. The tubes containing 0.2 ml of plant leaves extract, 2 ml of phosphor molybdenum reagent solution (0.6M Sulphuric acid, 28mM Sodium phosphate and 4mM Ammonium molybdate) were incubated at 95^0c for 90 minutes. After the mixture had cooled to room temperature the absorbance was measured at 695 nm using UV/VIS Spectrophotometer. The antioxidant capacity was expressed as ascorbic acid equivalent (Kumar et al., 2008).

3.7.4 Calculation

Total antioxidant activity is calculated by following formula

Ascorbic acid equivalent (µM/g) = (T/S)*C*(V/P)*(RS/E)*(1*MW)

T – OD of test solution.

S – OD of standard.

C – Concentration of test (µg).

V – Volume of solvent used for extraction (ml).

P – Amount of powder (g).

RS – Volume of reagent solution (ml).

E – Volume of extract (ml).

MW – Molecular weight of ascorbic acid (176-13 g/g mol).

Concentration (µg/ml)	20	40	60	80
Standard ascorbic acid (S)/ Plant extract (T) (ml)	0.2	0.2	0.2	0.2
Reagent solution (ml)	2.0	2.0	2.0	2.0
Incubation at 95^0C for 90 minutes				
OD value at 695 nm				
Ascorbic acid equivalent (µM/g)				

Table 3.4 Estimation of total antioxidant capacity

3.8 Estimation of Free Radical Scavenging Activity by DPPH Method (Yamaguchi *et al.*, 2008)

3.8.1 Principle

The DPPH free radical method is based on the determination of the concentration of 2,2-Diphenyl-1-Picryl Hydrazyl (DPPH) at steady state in a methanol solution, after adding the mixture of antioxidants. DPPH absorbs at 517 nm, and as its concentration is reduced by the existence of an antioxidant, the absorption gradually disappears with time.

3.8.2 Materials

- Standard ascorbic acid – stock: 10 mg in 1 ml distilled methanol
- Working Standard: 100µl of stock diluted to 900µl with methanol.
- Plant extract: extract was dissolved in methanol.
- DPPH (0.6mM): 0.0315g of DPPH dissolved in 100 ml methanol.

3.8.3 Procedure

3 ml of DPPH solution was mixed with 1 ml of samples of varying concentration (1-10mg/ml). The solution in the test tubes was vortexes and incubated in the dark for 30 minutes at room temperature. The decrease in the absorbance was measured at 517 nm. The control test tube contains equal volume of DPPH in methanol instead of extract (Shimada et al., 1992).

3.8.4 Calculation

The DPPH radical-scavenging activity in terms of percentage was calculated according to the following equation.

DPPH scavenging activity (%) =[(Acontrol – Asample) /Acontrol]*100%

Concentration of ascorbic acid/ plant extract (µg/ml)	**Volume of plant exract (ml)**	**Volume of DPPH (ml)**	**Incubate in dark for 30 minutes at room temperature**	**OD value at 517 nm**	**% Inhibition**
50	1	3			
100	1	3			
150	1	3			
200	1	3			
250	1	3			

Table 3.5 Estimation of free radical scavenging activity by DPPH method

3.9 Estimation of ABTS Cation Radical Scavenging Assay (Sanchez- Moreno, 2002)

3.9.1 Principle

The ABTS (2,2- azobis 3- ethylbenzothiazoline-6- sulfonic acid diammonium salt) assay is based on the inhibition of the absorbance of ABTS radical cation, which has a charesteric long wavelength absorption spectrum (Baskar *et al.*, 2007).

3.9.2 Reagents

1. ABTS (7 mM)
2. Ammonium persulfate (2.45 mM)
3. Methanol

3.9.3 Procedure

1. ABTS radical was produced by reacting ABTS solution (7 mM) with ammonium persulfate (2.45 mM).
2. The mixture is allowed to stand in the dark room temperature for 12-16 hours to give a dark colourd solution.
3. The absorbance was measured at 745 nm, this stock solution was diluted with methanol to give a final absorbance value of around 0.7 (+ or – 0.02) and equilibriated at 30^0C.
4. Different concentrations of the sample (50-250 µg/ml) were prepared by dissolving the extracts in water.
5. About 0.3 ml of the sample was mixed with 3 ml of ABTS working standard in a microcuvette.
6. The decrease in the absorbance was measured at 745 nm after mixing the solution in uniform time interval 3 minutes.
7. A solution of ABTS working standard and 0.3 ml of methanol was used as the control and about 3 ml of methanol was used as blank.

3.9.4 Calculation

The ABTS cation radical-scavenging activity in terms of percentage was calculated according to the following equation.

ABTS cation radical scavenging activity (%) =[(Acontrol – Asample) /Acontrol]*100%

Concentration of plant extract (µg/ml)	**Volume of plant exract (ml)**	**Volume of ABTS (ml)**	**OD value at 745 nm**	**% Inhibition**
50	0.3	3		
100	0.3	3		
150	0.3	3		
200	0.3	3		
250	0.3	3		

Table 3.6 Estimation of ABTS Cation Radical Scavenging Assay

3.10 Ferric ion reducing antioxidant power (FRAP) assay (Bharathi kumar et al., 2008)

3.10.1 Principle

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. In this method the Fe^{3+} is reduced to Fe^{2+} in the presence of antioxidants (Reductants) in the extracts. The blue colour formed is colorimetrically measured at 700 nm. The increase in the absorbance is directly proportional to the concentration of total antioxidants present in the sample.

3.10.2 Reagents

Sample preparation

Five gram of dried leaves and seeds (fresh leaves and seeds were air dried in the incubator at 37^0c for two days) was weighed and mixed with 50 ml of water in a conical flask. It was then incubated at room temperature in orbital shaker over night. The contents were filtered with whatman filter paper or centrifuged and the filtrate was collected. The solvent in the filtrate was evaporated and 50 mg of the dried powder was dissolved in 50 ml of distilled water. From this, the samples have taken for experimental analysis.

0.2M Phosphate buffer (pH 6.6)

Weighed 35.6g/L of disodium hydrogen phosphate (A) and 31.2 g/L of sodium dihydrogen phosphate (B). Mixed 26.5 ml of (A) and 73.5 mlof (B) and made upto 200 ml with distilled water and adjusted the pH to 6.6.

1% Potassium ferricyanide

Dissolved 1g of potassium ferricyanide in 100 ml of distilled water.

10% TCA

Dissolved 10g of trichloro acetic acid in 100 ml of distilled water.

0.1% Ferric chloride

Dissolve 0.1g of ferric chloride in 100 ml of distilled water.

3.10.3 Procedure

1. To 2.5 ml of the extract added 2.5 ml phosphate buffer (0.2M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide.
2. The mixture is boiled in a water bath at 50^0c for 20 minutes, then rapidly cooled, mixed with 2.5 ml of 10% trichloro acetic acid.
3. Centrifuged at 3000rpm for 10 minutes.
4. From this, pipette out 2.5 ml of supernatant, 2.5 ml distilled water and 0.5 ml of 0.1% ferric chloride.
5. Mixed well and allowed to stand for 10 minutes.
6. The increase in the absorbance at 700 nm was used to measure the reducing power of the plant extract.

