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FREE RADICAL SCAVENGING ACTIVITY OF LUTEIN ISOLATED FROM BANANA PEEL

A PROJECT REPORT

Submitted by

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BONAFIDE CERTIFICATE

Certified that this project report "**FREE RADICAL SCAVENGING ACTIVITY OF LUTEIN ISOLATED FROM BANANA PEEL**" is the bonafide work of **ANBURANI.C (0710204003), BANUPPRIYAA.K.P (0710204006), DIVYA.S (0710204011)** who carried out the project work under my supervision.

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ABSTRACT

Role of antioxidants in keeping a check on the free radical causing health complications has become inevitable with the rise in diseases like cancer, age related macular degeneration, arthritis, cardiovascular disorder and so on. To prevent their possible damages to biological molecules, especially to DNA, lipids and proteins all oxygen- consuming organisms are endowed with a well-integrated antioxidant system, including enzymatic and non-enzymatic components .The fruits are rich sources of various vitamins, minerals and fibres required by human body for optimal health. In the recent years, more attention has been paid to the antioxidants contained in fruits, because epidemiological studies reveal that high fruit intake was associated with reduced mortality and morbidity of cardiovascular disease and some type of cancer and one of possible mechanisms was attributed to the antioxidant activity presented by the fruits. Hence, different varieties of banana have been used for this study. Bananas have been proved to contain a potent antioxidant potential apart from other health benefits. The peel of banana is said to contain lutein , a xanthophyll and one of 600 known naturally-occurring carotenoids This study aimed at extraction, isolation ,identification and characterization of lutein from banana peel. In addition, the study also aims to determine antioxidant potential of purified lutein. Nearly seven local varieties of banana, viz., Kadhali, Karpooravalli, Nendran, Pachainadan, Poovan, Rasthali and Red. These varieties were screened for the total carotenoid content. The variety Pachainadan was found to have highest carotenoid content and thus it was selected for isolation of lutein. Free radicals scavenging activity of isolated lutein was assessed by DPPH radical scavenging assay, Ferric ion reducing assay, nitric oxide scavenging assay and superoxide scavenging assay. Total antioxidant assay to initially confirm the antioxidant potential was also carried out.

Thus, lutein obtained from these varieties may be useful to combat free radical-related diseases. The results prove that lutein isolated from peel of the variety Pachainadan has an efficient antioxidant activity. Hence banana peel could be used as the best source of lutein to prevent the free radical mediated diseases.

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LIST OF ABBREVIATIONS

µg	microgram
µl	microliter
µm	microgram
ATP	Adenosine triphosphate
DNA	Deoxyribose Nucleic Acid
DPPH	1, 1-diphenyl -2-picryl hydrazyl
EDTA	Ethylene Diamine Tetra Acetic acid
FDA	Food and Drug Administration
$FeCl_3$	Ferric Chloride
g	gram
H_2O_2	Hydrogen Peroxide
H_2SO_4	Sulphuric acid
LDL	Low Density Lipoprotein
MDA	Malondialdehyde
mg	milligram
min.	Minute
ml	milliliter
NaOH	Sodium hydroxide
NBT	Nitro Blue Tetrazolium
NO	Nitric oxide
O_2	Oxygen
OFR	Oxygen-Free Radicals
$^{\cdot}OH$	Hydroxyl radical
PUFA	Polyunsaturated fatty acid
ROS	Reactive Oxygen Species
SOD	Superoxide dismutase
TCA	Trichloro Acetic acid

INTRODUCTION

1. INTRODUCTION

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 ($O2^{\cdot}$), the hydroxyl radical ($OH^{\cdot}$), 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 $O_2^{-\cdot}$ 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. (Acworth *et al.*, 1997).

When free radicals and other reactive oxygen species accumulate in the body, they cause damage on cells, DNA, lipid, sugar, and protein. The damage caused by free radicals and reactive oxygen species, in plants and animals, could lead to deterioration of foods, cell membrane dysfunction, protein modification, enzyme inactivation, break of DNA strands, brain damage and dementia. Free-radical induced oxidative damages may be precursors to aging and diseases such as cancer, heart disease, diabetes mellitus, atherosclerosis, hypertension, sleep

apnea, brain damage and dementia related diseases such as Alzheimer's disease and Parkinson's disease.

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 (hydrophilic) or in lipids (hydrophobic). 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 the 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.

Lutein is one such multidimensional lipid soluble antioxidant and a natural colorant or pigment, present in plants especially in green vegetables such as spinach, plus various fruits and corn. Egg yolks are also sources of lutein. Even though more than 700 carotenoids have been isolated from natural sources, lutein is an important hydroxyl carotenoid antioxidant which is more efficient as free radical quencher (Southon, 2000). Epidemiological studies shown that, there is inverse relationship between vegetable intake and plasma lutein concentration(Yong *et al.*, 1994). Human plasma lutein has been inversely associeated with cytochrome activity and human cancer(Le Marchand *et al.*, 1997). In animal models it prevents colon (Nariswa. *et al.*, 1996 ; Kim *et al.*, 1998) and breast cancer(Chew *et al.*, 2003 and Park *et al.*, 1998) . Lutein can also enhanced the recovery of cells oxidative change by stimulating DNA strand break repair (Astley *et al.*, 2002). Lutein has been shown to enhance antibody production in response to Tdependent antigens in spleen cells in vitro, as well as in mice invivo (Jyonouchi *et al.*, 1994. Hence plants sources such as green vegetables, fruits can be used to derive the maximum health benefits because of their lutein contents.

Lutein provides nutritional support to our eyes and skin – the only organs of the body directly exposed to the outside environment. Lutein has been linked to promoting healthy eyes through reducing the risk of macular degeneration. Other studies suggest that a mixture of nutrients, including lutein, may provide supplemental antioxidant capacity to the skin, helping counteract free radical damage(Morganti Morganti *et al.*, 2002). Similar to our eyes, lutein is deposited throughout our skin through the lutein we consume(Gonzalez *et al.* 2003). Research suggests 10 mg of lutein from food or dietary supplements, may play a role in maintaining healthy skin (Van de Leun 1996, Podda *et al.*, 1998). A recent human clinical study showed 10 mg of lutein daily increased skin hydration, elasticity and skin lipid content (Morganti *et al.*, 2006). This is the first research to show improvement in skin health through lutein supplementation alone. Skin is the largest organ of the human body. Along with our eyes, it is the only organ of the body constantly exposed to the environment. Skin is "assaulted" by

- Light (especially ultraviolet and visible wavelengths)
- Environmental pollutants

Such exposure can create reactive oxygen species, leading to cell-damaging free radicals within skin. The skin provides numerous functions. It acts as a barrier of protection for the internal organs. It regulates body temperature. It plays an important role in immunological response. Therefore, it is important to protect the skin.

Among the fruits, banana has a lot of health benefits. It is a rich source of potassium, dietary fiber, manganese, Vitamin B_6 and C. A few suppliers claim that bananas are also a rich source of fructooligosaccharide. Bananas are not only a good source of B vitamins, they also contain vitamin C, A and high levels of potassium. Bananas provide antioxidants that can help keep your brain and heart healthy.

Although the body produces antioxidants of its own, it seems to benefit from extra antioxidants provided in the diet, especially from whole grains, vegetables and fruits. This is still an emerging science, but the evidence suggests that the banana is a key player. The peel of banana is a rich source of lutein.

Banana, a tropical plant may protect itself from the oxidative stress caused by strong sunshine and high temperature by producing large amounts of antioxidants. Banana should be considered to be a good source of natural antioxidant for foods and functional food source against cancer and heart disease. Therefore, attention in recent times has been focused on the isolation characterization and utilization of the natural antioxidants especially growing interest in polyphenols as potential disease preventive agents.

Bananas are one of the most popular foods on the world and it will be known that fruits contain various antioxidants compounds such as gallocatechin and dopamine. Since the bananas fruits are widely available, they have been used as food without apparent toxic effect. Not only the pulp, but the peel of banana is also found to contain lots of compounds that can serve as antioxidants.

Banana has been proved to contain antioxidant potential apart from health benefits. Though their potency as antioxidants and antimicrobial in the pulp has been studied extensively, scientific literature pertaining to presence of lutein in the banana peel is not yet documented. Hence, this project has been aimed to screen seven local varieties of banana viz., Kadali, Karpooravalli, Nendran, Pachinadan, Poovan, Rasthali, Red banana for carotenoids and to extract, isolate,identify and characterize lutein from banana peel and also to determine antioxitant potential of purified lutein.

OBJECTIVES

2.OBJECTIVES

- To screen seven varieties of banana for total carotenoids
- To extract, isolate, identify and characterize lutein from banana peel.
- To determine antioxidant potential of purified lutein.

LITERATURE REVIEW

3. LITERATURE REVIEW

3.1. Free radicals

Free radicals can be defined as 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 systems, 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.

Most of the molecular oxygen consumed by aerobic cells during metabolism is reduced to water by using cytochrome oxidase in mitochondria. However, when oxygen is partially reduced, it becomes activated and reacts readily with a variety of bimolecular. 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 $OH^{\cdot}$ This hydroxyl radical oxidizes lipids giving rise to lipid peroxidation. Hydrogen peroxide is known to cause DNA breaks in intact cells. 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). Lipid hydroperoxides may directly induce DNA chain breaking, and lipid peroxyl and alkoxyl radicals may cause base oxidation in DNA (Park, 1992).

3.2 Reactive oxygen species

There are many types of radicals, but those of most concern in biological systems are derived from oxygen, and known collectively as *reactive oxygen species*. Oxygen has two unpaired electrons in separate orbitals in its outer shell. This electronic structure makes oxygen especially susceptible to radical formation.

- **superoxide anion**
- **peroxide** (hydrogen peroxide)
- **hydroxyl radical**

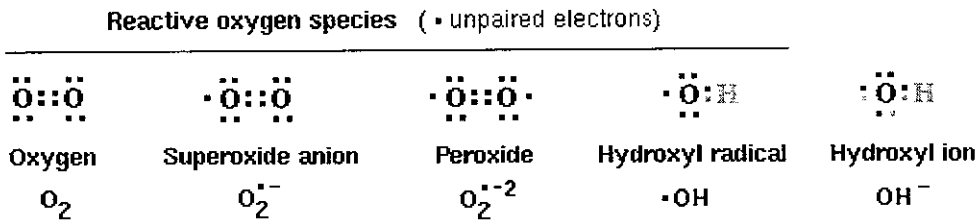

Figure 3.1: Reactive oxygen species

Reactive oxygen species (ROS) are free radicals that contain the oxygen atom. They are very small molecules that include oxygen ions and peroxides and can be either inorganic or organic. They are highly reactive due to the presence of unpaired valence shell electrons. ROS form as a natural by-product of the normal metabolism of oxygen and have important roles in cell signalling. However, during times of environmental stress (e.g. UV or heat exposure). ROS levels can increase dramatically, which can result in significant damage to cell structures. This cumulates into a situation known as oxidative stress. ROS are also generated by exogenous sources such as ionizing radiation.

3.3 Reactive oxygen system

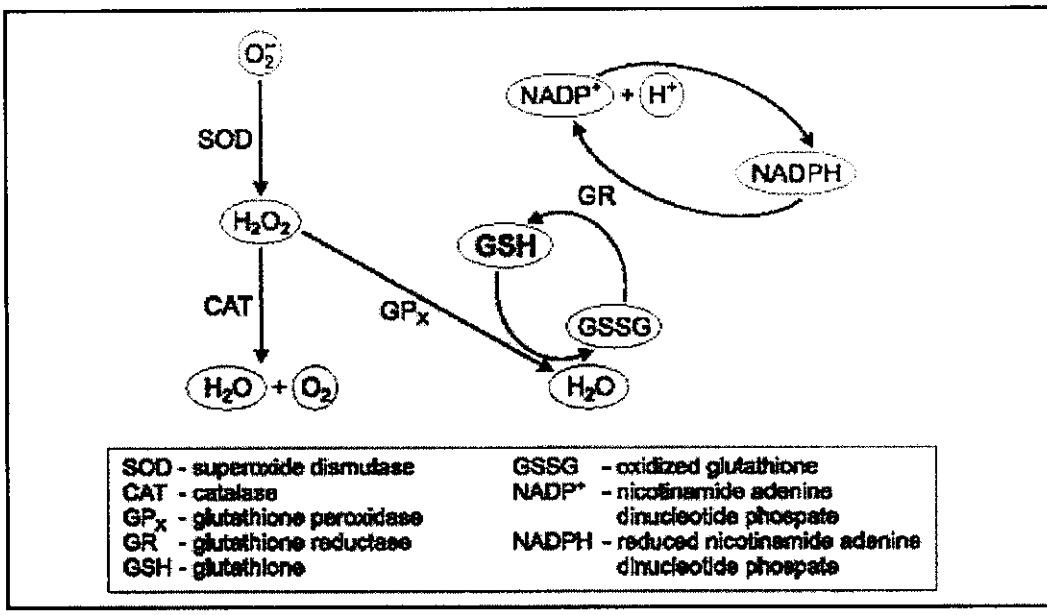

Figure 3.2: Reactive oxygen system

Three types of superoxide dismutase (SOD) can be distinguished: cytoplasmatic, mitochondrial and extracellular. SOD catalyzes the dismutation of superoxide radical anion ($O2^{\cdot}$) into less noxious hydrogen peroxide (H_2O_2), that is further degraded by catalase or glutathione peroxidase. Catalase is an enzyme which accelerates degradation of H_2O_2 into water and oxygen .The second pathway of H_2O_2 metabolism depends on activity of glutathione peroxidase (GPx) and cooperating glutathione reductase. The reduction of H_2O_2 into water by GPx is accompanied by the conversion of glutathione from reduced form (GSH) into oxidized form (GSSG)

3.4 Formation of free radicals

Free radicals and other reactive oxygen species are derived either from normal essential metabolic processes in the human body or from external sources such as exposure to X-rays, ozone, cigarette smoking, air pollutants and industrial chemicals.