Reagents	*Amaranthus gangetics*	*Basella alba*	*Solanum nigrum*	*Carica papaya*	*Citrullus lanatus*	*Citrus sinensis*
Volume of plant extracts (ml)	2.5	2.5	2.5	2.5	2.5	2.5
Volume of phosphate buffer (0.2 M, pH 6.6) (ml)	2.5	2.5	2.5	2.5	2.5	2.5
Volume of 1% potassium ferricyanide (ml)	2.5	2.5	2.5	2.5	2.5	2.5
Incubation for 20 minutes at 50^{0C}						
Volume of 10% trichloroacetic acid	2.5	2.5	2.5	2.5	2.5	2.5
Centrifugation at 3000 rpm for 10 minutes						
Volume of supernatant (ml)	2.5	2.5	2.5	2.5	2.5	2.5
Volume of distilled water (ml)	2.5	2.5	2.5	2.5	2.5	2.5
Volume of 0.1% ferric chloride (ml)	0.5	0.5	0.5	0.5	0.5	0.5
Mix and incubate at room temperature for 10 minutes						
OD value at 700 nm						

Table 3.7 Ferric ion reducing antioxidant power (FRAP) assay

3.11 Modified Ferric Ion Reducing/Antioxidant Power Assay (Modified FRAP Assay) (Apak et al., 2007)

3.11.1 Principle

The antioxidant present in the sample reduced the oxidant probe and the respective product interacted with some colouring agents to form a coloured complex. In this method, the antioxidants reduced the Fe^{3+} to Fe^{2+}. This ion then conjugated with the ferricyanide ion to form a Prussian blue coloured product, which was spectrophotometrically measured at 700 nm. The presence of SDS prevents the formation of turbidity in the solution.

3.11.2 Reagents

96% Ethanol

Dissolved 96 ml of absolute ethanol and made upto 100 ml with distilled water.

1% Potassium ferricyanide

Dissolved 1g of potassium ferricyanide in 100 ml of distilled water.

1M HCL

Dissolved 8.3 ml of concentrated HCL and made upto 100 ml with distilled water.

0.2% Ferric chloride

Dissolved 0.2g of ferric chloride in 100 ml of distilled water.

1% SDS

Dissolved 1g of sodium dodecyl sulphate in 100 ml of distilled water.

3.11.3 Procedure

1. To 0.1 ml of the extract added 0.9 ml of 96% ethanol, 5 ml of distilled water, 1.5 ml of 1M HCL, 1.5 ml of 1% potassium ferricyanide, 0.5 ml of 1% SDS and 0.2% ferric chloride.
2. The mixture is boiled in a water bath at 50^0c for 20 minutes, rapidly cooled and mixed well.
3. The increase in the absorbance at 750 nm was used to measure the reducing power of the plant extract.

Reagents	*Amaranthus gangetics*	*Basella alba*	*Carica papaya*	*Citrus sinensis*
Volume of plant extract (ml)	0.1	0.1	0.1	0.1
Volume of 96% ethanol (ml)	0.9	0.9	0.9	0.9
Volume of distilled water (ml)	5	5	5	5
Volume of 1M HCL (ml)	1.5	1.5	1.5	1.5
Volume of potassium ferricyanide (ml)	1.5	1.5	1.5	1.5
Volume of 1% SDS	0.5	0.5	0.5	0.5
Volume of 0.2% ferric chloride	0.5	0.5	0.5	0.5
Incubation for 20 minutes at 50^0C and rapidly cool				
OD value at 750 nm				

Table 3.8 Modified Ferric Ion Reducing/Antioxidant Power Assay

CHAPTER 4

RESULTS AND DISCUSSION

Some of the green leafy vegetables are under exploited, but are potent sources of natural antioxidants like vitamins, carotenoids, flavonoids, phenols, etc. The selected plant species include four green leafy vegetables Amaranthus gangetics, *Basella alba*, *Solanum nigrum, Sesbanic grandiflora* five unutilized seeds *Carica papaya*, *Cucumis melo*, *Citrullus lanatus*, *Benincasa hispida*, *Cucurbita maxima* and unutilized peels of *Citrus sinensis* has a potent antioxidant activity and its beneficial effects have been proved by experiments So natural antioxidans play a very important role in the protection of the body against various diseases.

The results obtained from the experiments conducted to the screening of commonly consumed green leafy vegetables and unutilized fruit parts for antioxidants status and the level of total phenols and flavonoids present in these plants are presented in this chapter. The results of the present investigation are also discussed on the basis of available information in the literature.

4.1 Qualitative phytochemical analysis of plant extracts

The preliminary phytochemical analysis of plant extracts was carried out and the phytochemicals present in the selected four green leafy vegetables and six unutilized fruit parts. A systematic survey of green leafy vegetables twelve commonest ones out of the twenty nine green leafy vegetables screened for phytochemicals (Mensah *et al*., 2008). The quantitative analyses of *Talinum triangulare* revealed the presence of bioactive compounds namely flavonoids, phenols, alkaloids, saponins (Aja *et al*., 2010). The result for the phytochemicals present in the plant extracts shown in Table 4.1.

Table 4.1 Preliminary phytochemical analysis of plant extracts

4.2 Estimation of total phenols

SAMPLE NAME	PHENOLS	FLAVONOIDS	SAPONINS	TERPENOIDS
Amaranthus gangetics	+	+	+	+
Basella alba	+	+	+	+
Solanum nigrum	+	+	+	+
Sebania grandiflora	+	+	+	+
Carica papaya	+	+	+	+
Cucumis melo	+	+	+	+
Citrullus lanatus	+	+	+	+
Benincasa hispida	+	+	+	+
Cucurbita maxima	+	+	+	+
Citrus sinensis	+	+	+	+

The amount of total phenols in extract was determined with Folin– Ciocalteu reagent according to the method of Singleton and Rossi (1965). The antioxidative properties of some vegetables and fruits are partly due to the low molecular weight phenolic compounds, which are known to be potent as antioxidants (Huda et al, 2009). The standard values are shown in Table 4.2 and Figure 4.2.

4.2.1 Green leafy vegetables

The results obtained indicate the level of total phenols is highest in *Sesbania grandiflora* having total phenol content of 28.18 mg TAE/ gDW**.** This suggests total phenols may be responsible for the free radical scavenging activity of the plant On the other hand, *Amaranthus gangetics* has the lowest level of phenols having total phenol content of 17.24

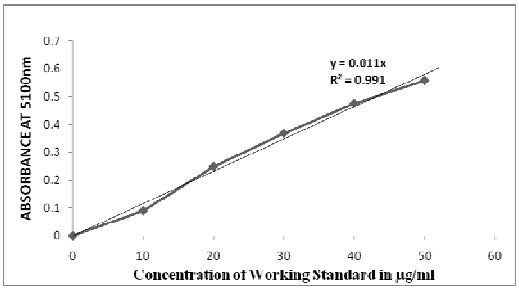

Figure 4.2 Standard graph for estimation of total phenols

Table 4.2.1 Levels of total phenols present in Green leafy vegetables

Species	OD value at 510 nm	Total phenols (mgTAE/gDW)
A.g	0.189	17.245
B.a	0.243	22.172
S.n	0.205	18.672
S.g	0.310	28.181

A.g: *Amaranthus grandiflora*; **B.a:** *Basella alba*; **S.n:** *Solanum nigrum*; **S.g:** *Sesbania grandiflora*

TAE: Tannic acid equivalents

DW: Dry weight

mg TAE/ g DW. The level of total phenols in *Sesbania grandiflora* has been reported as having 3.01 mg TAE/ g DW for ethanol extracts (Shyamala gowri and Vasantha, 2010*)*. The levels of total phenols present in Green leafy vegetables shown in Table 4.2.1 and Figure 4.2.1

4.2.2 Unutilized fruit parts

The results obtained indicate the level of total phenols is highest in *Citrus sinensis* having total phenol content of 32.236 mg GAE/100 g**.** This suggests total phenols may be responsible for the free radical scavenging activity of the plant On the other hand, *Cucumis melo* has the lowest level of phenols having total phenol content of 9.581 mg TAE/ g DW. The amount of total phenols in *Citrus sinensis* peels has been reported as 285.5 mg TAE/100 g DW (Zamantha Escobedo-Avellaneda *et al*., 2009). The levels of total phenols present in unutilized fruit parts shown in Table 4.2.2 and Figure 4.2.2.