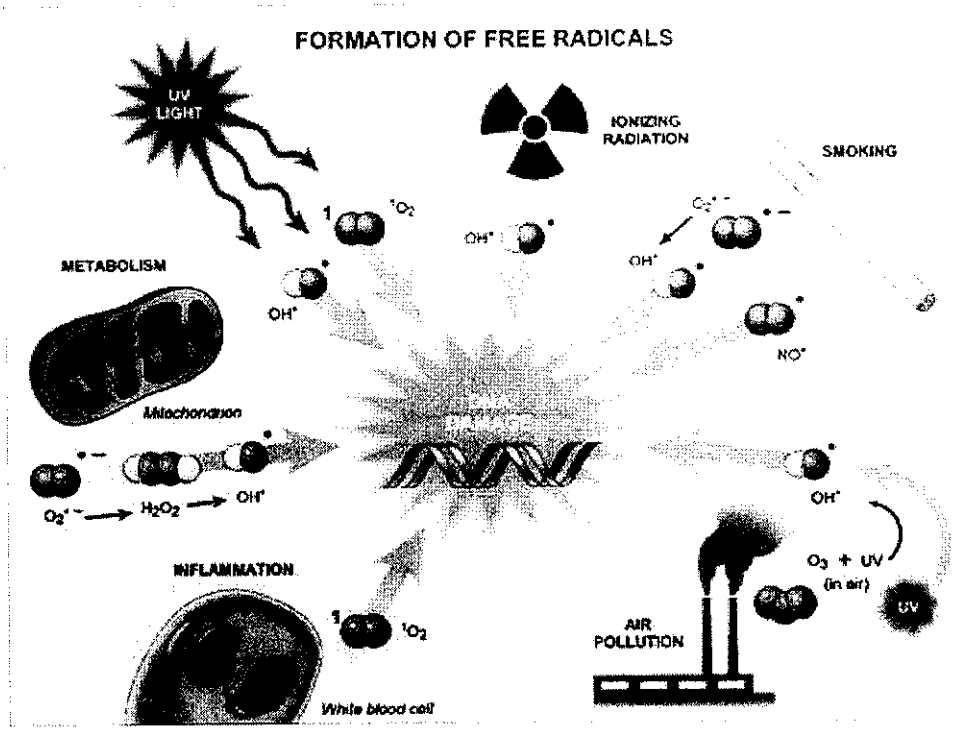

Figure 3.3: Formation of free radicals

3.5 Types of free radicals

Most free radicals are coming from oxygen atoms and are called Reactive Oxygen Species (ROS), such as superoxide ion, hydroxyl radical, hydrogen peroxide and singlet oxygen.

- **Superoxide ion (O_2^{-}.)** (or reactive oxygen species) is an oxygen molecule with an extra electron. This free radical can cause damage to mitochondria, DNA and other molecules. Our body can neutralize superoxide ions by producing superoxide dismutase.
- **Hydroxyl radical ($OH^{\cdot}$)** is formed by the reduction of an oxygen molecule in the electron transport chain. Because of its high reactivity it will damage most organic molecules such as carbohydrates, DNA, lipids and proteins.
- **Singlet oxygen** is formed by our immune system. Singlet oxygen causes oxidation of LDL cholesterol.

- **Hydrogen peroxide** is not a free radical but it is involved in the production of many reactive oxygen species. Hydrogen peroxide is a byproduct of oxygen metabolism and is neutralized by peroxidases.

Sometimes reactive nitrogen atoms are involved and these free radicals grouped under Reactive Nitrogen Species (RNS). Nitric acid is the most important RNS. Some transitional metals, such as iron and copper, have many numbers of unpaired electrons and can also act as free radicals. These metals do not have that strong electron affinity, but can easily accept and donate electrons.

3.6 Free radicals and human diseases

Free radicals are involved in both the process of aging and the development of cancer (Halliwell, *et al.,* 1989). They attack many cellular targets including membranes, proteins and nucleic acids, (Cerutti *et al.,* 1994) and cause structural damage to the cellular DNA. These structural changes manifest as point mutations and chromosomal alterations in cancer-related genes. (Cerutti *et al.,* 1994).Consequently, elderly people are predisposed to the development of cancer. Fortunately, certain antioxidant supplements like vitamins C and E, can prevent much oxidative damage to DNA and thus reduce the ability of the oxidants to induce cancer. (Shingenaga *et al.,*1993). Lipids in cell membranes are very prone to oxidative damage because some free radicals tend to concentrate in the membrane and cause oxidative damage, known as lipid peroxidation. Other diseases such as atherosclerosis, Parkinson's disease and Alzheimer's are also attributed to free radicals

3.7 Antioxidant as Scavengers

To deal with the free radicals or so called ROS, the body is equipped with an effective defense system which includes various enzymes and high and low molecular weight antioxidants. Antioxidants neutralize free radicals by donating one of their own electrons, ending the electron stealing reaction. The antioxidants do not themselves become free radical by donating electrons because they are stable in other form. These act as scavenger and play the housekeeper's role by mopping up free radicals before they get a chance to create havoc in a body. Thus, they may be well defined as the substances that are capable of quenching or stabilizing free radicals

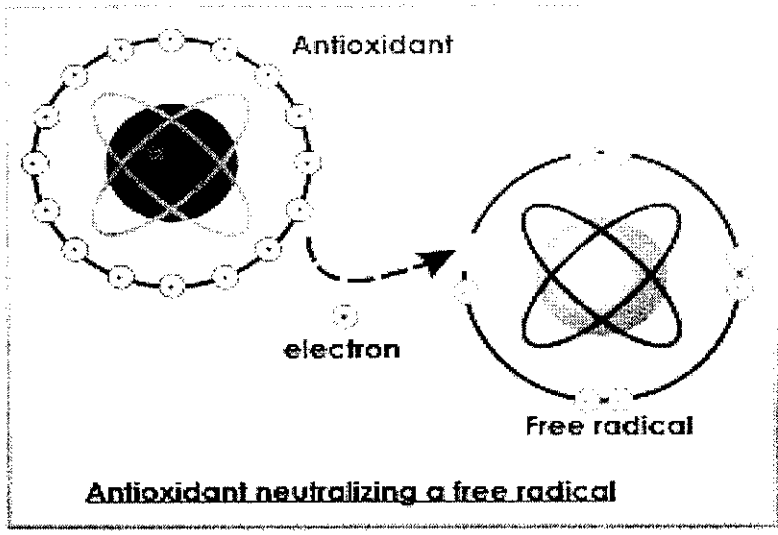

Figure 3.4: Antioxidant neutralizing free radical

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

In recent years, the use of some synthetic antioxidants has been restricted because of their possible toxic and carcinogenic effects. This concern has resulted in an increased interest in the investigation of the effectiveness of naturally occurring compounds with antioxidant properties. Foods rich in antioxidants have been shown to play an essential role in the prevention of cardiovascular diseases; cancers, neurodegenerative diseases, the most well known of which are Parkinson's and Alzheimer's diseases, inflammation and problem caused by cell and cutaneous aging (Shahidi and Wanasundara, 1992).

3.8 Types of antioxidants

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

3.8.1 Enzymatic antioxidants

As with the chemical antioxidants, cells are protected against oxidative stress by an interactive network of antioxidant enzymes (Seis, 1997 and Vertuani *et al.,* 2004).The enzymes responsible for the defense against the free radical damage include superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, etc.

3.8.1.1 Superoxide Dismutase

Superoxide Dismutase (SOD) is an enzyme that repairs cells and reduces the damage done to them by superoxide, the most common free radical in the body. Studies have shown that SOD acts as both an antioxidant and anti-inflammatory in the body, neutralizing the free radicals that can lead to wrinkles and precancerous cell changes. Researchers are currently studying the potential of superoxide dismutase as an anti-aging treatment, since it is now known that SOD levels drop while free radical levels increase as age.

SOD

$2O_2^- + 2H$ ----------> $H_2O_2 + O_2$

3.8.1.2 Catalase

It helps the body to convert hydrogen peroxide into water and oxygen, thus preventing the formation of carbon dioxide bubbles in the blood. Catalase works closely with superoxide dismutase to prevent free radical damage to the body. SOD converts the dangerous superoxide radical to hydrogen peroxide, which catalase converts to harmless water and oxygen.

Catalase

$2H_2O_2$ ----------> $H_2O + O_2$

3.8.1.3 Glutathione peroxidase

Glutathione peroxidase also known as gamma - glutamylcysteinylglycine or GSH, the [illegible] which is in virtually every cell, is one of the most powerful free

radical fighters that the body has in its arsenal. Glutathione helps maintain the integrity of red blood cells, as well as protecting white blood cells, assists in carbohydrate metabolism and breaking down oxidized fats.

$$H_2O_2 + 2 \text{ glutathione} \xrightarrow{GPx} \text{oxidized glutathione} + 2H_2O$$

3.8.1.4 Glutathione-S-transferase

A family of enzymes that utilize glutathione in reactions contributing to the transformation of a wide range of compounds, including carcinogens, therapeutic drugs, and products of oxidative stress. These enzymes play a key role in the detoxification of such substances.

3.8.1.5 Glutathione reductase

Also known as GSR or 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.

3.8.1.6 Polyphenol oxidase

The enzyme polyphenol oxidase (PPO) may be the most primitive, nonspecific defense system found in eukaryotes. In plants, it is responsible for the browning of damaged tissues as seen in apple, banana, and potato.

3.8.1.7 Glucose-6-phosphate dehydrogenase (G6PD)

It is a cytosolic enzyme in the pentose phosphate pathway, 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.

3.8.2 Non enzymatic antioxidants

3.8.2.1 Vitamin A (β – Carotene)

Beta-carotene is a member of the carotenoid family, a group of powerful antioxidants that also includes alpha-carotene, lycopene, zeaxanthin, and lutein. However, of all the carotenoids, only alpha-carotene and beta-carotene are converted to significant amounts of vitamin A in the

body, and beta-carotene is by far the most plentiful carotenoid found in fruits and vegetables. Beta-carotene acts as a precursor of vitamin A, and is therefore called a provitamin A compound. Foods or supplements containing beta-carotene are converted to vitamin A for the maintenance of healthy skin, good vision, and a robust immune system. Beta-carotene is also a powerful antioxidant, and has been shown to help guard against cancer and heart disease.

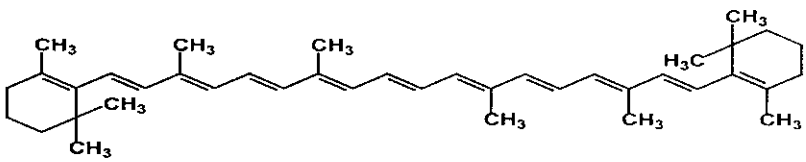

Figure 3.5: Vitamin A

3.8.2.2 Vitamin C (Ascorbic acid)

Vitamin C is an important dietary antioxidant, it significantly decreases the adverse effect of reactive species such as reactive oxygen and nitrogen species that can cause oxidative damage to macromolecules such as lipids, DNA and proteins which are implicated in chronic diseases including cardiovascular disease, stroke, cancer, neurodegenerative diseases and cataractogenesis (Halliwell & Gutteridge, 1986). Ascorbic acid is a potent water soluble antioxidant capable of scavenging or neutralizing an array of reactive oxygen species viz., hydroxyl, alkoxyl, peroxyl, superoxide anion, hydroperoxyl radicals and reactive nitrogen radicals such as nitrogen dioxide, nitroxide, peroxynitrite at very low concentrations. In addition ascorbic acid can regenerate other antioxidants such as α-tocopheroxyl, urate and β-carotene radical cation from their radical species (Halliwell & Gutteridge, 1986). Thus, ascorbic acid acts as co-antioxidant for α-tocopherol by converting α-tocopheroxyl radical to α-tocopherol and helps to prevent the α-tocopheroxyl radical mediated peroxidation reactions (Neuzil, 1997)

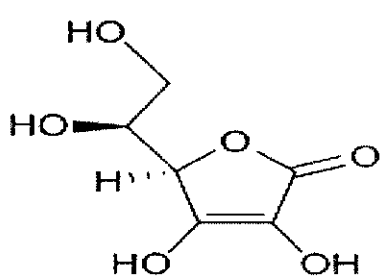

Figure 3.6: Vitamin C

3.8.2.3 Vitamin E (α-Tocopherol)

Vitamin E is a fat-soluble antioxidant that stops the production of reactive oxygen species formed when fat undergoes oxidation (Herrera *et al.*, 2001 and Packer *et al.*, 2001).Of these, α-tocopherol (also written as alpha-tocopherol) has been most studied as it has the highest bioavailability (Brigelius and Traber, 1999). It has been claimed that α-tocopherol is the most important lipid-soluble antioxidant, and that it protects cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction (Traber and Atkinson, 2007). 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.

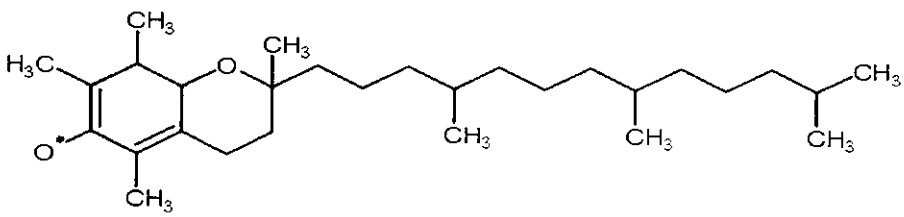

Figure 3.7: VitaminE

3.8.2.4 Glutathione

Glutathione is body's master antioxidant and one of the most important cleansing and healing agents. Glutathione blocks free radical damage and help to recycle Vitamins E and C, therefore plays a key role in their function. Because Glutathione exists within the cells, it is in a prime position to neutralize free radicals. The highest concentration of glutathione is found in the liver which is the principal organ involved in the detoxification and elimination of toxic materials, and prevents the buildup of oxidized fats that may contribute to atherosclerosis.