Table 4.2 Standard values for estimation of total phenols

Standard concentration (µg/ml)	OD value at 510 nm
10	0.09
20	0.249
30	0.368
40	0.476
50	0.558

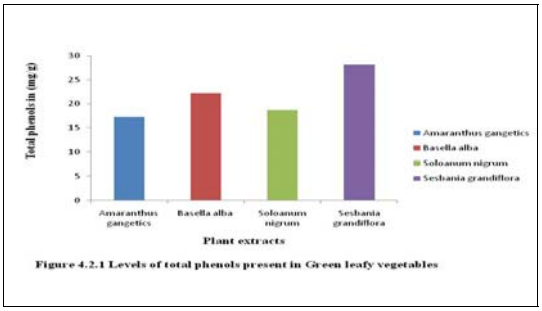

Figure 4.2.1 Levels of total phenols present in Green leafy vegetables

Table 4.2.2 Levels of total phenols present in unutilized fruit parts

Species	OD value at 510 nm	Total phenols (mgTAE/gDW)
C.p	0.112	10.218
C.m	0.105	9.581
C.l	0.118	10.754
B.h	0.115	10.490
C.max	0.192	17.472
C.s	0.354	32.236

C.p: *Carica papaya*; **C.m:** *Cucumis melo*; **C.l:** *Citrullus lanatus*; **B.h:** *Benincasa hispida*; **C.max:** *Cucurbita maxima*; **C.s:** *Citrus sinensis*

TAE: Tannic acid equivalents

DW: Dry weight

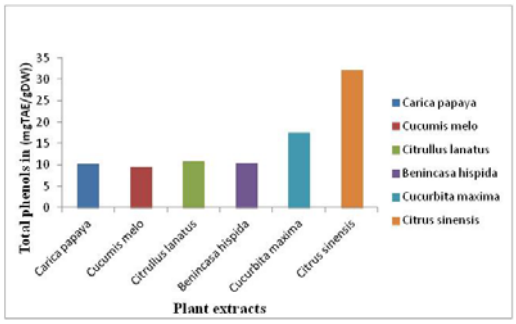

Figure 4.2.2 Levels of total phenols present in unutilized fruit parts

4.3 Estimation of total flavonoids

Flavonoids are large compounds occurring in all plants. They occur as glycosides and contain several phenolic hydroxyl groups on their ring structures. Many flavonoids are found to be very good free radical scavengers and antioxidants (Scott and Slater, 1981; Robak and Gryglewski, 1988). Food-derived flavonoids such as the flavonols like Quercetin, Myrecetin have been reported to reduce the risk of cancer (Verma *et al*., 1988). Flavonols are known to act as both free radical scavengers and also as metal chelators. Hence they can play a crucial role in the prevention of many deadly diseases. The standard values are shown in Table 4.3 and Figure 4.3

4.3.1 Green leafy vegetables

The results obtained indicate the levels of Flavonoids are the highest in *Sesbania grandiflora* of 13.54 mg/g. This suggests that flavonoids may be responsible for the free radical scavenging activity of the plant. On the other hand, *Basella alba* has the lowest levels of flavonoids of 5.7 mg RE/g DW, it might be inferred that the former plays a major role in the plant's antioxidant activity. The amount of total flavonoids in *Basella alba* has been reported as 26.53 mg RE/ g DW (Olajire and Azeez 2011). The levels of total flavonoids present in Green leafy vegetables shown in Table 4.3.1 and Figure 4.3.1.

4.3.2 Unutilized fruit parts

The results obtained indicate the levels of Flavonoids are the highest in *Carica papaya* of 30.25 mg RE/ g DW**.** This suggests that flavonoids may be responsible for the free radical scavenging activity of the plant. On the other hand, *Citrus sinensis* has the lowest levels of flavonoids of 8.39 mg RE/ g DW, it might be inferred that the former plays a major role in the plant's antioxidant activity. The amount of total flavonoids in *Carica papaya* has been reported as

22.47 mg RE/ g DW (Kaibing Zhou *et al.*, 2011). The amount of total flavonoids in *Citrus sinensis* has been reported as 2.99 mg RE/ g DW. The levels of total phenols present in unutilized fruit parts shown in Table 4.3.2 and Figure 4.3.2.

Table 4.3 Standard values for estimation of total flavonoids

Standard concentration (µg/ml)	OD value at 510 nm
50	0.097
100	0.185
150	0.206
200	0.282
250	0.351

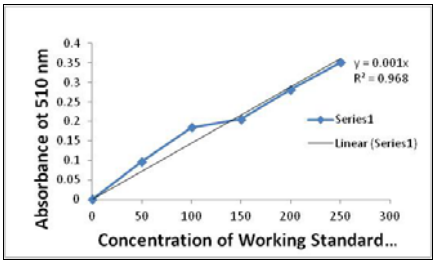

Figure 4.3 Standard graph for estimation of total flavonoids

Table 4.3.1 Levels of flavonoids present in Green leafy vegetables

Species	OD value at 510 nm	Total flavonoids (mgRE/gDW)
A.g	0.013	13.300
B.a	0.005	5.700
S.n	0.008	8.920
S.g	0.013	13.540

A.g: *Amaranthus grandiflora*; **B.a:** *Basella alba*; **S.n:** *Solanum nigrum*;
S.g: *Sesbania grandiflora*
RE: Rutin equivalents
DW: Dry weight of extract

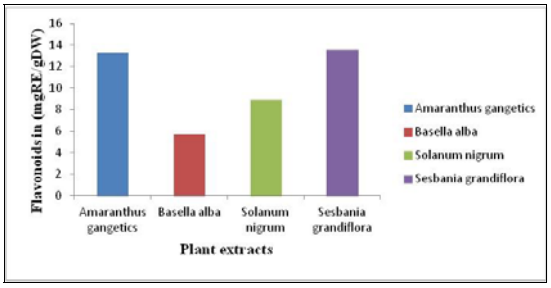

Figure 4.3.1 Levels of flavonoids present in Green leafy vegetables

Table 4.3.2 Levels of flavonoids present in unutilized fruit parts

Species	OD value at 510 nm	Total flavonoids (mgRE/gDW)
C.p	0.030	30.250
C.m	0.013	13.010
C.l	0.010	10.280
B.h	0.011	11.220
C.max	0.018	18.610
C.s	0.008	8.390

C.p: *Carica papaya*; **C.m:** *Cucumis melo*; **C.l:** *Citrullus lanatus*; **B.h:** *Benincasa hispida*; **C.max:** *Cucurbita maxima*; **C.s:** *Citrus sinensis*
RE: Rutin equivalents
DW: Dry weight of extract

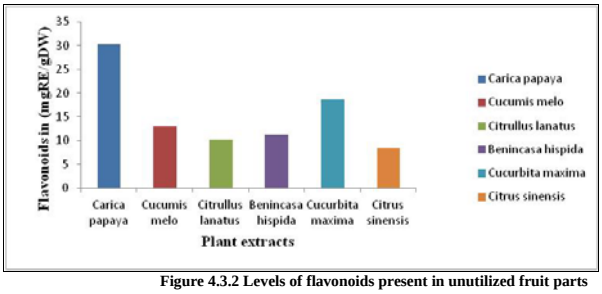

Figure 4.3.2 Levels of flavonoids present in unutilized fruit parts

4.4 Total antioxidant capacity assay

The total antioxidant assay gives an estimate of the overall antioxidant potential of the plant. There is a formation of phosphomolybdenum complex the intensity of which indicates the potential of the plant as a scavenger of free radicals (Kumar et al., 2008). The standard values for ethanol extracts are shown in Table 4.4.1 and Figure 4.4.1.

4.4.1 Green leafy vegetables

Total antioxidant capacity of Ethanol extracts

The results obtained indicate that the total antioxidant capacity of *Sesbania grandiflora* is highest of 6.009 (µM/g). On the other hand, *Basella abla* has the lowest of 1.914 (µM/g). The total antioxidant capacity of *Basella alba* has been reported as 4.74 (µM/g) (Olajire and Azeez 2011). The total antioxidant capacity of Ethanol extracts of Green leafy vegetables shown in Table 4.4.1 and Figure 4.4.1.

Total antioxidant capacity of Aqueous extracts

The results obtained indicate that the total antioxidant capacity of *Amaranthus gangetics* is highest of 4.458 (µM/g). On the other hand, *Basella abla* has the lowest of 0.669 (µM/g). The total antioxidant capacity of Ethanol extracts of Green leafy vegetables shown in Table 4.4.2 and Figure 4.4.2.

4.4.2 Unutilized fruit parts

Total antioxidant capacity of Ethanol extracts

The results obtained indicate that the total antioxidant capacity of *Citrus sinensis* is highest of 10.23 (µM/g). On the other hand, *Carica papaya* has the lowest of 0.582 (µM/g). The total antioxidant capacity of *Carica papaya* has been reported as 2.08 (µM/g) (Kaibing Zhou *et al.*, 2011). The total antioxidant capacity of Ethanol extracts of Unutilized fruit parts shown in Table 4.4.3 and Figure 4.4.3.

Total antioxidant capacity of Aqueous extracts

The results obtained indicate that the total antioxidant capacity of *Citrus sinensis* is highest of 4.337 (µM/g). On the other hand, *Cucumis melo* has the lowest of 0.619 (µM/g). The total antioxidant capacity of *Carica papaya* has been reported as 0.29 (µM/g) (Kaibing Zhou *et al.*, 2011). The total antioxidant capacity of Aqueous extracts of Unutilized fruit parts shown in Table 4.4.4 and Figure 4.4.4.

Table 4.4 Standard values for total antioxidant capacity

Concentration of standard (µg/ml)	OD values at 695 nm
20	0.119
40	0.173
60	0.237
80	0.283
100	0.424
120	0.496
140	0.567
160	0.664
180	0.730

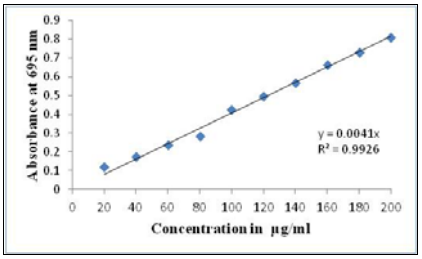

Figure 4.4 Standard graph for total antioxidant capacity

Table 4.4.1 Total antioxidant capacity of Ethanol extracts of Green leafy vegetables

Species	OD value at 695 nm for 20 µg/ml			Mean±Standard deviation	Ascorbic acid equivalents (µM/g)
	R1	R2	R3		
A.g	0.448	0.386	0.456	0.430±0.038	2.139
B.a	0.394	0.362	0.418	0.391±0.028	1.914
S.n	0.636	0.557	0.662	0.618±0.055	3.072
S.g	1.300	1.317	1.306	1.308±0.009	6.009

A.g: *Amaranthus grandiflora*; **B.a:** *Basella alba*; **S.n:** *Solanum nigrum*; **S.g:** *Sesbania grandiflora*

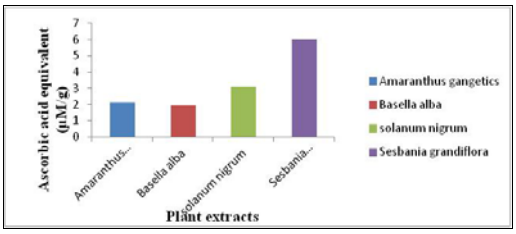

Figure 4.4.1 Total antioxidant activity of Ethanol extracts of Green leafy vegetables

Table 4.4.2 Total antioxidant capacity of Aqueous extracts of Green leafy vegetables

Species	OD value at 695 nm for 20 µg/ml			Mean±Standard deviation	Ascorbic acid equivalents (µM/g)
	R1	R2	R3		
C.p	0.103	0.116	0.128	0.116±0.013	0.582
C.m	0.157	0.125	0.179	0.154±0.027	0.822
C.l	0.299	0.376	0.381	0.352±0.046	1.817
B.h	0.244	0.218	0.238	0.233±0.014	1.126
C.max	0.414	0.534	0.540	0.363±0.045	1.857
C.s	2.215	2.216	2.115	2.182±0.058	10.230

A.g: *Amaranthus grandiflora*; **B.a:** *Basella alba*; **S.n:** *Solanum nigrum*; **S.g:** *Sesbania grandiflora*

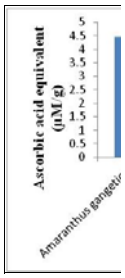

Figure 4.4.2 Total antioxidant capacity of Aqueous extracts of Green leafy vegetables

Table 4.4.3 Total antioxidant capacity of Ethanol extracts of Unutilized fruit parts

Species	OD value at 695 nm for 20 µg/ml			Mean±Standard deviation	Ascorbic acid equivalents (µM/g)
	R1	R2	R3		
C.p	0.153	0.189	0.174	0.172±0.018	0.868
C.m	0.100	0.135	0.121	0.119±0.018	0.619
C.l	0,410	0.383	0.372	0.378±0.008	1.861
B.h	0.586	0.288	0.298	0.391±0.169	2.554
C.max	0.439	0.194	0.215	0.285±0.136	1.908
C.s	0.946	0.929	0.892	0.922±0.028	4.337

C.p: *Carica papaya*; **C.m:** *Cucumis melo*; **C.l:** *Citrullus lanatus*; **B.h:** *Benincasa hispida*; **C.max:** *Cucurbita maxima*; **C.s:** *Citrus sinensis*

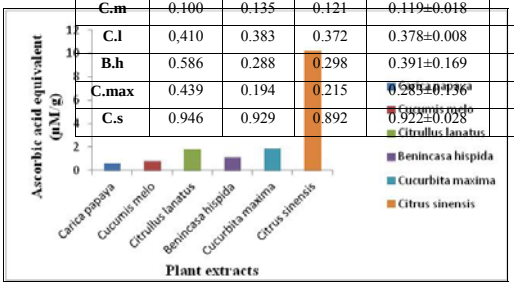

Figure 4.4.3 Total antioxidant capacity of Ethanol extracts of Unutilized fruit parts

Table 4.4.4 Total antioxidant capacity of Aqueous extracts of Unutilized fruit parts

C.p: *Carica papaya*; **C.m:** *Cucumis melo*; **C.l:** *Citrullus lanatus*; **B.h:** *Benincasa hispida*; **C.max:** *Cucurbita maxima*; **C.s:** *Citrus sinensis*

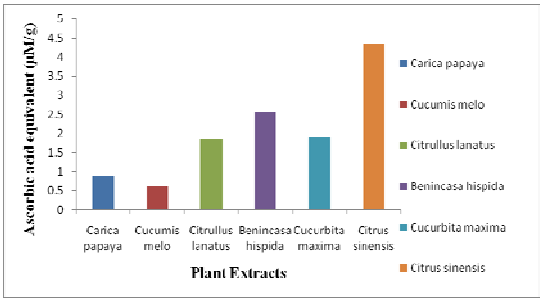

Table 4.4.4 Total antioxidant capacity of Aqueous extracts of Unutilized fruits parts

4.5 DPPH radical scavenging activity

DPPH is a relatively stable free radical. The assay is based on the measurements of the antioxidants' ability to scavenge the stable radical DPPH. DPPH radicals react with suitable reducing agents. The electrons become paired off and the solution loses color stoichiometrically depending on the number of electrons taken up.