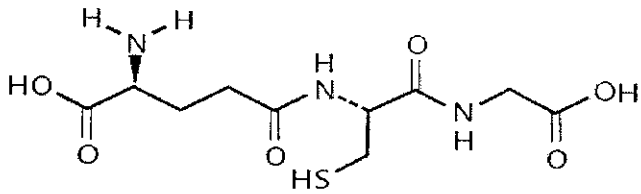

Figure 3.8: Glutathione

3.8.2.5 Melatonin

Melatonin is an antioxidant that can easily cross cell membranes and the blood-brain barrier (Hardeland, 2005). Melatonin is a direct scavenger of OH, O_2-, and NO (Poeggeler *et al.*, 1994). Unlike other antioxidants, melatonin does not undergo redox cycling, the ability of a molecule to undergo reduction and oxidation repeatedly. Redox cycling may allow other antioxidants (such as vitamin C) to regain their antioxidant properties. Melatonin, on the other hand, once oxidized, cannot be reduced to its former state because it forms several stable end-products upon reacting with free radicals. Therefore, it has been referred to as a terminal (or suicidal) antioxidant (Tan *et al.*, 2000)

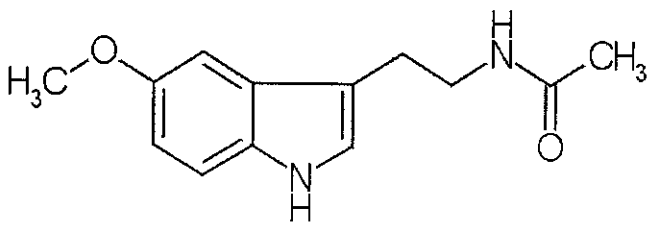

Figure 3.9: Melatonin

3.9 Phytochemicals

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. There are more than thousand known phytochemicals. It is well-known that plants produce these chemicals to protect itself, but recent research demonstrate that they can protect humans against diseases. Some of the well-known phytochemicals are lycopene in

tomatoes, isoflavones in soy and flavonoids in fruits. They are not essential nutrients and are not required by the human body for sustaining life.

3.9.1 Flavonoids

Flavonoids are polyphenols abundantly found in fruits, vegetables, and herbs. Flavonoids are synthesized only in plants. They are a diverse group of phytochemicals, exceeding four thousand in number. From human nutrition perspective, flavonoids are important components of a healthy diet because of their antioxidant activity. Nevertheless, the antioxidant potency and specific effect of flavonoids in promoting human health varies depending on the flavonoid type (chemical, physical, and structural properties). Among the potent antioxidant flavonoid types are quercetin, catechins and xanthohumol. Because of the antioxidative property, it is suggested that flavonoids may delay or prevent the onset of diseases (such as cancer) induced by free radicals. They also inhibit low density lipoprotein (LDL) oxidation by free radicals. Flavonoids have been reported to have negative correlation with incidence of coronary heart disease. Furthermore, flavonoids have anti-bacterial, anti-viral, anti-tumor, anti-inflammatory, antiallergenic and vasodilatory effect. They also inhibit platelet aggregation. (Verena *et al.*, 2006 and Subramani Sellappan *et al.*,2002)

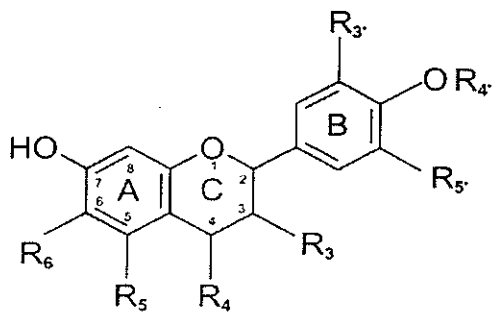

Figure 3.10: General structure of flavonoids

3.9.2 Alkaloids

Alkaloids are a class of compounds that typically contain nitrogen and have complex ring structures. They naturally occur in seed-bearing plants and are found in berries, bark, fruit, roots, and leaves. Often, they are bases that have some physiological effect.

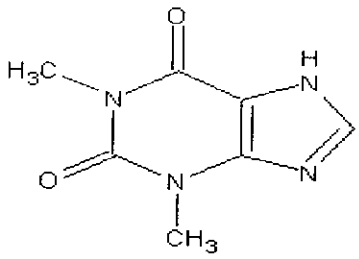

Figure 3.11: General structure of alkaloids

3.9.3 Tannins

Tannins are polyphenolics that make cranberries and pomegranates bitter. Tannins, along with Vitamin C, help build and strengthen collagen. Tannins prevent urinary tract infection by preventing bacteria from adhering to the walls. Combination of tannin plus anthocyanins (as in pomegranate juice) can break-down oxidized cholesterol in the bloodstream and in atherosclerotic plaques. Most of the active compounds in black tea are tannins which are 90% catechins. Epicatechin is the major component of natural tannin in grapes.

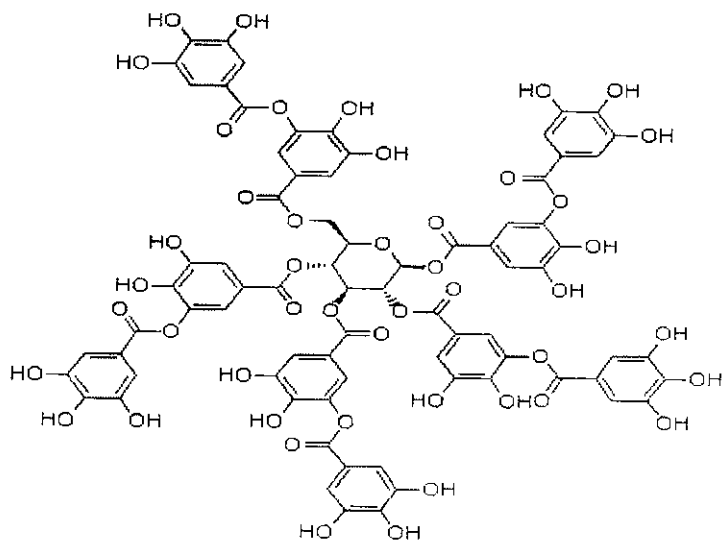

Figure 3.12: General structure of Tannin

3.9.4 Carotenoids

Carotenoids represent one of the most widespread groups of naturally occurring pigments. These compounds are largely responsible for the red, yellow, and orange color of fruits and

vegetables, and are also found in many dark green vegetables. In recent years, carotenoids have received a tremendous amount of attention as potential anti-cancer and anti-aging compounds. Carotenoids are powerful antioxidants, protecting the cells of the body from damage caused by free radicals. Carotenoids, and specifically beta-carotene, are also believed to enhance the function of the immune system.

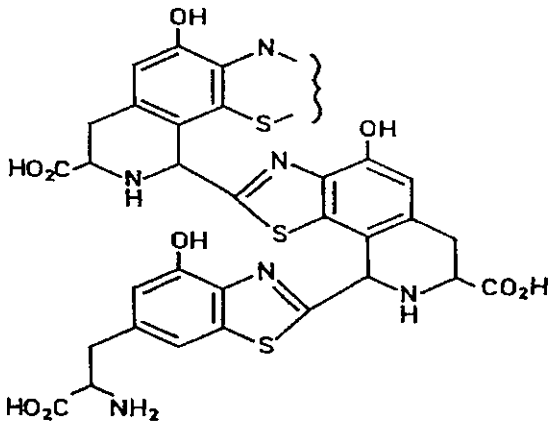

Figure 3.13: General structure of Carotenoids

3.9.5 Lycopene

Lycopene is a bright red carotene and carotenoid pigment and phytochemical found in tomatoes and other red fruits and vegetables, such as red carrots, watermelons and papayas. Lycopene has a structure similar to that of the well-known antioxidant beta-carotene, but its antioxidant activity is much stronger. Lycopene is especially effective at quenching a free radical called singlet oxygen. Singlet oxygen is a highly reactive free radical formed during normal metabolic processes that reacts with polyunsaturated fatty acids, which are major constituents of cell membranes. Due to the fact that lycopene is commonly located in cell membranes, it plays an important role in preventing oxidative damage to the membrane lipids, thereby influencing the thickness, strength, and fluidity of the membranes. Cell membranes are the gatekeepers of the cell, allowing nutrients in, while preventing toxins from entering and facilitating the removal of cellular garbage. Maintaining the integrity of cell membranes is a therefore key factor in the prevention of diseases.