The proton radical scavenging action is known as an important mechanism of auto-oxidation. DPPH was used to determine the proton radical scavenging action of the methanol and acetone fractions of the plants. It shows a characteristic absorbance at 517 nm. The purple color of the DPPH solution fades rapidly when it encounters proton radical scavengers (Yamaguchi *et al*., 1998). The standard values for DPPH free radical scavenging activity shown in Table 4.5 and Figure 4.5.

4.5.1 Green leafy vegetables

The values across the concentrations indicate that the ethanol extracts of the plant *Solanum nigrum* shows more potent in neutralizing DPPH free radical. The ethanol extract shows an inhibition of 64-82 % in the concentration range of 50-250 µg/ml, while the ethanol extracts of *Basella alba* shows least inhibition of 25-50 % in the concentration range of 50-250 µg/ml. The free radicals scavenging activity of *Sesbania grandiflora* has been reported as 18.03%-53.12% for the concentration range (50-250) µg/ml (Shyamala gowri and Vasantha, 2010). The free radicals scavenging activity of DPPH of Ethanol extracts of Green leafy vegetables shown in Table 4.5.1 and Figure 4.5.1.

4.5.2 Unutilized fruit parts

The values across the concentrations indicate that the ethanol extracts of the plant *Citrus sinensis* shows more potent in neutralizing DPPH free radical. The ethanol extract shows an inhibition of 56-84 % in the concentration range of 50-250 µg/ml, while the ethanol extracts of *Benincasa hispida* shows least inhibition of 4-26 % in the concentration range of 50-250 µg/ml. The free radicals scavenging activity of Citrus sinensis has been reported as 34-92 % in the concentration range of 50-250 µg/ml (Mohammed Fadlinizal Abd Ghafar *et al.,* 2010) The free radicals scavenging activity of DPPH of Ethanol extracts of Unutilized fruit parts shown in Table 4.5.2 and Figure 4.5.2.

Table 4.5 Standard values for free radical scavenging activity of DPPH

Concentration of standard (µg/ml)	OD value at 517 nm
10	3.165
20	2.96
30	2.791
40	2.484
50	2.204
60	1.492
70	1.67
80	1.304
90	0.984
100	0.735

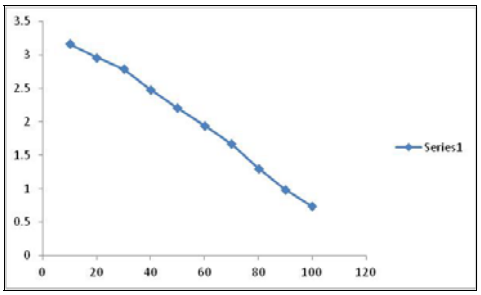

Figure 4.5 Standard graph for free radical scavenging activity of DPPH

Table 4.5.1 Free radicals scavenging activity of DPPH of Ethanol extracts of Green leafy vegetables

Concentration of *Amaranthus gangetics* (µg/ml)	OD value at 517 nm			Standard deviation	Mean±Standard deviation	% Inhibition
	R1	R2	R3			
50	2.013	1.922	2.116	0.123	2.017±0.097	42.818
100	1.987	1.715	1.907	0.139	1.870±0.140	46.225
150	1.712	1.631	1.654	0.041	1.666±0.042	54.383
200	1.523	1.457	1.479	0.033	1.486±0.034	59.392
250	1.311	1.256	1.386	0.065	1.318±0.065	63.053
Concentration of *Basella abla* (µg/ml)						
50	2.632	2.783	2.548	0.119	2.654±0.119	25.903
100	2.312	2.324	2.496	0.102	2.377±0.103	33.726
150	2.301	2.086	2.269	0.124	2.219±0.116	39.800
200	1.921	2.086	2.003	0.043	2.003±0.083	46.198
250	1.589	1.987	1.846	0.149	1.807±0.202	50.790
Concentration of *Solanum nigrum* (µg/ml)						
50	1.321	1.231	1.267	0.045	1.273±0.045	64.779
100	1.231	1.097	1.149	0.067	1.159±0.068	67.230
150	1.151	0.915	1.086	0.121	1.051±0.122	68.672
200	0.921	0.753	0.963	0.111	0.879±0.111	73.547
250	0.645	0.532	0.568	0.057	0.582±0.058	82.917
Concentration of *Sesbania grandiflora* (µg/ml)						
50	1.312	1.640	1.218	0.221	1.390±0.222	56.944
100	1.423	1.321	1.168	0.128	1.304±0.128	61.732
150	1.215	1.089	1.036	0.091	1.113±0.092	67.799
200	1.015	0.771	0.985	0.133	0.924±0.133	71.713
250	0.816	0.568	0.759	0.129	0.714±0.130	77.445

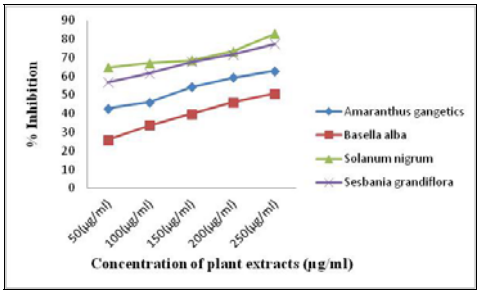

Figure 4.5.1 Free radicals scavenging activity of DPPH of Ethanol extracts of Green leafy vegetables

Table 4.5.2 Free radicals scavenging activity of DPPH of Ethanol extracts of Unutilized fruit parts

Concentration of *Carica papaya* (µg/ml)	OD value at 517 nm			Standard deviation	Mean±Standard deviation	% Inhibition
	R1	R2	R3			
50	2.915	3.047	2.915	0.076	2.959±0.076	18.909
100	2.645	2.869	2.741	0.112	2.752±0.112	23.482
150	2.410	2.531	2.432	0.064	2.458±0.064	32.617
200	2.152	2.317	2.118	0.106	2.196±0.106	38.492
250	2.065	2.118	2.019	0.049	2.067±0.050	43.444
Concentration of *Cucumis melo* (µg/ml)						
50	2.651	2.891	2.851	0.128	2.798±0.129	21.820
100	2.529	2.741	2.658	0.106	2.643±0.107	26.543
150	2.431	2.678	2.531	0.124	2.547±0.124	28.642
200	2.152	2.312	2.329	0.097	2.264±0.098	36.895
250	1.987	2.213	2.191	0.124	2.130±0.125	39.755
Concentration of *Citrullus lanatus* (µg/ml)						
50	2.815	2.291	2.872	0.320	2.659±0.320	20.395
100	2.781	2.563	2.658	0.109	2.667±0.109	25.817
150	2.431	2.172	2.315	0.129	2.306±0.130	34.925
200	2.115	1.896	2.128	0.130	2.046±0.130	41.846
250	1.985	1.532	1.856	0.233	1.791±0.233	45.915
Concentration of *Benincasa hispida* (µg/ml)						
50	3.341	3.513	3.521	0.101	3.458±0.102	4.888
100	3.142	3.247	3.215	0.053	3.201±0.054	13.033
150	2.852	3.012	3.215	0.133	3.026±0.182	16.433
200	2.561	2.938	2.853	0.197	2.784±0.198	20.338
250	2.732	2.741	2.681	0.032	2.718±0.032	26.519
Concentration of *Cucurbita maxima* (µg/ml)						
50	2.781	2.561	2.768	0.123	2.703±0.123	24.478
100	2.402	2.323	2.531	0.104	2.419±0.105	32.576
150	2.115	2.106	2.245	0.077	2.155±0.078	40.338
200	1.953	1.987	2.087	0.069	2.009±0.070	44.465
250	1.745	1.640	1.865	0.112	1.750±0.113	50.238
Concentration of *Citrus sinensis* (µg/ml)						
50	1.467	1.579	1.631	0.083	1.559±0.084	56.109
100	1.208	1.267	1.321	0.056	1.265±0.057	64.684