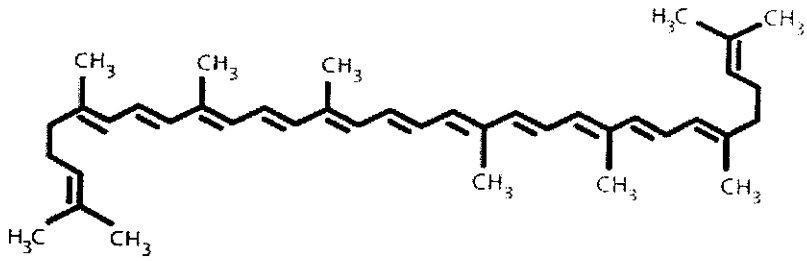

Figure 3.14: General structure of Lycopene

3.10 Antioxidant capacity assays

3.10.1 DPPH radical scavenging activity

The 1,1-diphenyl -2-picryl hydroxyl (DPPH) radical was widely used as the model system to investigate the scavenging activities of several natural compounds such as phenolic and anthocyanins or crude mixtures such as the ethanol extract of plants. DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH. When DPPH is placed in an assay system containing free radical scavengers such as flavonoids, the color vanishes. The color changes from purple to yellow after reduction, which can be quantified by its decrease of absorbance at wavelength 517nm. Radical scavenging activity increased with increasing percentage of the free radical inhibition.

3.10.2 Nitric oxide scavenging assay

Nitric oxide is a potent phototropic mediator of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity.

3.10.3 Ferric Reducing Antioxidant Potential Assay

The reducing capacity was investigated by measuring Fe^{3+}- Fe^{2+} conversion. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The antioxidant activities of putative antioxidants have been attributed to various

mechanisms such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued proton abstraction and radical scavenging.

3.11 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.

3.12 Antioxidants from natural dietary sources

A variety of fruits, vegetables, nuts, whole grain cereals and legumes, tea, red wine, and herbs (eg. rosemary extract) are rich sources of natural antioxidants. The benefit of eating whole fruits, vegetables, and whole grain foods, in contrast to antioxidant supplements, is that they contain a variety of natural antioxidants. A combination of multiple antioxidants has greater health benefit than when an antioxidant is taken individually. Furthermore, the antioxidant content tends to be high on their outer layer of the plant products. For example, in cereals, the bran is richer in antioxidants such as phenolic acids and phytic acid than the inner part of the grain. A study comparing high bran, whole grain cereals and refined wheat cereals documented that the high bran and whole grain cereals contained higher antioxidants content than the refined wheat cereal.

Foods high in antioxidants include fruits such as apple, grape, grapefruit, crane berry, black berry and blueberry. Even though, comprehensive studies on antioxidant content of foods are yet to come, among the best antioxidant sources are crane berry, black berry and blue berry. Vegetables are also rich antioxidant sources. Red bean, pinto bean, kidney bean, carrot, tomato, garlic and Russet potato are among the vegetables high in antioxidant content and categorized among the best antioxidant sources. Other important sources of antioxidants are green tea, black tea, herbal tea, spices, red wine, ginger, and garlic.

3.13 Importance of Antioxidant rich dietary sources

The best known sources of antioxidants are fruits and vegetables. Natural antioxidants are as important to plants as they are to humans for preventing oxidative stress and damage from UV light. Multiple types of natural antioxidants are needed to maintain the complex system that prevents cell damage and death. The best antioxidant diet should include a variety of different colored fruits and vegetables. The colors are, in some cases, the source of antioxidants. Antioxidants are vitamins and compounds that reduce the effects of oxidative stress on the human body. Oxidative stress can lead to the formation of free radicals, which through a chain reaction can lead to the growth of cancerous cells and tumors.

3.14 BANANA SPECIES

The bananas all belong to the genus ***Musa*** in the family ***Musaceae***.

Kingdom:Plantae

Division:Magnoliophyta

Class:Liliopsida

Order:Zingiberales

Family:Musaceae

Genus: *Musa*

Bananas are a large, monocotyledonous herb belongs to the Musaceae family of the order *Zingiberales*. The edible bananas cultivars are mostly derived from two wild species of genus *Musa* (section Eumusa) namely *Musa acuminate* and *Musa balbisiana.*

There are over 300 varieties of bananas worldwide and is eaten around the world. Bananas are rich in potassium, calcium, magnesium phosphorus and iron. They are also a good source of Vitamins A and C as well as thiamine, riboflavin and niacin. Bananas also contain all 8 essential amino acids. Bananas are a good fuel for the brain because the potassium helps you concentrate and think clearer. Potassium is perhaps the best fuel for the brain. Bananas are rich in fiber, which absorbs water giving you a full feeling - great for those trying to lose weight. It is rich in magnesium (helps protect circulatory system), potassium and slowly-absorbed sugars. Good source of pectin (a soluble fiber). Prevents radical swings in blood sugar.

3.14.1 Calories in a Banana

The number of calories in a banana can be determined only by its size. The major source of calories in bananas comes from carbohydrates. These carbohydrates contain sugar and starch; however, the level of sugar rises and that of starch lowers when the banana starts ripening. It has been found that more than half of the calories of a banana come from sugar, while the rest of the calories come from proteins and a little from fats. Moreover, because bananas contain lots of water and are free from saturated fat, they are really good for health. The calories in a banana provide our body with the energy required for performing daily activities. Body builders, athletes, and those who work out to remain fit are recommended to eat at least one banana an hour before they start their exercise regime. Even a single banana can give them the energy to work out continuously for more than ninety minutes. As bananas contain very less fat, they are also a necessary food item for people who want to lose weight.

3.14.2 Nutritional values of Banana

Macronutrients	Banana / 1 fruit
Water	88.39
Calories	109
Protein (g)	1
Carbohydrates (g)	28
Dietary fiber (g)	3.1
Sugars(g)	14.43
Total fat (g)	0.6
Saturated fat (g)	0.2
Monounsaturated fat(g)	0.1
Polyunsaturated fat (g)	0.1

Table 3.1 Macronutrient values of banana

Micronutrients	**Banana/ 1 fruit**
Potassium (mg)	467
Sodium (mg)	1
Calcium (mg)	9.2
Magnesium (mg)	44.1
Phosphorus (mg)	25
Zinc (mg)	18
Iron (mg)	0.31
Vitamin C (mg)	10.3
Thiamin (mg)	0.037
Riboflavin (mg)	0.086
Niacin (mg)	0.785
Pantothenic acid (mg)	0.394
Vitamin B6 (mg)	0.433

Vitamin B12 (mcg)	0
Folate (mcg)	24
Vitamin A (IU)	76
Vitamin E (mg)	0.12
Vitamin K (mcg)	0.6
Beta carotene (mcg)	31

Table 3.2: Micronutrient Values of Banana

3.15 Lutein

Lutein is a carotenoid, found in dark green leafy vegetables such as spinach, plus various fruits and corn. Egg yolks are also sources of lutein. Lutein provides nutritional support to our eyes and skin – the only organs of the body directly exposed to the outside environment. Lutein has been linked to promoting healthy eyes through reducing the risk of macular degeneration. Other studies suggest that a mixture of nutrients, including lutein, may provide supplemental antioxidant capacity to the skin, helping counteract free radical damage (Morganti Morganti *et al.* 2002).

Lutein is an antioxidant that appears to quench or reduce harmful free radicals in various parts of the body. Free radicals can play a role in a variety of chronic diseases.

Lutein also filters the high-energy, blue wavelengths of light from the visible-light spectrum by as much as 90% (Krinsky et al.,2003) Blue light, in both indoor lighting and sunlight, is believed to induce oxidative stress and possible free-radical damage in human organs exposed to light, such as the eyes and skin. Blue light is not the same as the commonly known ultraviolet A and ultraviolet B wavelengths of the invisible spectrum.

3.15.1. Dosage

Research suggests a minimum of 6-10 mg per day of lutein from dark green leafy vegetables and other sources is necessary to realize lutein's health benefits. Lutein is widely available in a variety of nutritional supplements and fortified foods and beverages for people wanting to supplement their dietary intake of lutein, making their diet even better for their eyes and skin.

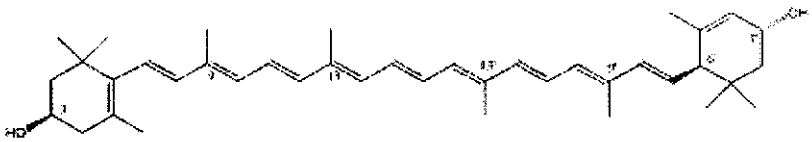

Fig. 3.15 Structure of lutein

3.15.2 Mechanism of action

Lutein is an important compound in the human body, but the body does not manufacture lutein. Eating foods containing lutein or consuming dietary supplements that contain lutein is the only way for your body to get lutein. Lutein is present in the eye, blood serum, skin, cervix, brain and breast. Within the eye, lutein is highly concentrated in the macular region of the retina and is dispersed in lower amounts throughout the retina and lens. Within the skin, lutein appears to be deposited in the epidermis (outer layers) and dermis (inner layers), providing its antioxidant and blue-light absorption functions throughout the depth of the skin.

3.16 Therapeutic uses of lutein:

3.16.1 Age-related macular degeneration (AMD)

Macular degeneration, commonly referred to as age-related macular degeneration (AMD), is an eye disease affecting the central retina (the macula), which can lead to permanent vision loss. AMD has no cure, and only limited treatments are available, but lutein might be a possible treatment for AMD patients. AMD is the number cause of permanent vision loss among patients over the age of 60, according to All AboutVision.com. About 85 to 90 percent of patients with AMD have the "dry" form of the disease that has not yet advanced to its serious

vision-loss-threatening phase; these patients have a 10 percent chance of the disease advancing and leading to vision loss.

Lutein is a natural antioxidant found throughout the body, and is particularly abundant in the eye. It benefits the eye with protective capabilities, such as filtering harmful light, and strengthens the eye's macula. Loss and weakening of natural lutein occurs with age. The National Eye Institute (NEI) concluded a study of the efficacy of potent vitamin and antioxidant supplements on AMD patients. The results showed a 25 percent risk-reduction for AMD disease progression to vision-loss stages. The NEI's second study, currently in progress as of late 2009, is researching the effects of lutein (among other nutrients) on AMD, to prove it can help prevent vision loss.

3.16.2 Atherosclerosis

Atherosclerosis is a condition in which fatty material collects along the walls of arteries. This fatty material thickens, hardens (forms calcium deposits), and may eventually block the arteries.Research suggests lutein plays a significant role in human health. For cardiovascular health, lutein present in blood serum may favorably impact arterial wall thickening, a component of atherosclerosis. At least one published study produced findings that suggested higher levels of lutein in the serum may be linked with less thickening of arterials walls (Dwyer., Navab *et al.* 2001).

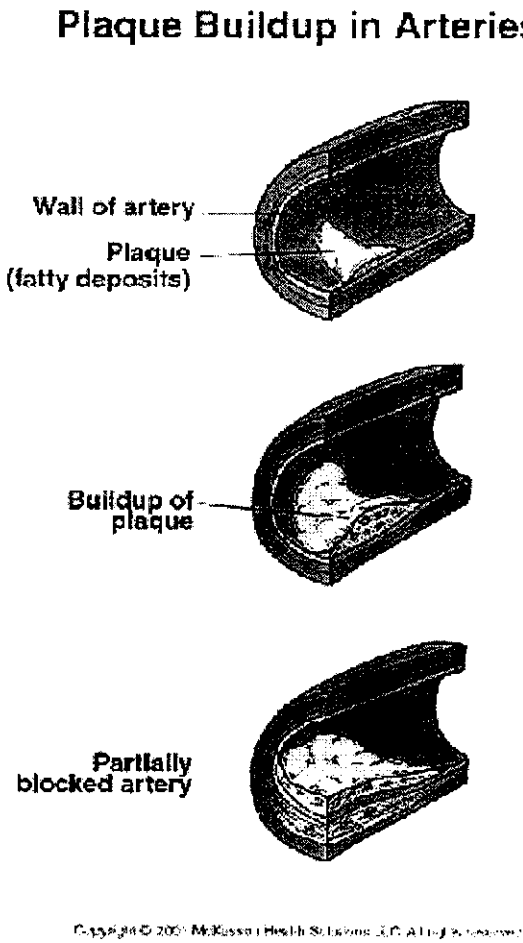

Figure 3.16 Plaque formation in arteries

3.16.3 Cataract

A cataract is a clouding that develops in the crystalline lens of the eye or in its envelope, varying in degree from slight to complete opacity and obstructing the passage of light. Lutein and zeaxanthin, carotenoids found in dark leafy green vegetables, were nearly 10 times more powerful than the antioxidant vitamin E in protecting human eye cells from UV-induced damage

The report from a research project called the Beaver Dam Eye Study, which involves adults from 43-84 years of age, suggests that lutein and zeaxanthin intake may reduce the incidence of cataracts (Lyle *et al.* 1999). Lutein and zeaxanthin are the only carotenoids found in the lens (Yeum *et al.*, 1995).

Chasan-Taber and co-workers conducted a prospective study of 77,466 female nurses 45-71 years old, from 1980 through 1992. The results showed that nurses with the highest intake of lutein and zeaxanthin had 22 percent lower risk of cataract extraction compared to those in the lowest quintile of intake. This study also showed that high intake of spinach and kale, green vegetables rich in lutein, may reduce the risk of cataract extraction (Chasan-Taber,Willett, *et al.* 1999).