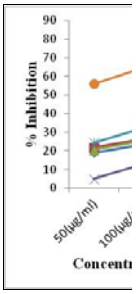

Figure 4.5.2 Free radicals scavenging activity of DPPH of Ethanol extracts of Unutilized fruit parts

4.6 ABTS cation radical scavenging activity

ABTS assay is based on the inhibition of the absorbance of the radical cation $ABTS^{+}$, which has a characteristic long wavelength absorption spectrum (Sanchez- Moreno, 2002).

4.6.1 Green leafy vegetables

The values across the concentrations indicate that the ethanol extract of *Basella alba* is more potent in neutralizing ABTS cation free radical. The ethanol extract of *Basella alba* shows an inhibition of 37-85% in the concentration range of 50-250 µg/ml, while *Solanum nigrum* shows lowest inhibition of 28-84 in the same concentration range. The ABTS cation radical scavenging activity of Ethanol extracts of The ABTS cation radical scavenging activity of Ethanol extracts of Green leafy vegetables shown in Table 4.6.1 and Figure 4.6.1.

4.6.2 Unutilized fruit parts

The values across the concentrations indicate that the ethanol extract of *Citrullus lanatus* is more potent in neutralizing ABTS cation free radical. The ethanol extract of *Citrullus lanatus* shows an inhibition of 34-96% in the concentration range of 50-250 µg/ml, while *Citrus sinensis* shows lowest inhibition of 11-67% in the same concentration range. The ABTS cation radical scavenging activity of Ethanol extracts of Unutilized fruit parts shown in Table 4.6.2 and Figure 4.6.2.

Table 4.6.1 ABTS cation radical scavenging activity of Ethanol extracts of Green leafy vegetables

Concentration of *Amaranthus gangetics* (µg/ml)	OD value at 745 nm			Standard deviation	Mean±Standard deviation	% Inhibition
	R1	R2	R3			
50	0.811	0.854	0.883	0.036	0.849±0.036	51.714
100	0.712	0.695	0.737	0.021	0.715±0.021	59.880
150	0.443	0.519	0.532	0.048	0.498±0.048	70.225
200	0.327	0.391	0.354	0.032	0.357±0.032	78.764
250	0.226	0.278	0.251	0.026	0.252±0.026	84.859

Concentration of *Basella abla* (µg/ml)						
50	0.922	1.092	1.119	0.106	1.044±0.107	37.233
100	0.692	0.721	0.743	0.025	0.719±0.026	59.419
150	0.458	0.479	0.512	0.027	0.483±0.027	72.179
200	0.334	0.421	0.562	0.115	0.439±0.115	69.789
250	0.239	0.253	0.274	0.017	0.255±0.018	85.117
Concentration of *Solanum nigrum* (µg/ml)						
50	1.059	1.112	1.321	0.138	1.164±0.139	28.979
100	0.743	0.789	0.804	0.031	0.779±0.032	55.809
150	0.521	0.543	0.579	0.029	0.548±0.029	68.541
200	0.358	0.408	0.414	0.030	0.393±0.031	76.876
250	0.246	0.251	0.278	0.017	0.258±0.017	84.975
Concentration of *Sesbania grandiflora* (µg/ml)						
50	0.831	0.975	1.003	0.092289	0.936±0.092	43.9137005
100	0.652	0.687	0.694	0.022502	0.678±0.023	61.8228725
150	0.508	0.519	0.534	0.013051	0.520±0.013	70.9168749
200	0.387	0.391	0.418	0.016862	0.399±0.017	77.3430287
250	0.275	0.284	0.295	0.010017	0.285±0.010	83.9322072

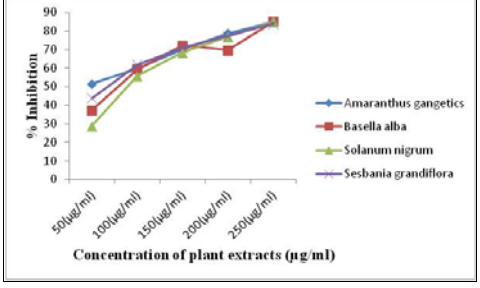

Figure 4.6.1 ABTS cation radical scavenging activity of Ethanol extracts of Green leafy vegetables

Table 4.6.2 ABTS cation radical scavenging activity of Ethanol extracts of Unutilized fruit parts

Concentration of *Carica papaya* (µg/ml)	OD value at 517 nm			Standard deviation	Mean±Standard deviation	% Inhibition
	R1	R2	R3			
50	0.952	0.987	1.032	0.040	0.952 ±0.040	43.814
100	0.693	0.701	0.721	0.014	0.693±0.014	60.773
150	0.467	0.484	0.493	0.013	0.467±0.013	73.035
200	0.225	0.278	0.312	0.043	0.225±0.044	82.796
250	0.094	0.1008	0.129	0.018	0.094±0.019	93.102
Concentration of *Cucumis melo* (µg/ml)						
50	1.122	1.145	1.311	0.103	1.193±0.103	29.346
100	0.815	0.832	0.845	0.015	0.831±0.015	53.887
150	0.604	0.623	0.645	0.020	0.624±0.021	64.857
200	0.389	0.413	0.423	0.017	0.408±0.017	76.782
250	0.056	0.087	0.098	0.021	0.080±0.022	94.432
Concentration of *Citrullus lanatus* (µg/ml)						
50	0.911	1.082	1.198	0.144	1.064±0.144	34.130
100	0.623	0.642	0.689	0.033	0.651±0.034	62.633
150	0.392	0.409	0.423	0.015	0.408±0.016	76.907
200	0.158	0.231	0.25	0.048	0.213±0.049	85.737
250	0.028	0.043	0.056	0.014	0.042±0.014	96.927
Concentration of *Benincasa hispida* (µg/ml)						
50	0.897	0.932	1.22	0.177	1.016±0.177	34.919
100	0.591	0.608	0.626	0.017	0.608±0.018	65.875
150	0.352	0.389	0.392	0.022	0.378±0.022	78.192
200	0.182	0.199	1.002	0.468	0.461±0.469	49.313
250	0.092	0.109	0.123	0.015	0.108±0.016	93.264
Concentration of *Cucurbita maxima*						

(µg/ml)						
50	0.811	0.843	0.992	0.096	0.882±0.097	46.641
100	0.727	0.74	0.776	0.025	0.748±0.025	57.848
150	0.487	0.4992	0.509	0.011	0.498±0.011	72.223
200	0.293	0.301	0.321	0.014	0.305±0.014	82.583
250	0.137	0.167	0.199	0.031	0.168±0.031	89.167
Concentration of *Citrus sinensis* (µg/ml)						
50	1.222	1.423	1.63	0.204	1.425±0.204	11.177
100	0.942	0.1003	0.132	0.477	0.391±0.477	52.644
150	0.742	0.887	0.998	0.128	0.876±0.128	45.253
200	0.562	0.663	0.716	0.078	0.647±0.078	60.456
250	0.356	0.467	0.59	0.117	0.471±0.117	67.936

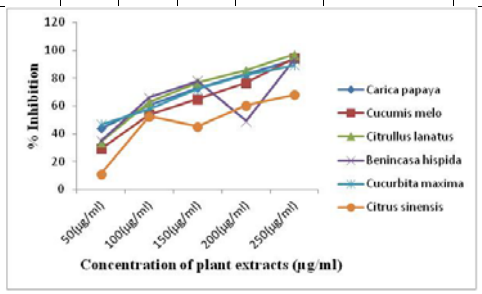

Figure 4.6.2 ABTS cation radical scavenging activity of Ethanol extracts of Unutilized fruit parts

4.7 Antioxidant activity of FRAP Assay

The ferric reducing antioxidant power assay is carried out to determine the ability of the plant extracts to scavenge free radicals by donating electrons. The greater the absorbance, the greater the reducing potential of the plant extract.

4.7.1 Green leafy vegetables

Among the four green leafy vegetables *Sesbania grandiflora* showed relatively higher antioxidant activity of absorbance at 700 nm of 2.471, while *Amaranthus gangetics* showed lowest of 0.427 in the concentration of 100 (µg/ml). The Antioxidant activity of FRAP Assay by Ethanol extracts of Sesbania grandiflora has been reported as having an absorbance at 700nm of 1.432 (Shyamala gowri and Vasantha, 2010). The Antioxidant activity of FRAP Assay by Ethanol extracts of Green leafy vegetables in the concentration of 100 (µg/ml) shown in Table 4.7.1 and Figure 4.7.1.

4.7.2 Unutilized fruit parts

Among the six unutilized fruit seeds *Citrullus lanatus* showed relatively higher antioxidant activity of absorbance at 700 nm of 0.824, while *Cucumis melo* showed lowest of 0.196 in the concentration of 100 (µg/ml). The Antioxidant activity of FRAP Assay by Ethanol extracts of *Carica papaya* has been reported as having an absorbance at 700 nm of 1.026 (Kaibing Zhou et al., 2011). The Antioxidant activity of FRAP Assay by Ethanol extracts of Unutilized fruit parts in the concentration of 100 (µg/ml) shown in Table 4.7.2 and Figure 4.7.2.

Table 4.7.1 Antioxidant activity of FRAP Assay by Ethanol extracts of Green leafy vegetables

Species	OD value at 700 nm for 100 µg/ml			Mean±Standard deviation
	R1	R2	R3	
A.g	0.394	0.412	0.427	0.411+0.017
C.p	0.324	0.301	0.348	0.324±0.024
B.a	1.155	1.143	1.139	1.146±0.008
C.m	0.195	0.189	0.176	0.187±0.010
S.n	2.057	2.04	2.13	2.076+0.048
C.l	0.81	0.796	0.825	0.810±0.015
S.g	2.481	2.342	2.375	2.399±0.073
B.h	0.227	0.209	0.218	0.218±0.009
C.max	0.205	0.195	0.211	0.204±0.008
C.s	0.591	0.576	0.534	0.567±0.030

A.g: Amaranthus alba; **S.n:** Solanum **S.g:** grandiflora; **B.a:** Basella nigrum; Sesbania grandiflora

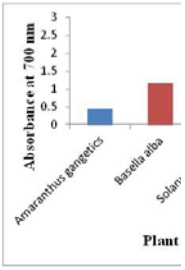

Figure 4.7.1 Antioxidant activity of FRAP Assay by Ethanol extracts of Green leafy vegetables

Table 4.7.2 Antioxidant activity of FRAP Assay by Ethanol extracts of Unutilized fruit parts

C.p: Carica papaya; **C.m:** Cucumis melo; **C.l:** Citrullus lanatus; **B.h:** Benincasa hispida; **C.max:** Cucurbita maxima; **C.s:** Citrus sinensis

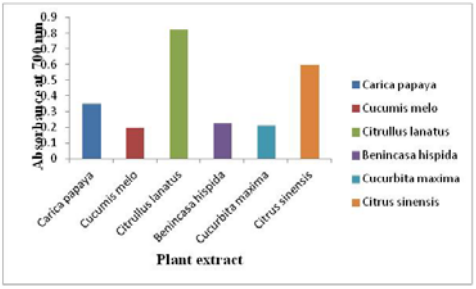

Figure 4.7.2 Antioxidant activity of FRAP Assay by Ethanol extracts of Unutilized fruit parts

4.8 Antioxidant activity of Modified FRAP Assay

The modified ferric reducing antioxidant power assay is carried out to determine the ability of the plant extracts to scavenge free radicals by donating electrons. The greater the absorbance, the greater the reducing potential of the plant extract.

4.8.1 Green leafy vegetables

Among the four green leafy vegetables *Sesbania grandiflora* showed relatively higher antioxidant activity of absorbance at 750 nm of 3.535, while *Amaranthus gangetics* showed lowest of 1.413 in the concentration of 100 (µg/ml). The Antioxidant activity of Modified FRAP Assay by Ethanol extracts of Green leafy vegetables in the concentration of 100 (µg/ml) shown in Table 4.8.1 and Figure 4.8.1.

4.8.2 Unutilized fruit seeds

Among the six unutilized fruit seeds *Citrus sinensis* showed relatively higher antioxidant activity of absorbance at 750 nm of 3.057, while *Cucumis melo* showed lowest of 0.382 in the concentration of 100 (µg/ml). The Antioxidant activity of Modified FRAP Assay by Ethanol extracts of Unutilized fruit parts in the concentration of 100 (µg/ml) shown in Table 4.8.2 and Figure 4.8.2.

Table 4.8.1 Antioxidant activity of Modified FRAP Assay by Ethanol extracts of Green leafy vegetables

Species	OD value at 750 nm for 100 µg/ml			Mean±Standard deviation
	R1	R2	R3	
A.g	1.389	1.346	1.409	1.389±1.346
B.a	1.42	1.458	1.473	1.42±1.458
S.n	1.845	1.793	1.813	1.845±1.793
S.g	3.497	3.534	3.519	3.497±3.534

A.g: Amaranthus grandiflora; **B.a:** Basella alba; **S.n:** Solanum nigrum; **S.g:** Sesbania grandiflora

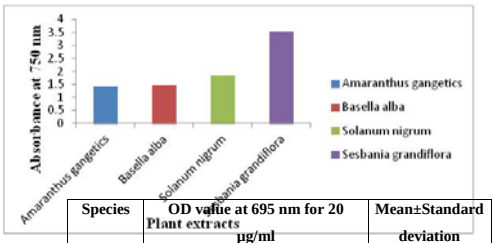

Figure 4.8.1 Antioxidant activity of Modified FRAP Assay by Ethanol extracts of Green leafy vegetables

Species	OD value at 695 nm for 20 µg/ml			Mean±Standard deviation
	R1	R2	R3	
C.p	0.954	0.979	0.991	0.975±0.019
C.m	0.348	0.352	0.386	0.362±0.021
C.l	1.133	1.157	1.195	1.162±0.031
B.h	0.734	0.756	0.774	0.755±0.020
C.max	0.8	0.769	0.815	0.795±0.023
C.s	3.012	3.058	3.032	3.034±0.023

Table 4.8.2 Antioxidant activity of Modified FRAP Assay by Ethanol extracts of Unutilized fruit parts

C.p: Carica papaya; **C.m:** Cucumis melo; **C.l:** Citrullus lanatus; **B.h:** Benincasa hispida; **C.max:** Cucurbita maxima; **C.s:** Citrus sinensis

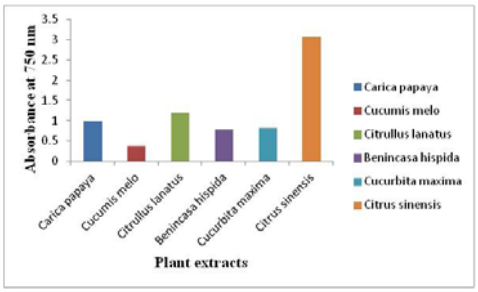

Figure 4.8.2 Antioxidant activity of Modified FRAP Assay by Ethanol extracts of Unutilized fruit parts

4.9 Comparison of Total antioxidant capacity and Total phenol content for plant extracts

The results obtained indicate that among the plant extracts the level of total phenols is highest in *Citrus sinensis* having total phenol content of 28.18 mgTAE/gDW followed by Solanum nigrum of 18.672 mgTAE/gDW. This suggests total phenols and flavonoids may be responsible for the free radical scavenging activity of *Citrus sinensis* of 10.231 (µM/g). On the other hand, *Cucumis melo* showed the lowest level of phenols of 9.581 mgTAE/gDW may responsible for the lowest free radical scavenging activity of the plant of 0.822 (µM/g). The comparison between total antioxidant capacity and total phenol content for plant extracts shown in Table 4.9 and Figure 4.9.

Table 4.9 Comparison of Total antioxidant capacity and Total phenol content for plant extracts

Species	Total antioxidant capacity (µM/g)	Total phenol content mgTAE/gDW
A.g	2.139	17.245
B.a	1.914	22.172
S.n	3.072	18.672
S.g	6.009	28.181
C.p	0.582	10.218
C.m	0.822	9.581
C.l	1.817	10.754
B.h	1.126	10.490
C.max	1.857	17.472
C.s	10.231	32.236

A.g: Amaranthus grandiflora; **B.a:** Basella alba; **S.n:** Solanum nigrum; **S.g:** Sesbania grandiflora; **C.p:** Carica papaya; **C.m:** Cucumis melo; **C.l:** Citrullus lanatus; **B.h:** Benincasa hispida; **C.max:** Cucurbita maxima; **C.s:** Citrus sinensis

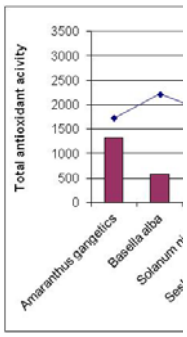

Figure 4.9 Comparison of Total antioxidant capacity and Total phenol content for plant extracts

TAC: Total antioxidant capacity

TP: Total phenol content

4.10 Comparison of Total antioxidant capacity and Total flavonoids content for plant extracts

The results obtained indicate that among the unutilized fruit seeds the level of total phenols is highest in *Carica papayas* having total flavonoid content of 30.25 mgRE/gDW followed by *Cucurbita maxima* of 18.61 mgRE/gDW**.** This suggests lavonoids may responsible for the free radical scavenging activity of the plant of 10.23 (µM/g). On the other hand, *Basella alba* showed the lowest level of flavonoids of 5.7 mgRE/gDW may responsible for the lowest free radical scavenging activity of the plant of 1.914 (µM/g). The comparison between total antioxidant capacity, total phenol content and total flavonoid content shown in Table 4.10 and Figure 4.10.

Table 4.10 Comparison of Total antioxidant capacity and Total flavonoids content for plant extracts

Species	Total antioxidant capacity (µM/g)	Total flavonoid content mgRE/gDW
A.g	2.139	13.3
B.a	1.914	5.7
S.n	3.072	8.92
S.g	6.009	13.54
C.p	0.582	30.25
C.m	0.822	13.01
C.l	1.817	10.28
B.h	1.126	11.22
C.max	1.857	18.61
C.s	10.231	8.39

A.g: Amaranthus grandiflora; **B.a:** Basella alba; **S.n:** Solanum nigrum; **S.g:** Sesbania grandiflora; **C.p:** Carica papaya; **C.m:** Cucumis melo; **C.l:** Citrullus lanatus; **B.h:** Benincasa hispida; **C.max:** Cucurbita maxima; **C.s:** Citrus sinensis

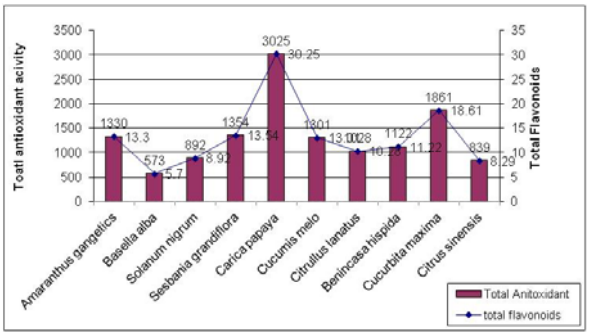

Figure 4.10 Comparison of Total antioxidant capacity and Total flavonoids content for plant extracts

TAC: Total antioxidant capacity

TF: Total flavonoid content

CHAPTER 5

CONCLUSION

- The present work has been undertaken to study about the free radical scavenging ability of four species of green leafy vegetables, namely, ***Amaranthus gangetics, Basella alba, Solanum nigrum*** and ***Sesbania grandiflora*** and six unutilized fruit parts, namely, ***Carica papaya, Cucumis melo, Citrullus lanatus, Benincasa hispida, Cucurbita maxima*** and ***Citrus sinensis***.
- The preliminary study carried out in our work has confirmed the presence of phytochemicals like Flavonoids,Phenols,Saponins which are responsible for the free radical scavenging potential of the plant extracts.
- The plant species were analyzed for the presence of the various non-enzymatic antioxidants like Flavonoids, Total Phenols.
- Among the selected green leafy vegetables and unutilized fruit parts, ***Citrus sinensis*** peel extract showed highest potential free radical scavenging activity and have highest phenol and flavonoid content, which is the cause for the free radical scavenging activity.

APPENDICES

Appendix 1

Preparation of Dragendroff's reagent

1. 1.7g of Bismuth nitrate dissolved in 100 ml of water/acetic acid in 4:1 ratio (Solution A).
2. 40g of potassium iodide dissolved in 100 ml distilled water (Solution B).
3. 5 ml of solution A added with 5 ml of solution B, 20 ml of acetic acid and 70 ml of distilled water.

Appendix 2

Preparation of Wagner's reagent

2g of iodine added with 6g of potassium iodide in 100 ml distilled water.

Appendix 3

Preparation of Millon's reagent

1. Dissolved one part of mercury in one part of cold fuming nitric acid.
2. Dissolved with twice the volume of distilled water and decant the clear solution after 7 hours.

Appendix 4

Preparation of Bradford's reagent

Dissolved 66g of cupric acetate and 10 ml of glacial acetic acid in water and dilute it to one litre with distilled water.

Appendix 5

Preparation of Fehling's reagent

1. Dissolved 34.66g of copper sulphate in 500 ml water (Solution A).
2. Dissolved 17.3g of potassium sodium tartarate and 50g of sodium hydroxide in 500 ml cold water.
3. Mix equal volume of Solution A and Solution B.

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