3.16.4 Diabetes mellitus

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia. It is often associated with complications, such as cataracts and increased susceptibility to frequent and protracted infections. High glucose levels induce oxidative stress in immune system cells, both *in vitro* and *in vivo*, as well as an increase in their Nuclear Factor-kappa B activity. A study conducted by Muriach and co-workers (2008) demonstrated that lutein treatment of lymphocytes from diabetic rats inhibited oxidative stress and reduced the Nuclear Factor-kappa B activity. They concluded that lutein is a potential candidate for the reduction of susceptibility to infections of diabetic patients.

3.16.5 Skin health

Skin is the largest organ of the human body. Along with our eyes, it is the only organ of the body constantly exposed to the environment. Skin is "assaulted" by

- Light (especially ultraviolet and visible wavelengths)
- Environmental pollutants

Such exposure can create reactive oxygen species, leading to cell-damaging free radicals within skin. The skin provides numerous functions. It acts as a barrier of protection for the internal organs. It regulates body temperature. It plays an important role in immunological response. Therefore, it is important to protect the skin.

A recent human clinical study showed 10 mg of lutein daily increased skin hydration. elasticity and skin lipid content (Morganti *et al.* 2006). This is the first research to show improvement in skin health through lutein supplementation alone.

3.16.6 Genital warts

Genital warts (or Condylomata acuminata, venereal warts, anal warts and anogenital warts) is a highly contagious sexually transmitted disease caused by some sub-types of human papillomavirus (HPV). It is spread through direct skin-to-skin contact during oral, genital, or anal sex with an infected partner. Warts are the most easily recognized symptom of genital HPV infection, where types 6 and 11 are responsible for 90% of genital warts cases. Lutein found in banana peel is effective in treating genital warts. A common method of curation involves direct application of banana peel to the skin (O'Mahony, 2005).

3.17 Thin layer chromatography

TLC is a form of liquid chromatography consisting of a mobile phase (developing solvent) and a stationary phase (a plate or strip coated with a form of silica gel). Analysis is performed on a flat surface under atmospheric pressure and room temperature. TLC is one of the simplest, fastest, easiest and least expensive of several chromatographic techniques used in qualitative and quantitative analysis to separate organic compounds. Michael Tswett is credited as being the father of liquid chromatography. Tswett developed his ideas in the early 1900's.The mode of separation is generally by adsorption or partition. The more polar components will be adsorbed preferentially by the polar layer. Hydrogen Bonding is the main force controlling adsorption between the silica gel surface and the analyte functional groups (Pratheesh,*et al.*2009).

3.18 Column chromatography

It is a method used to purify individual chemical compounds from mixtures of compounds. It is often used for preparative applications on scales from micrograms up to kilograms. The main advantage of column chromatography is the relatively low cost and disposability of the stationary phase used in the process. Two methods are generally used to prepare a column; the dry method, and the wet method.. For the dry method, the column is first filled with dry stationary phase powder, followed by the addition of mobile phase, which is flushed through the column until it is completely wet, and from this point is never allowed to run

dry. For the wet method, a slurry is prepared of the eluent with the stationary phase powder and then carefully poured into the column. Care must be taken to avoid air bubbles (Thammanna, *et al*.2010).

A solution of the organic material is pipetted on top of the stationary phase. This layer is usually topped with a small layer of sand or with cotton or glass wool to protect the shape of the organic layer from the velocity of newly added eluent. Eluent is slowly passed through the column to advance the organic material. Often a spherical eluent reservoir or an eluent-filled and stoppered separating funnel is put on top of the column. The stationary phase or adsorbent in column chromatography is a solid. The most common stationary phase for column chromatography is silica gel followed by alumina. There is an optimum flow rate for each particular separation. A faster flow rate of the eluent minimizes the time required to run a column and thereby minimizes diffusion, resulting in a better separation (Laurence, *et al.2003).*

3.19 BANANA VARIETIES

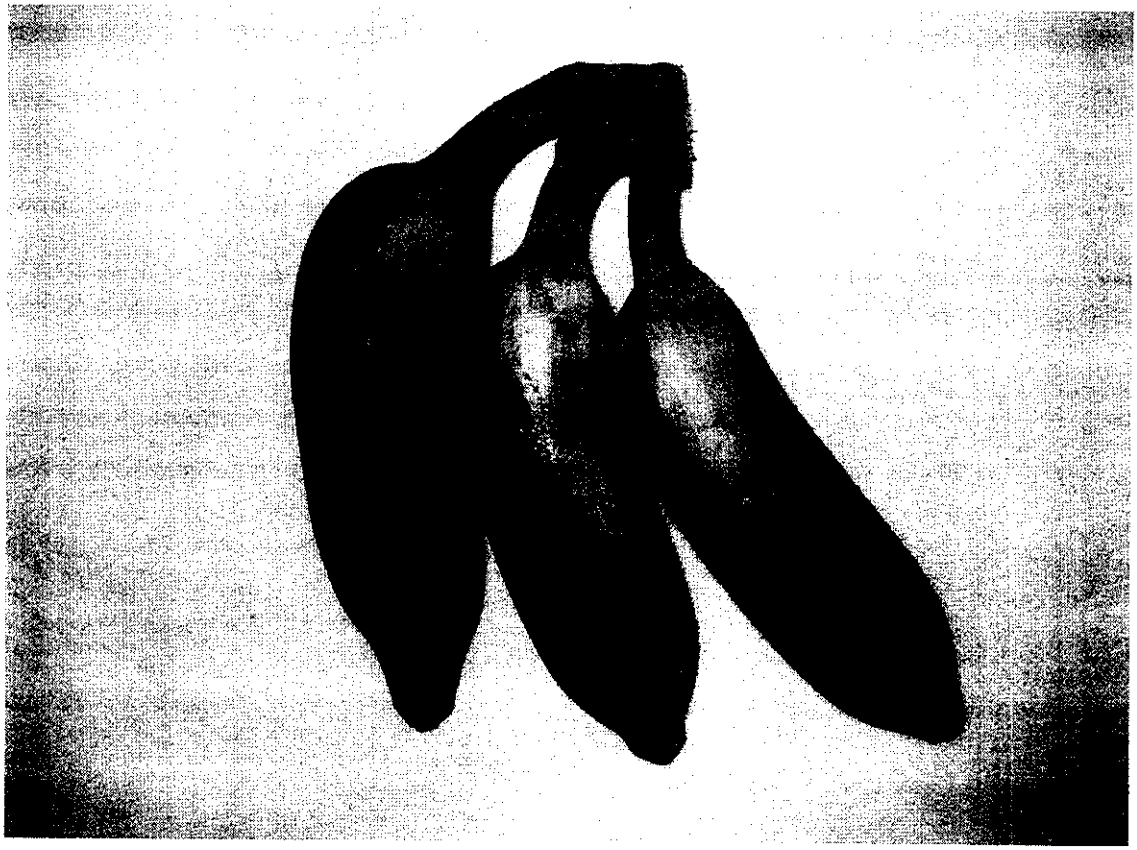

Figure 3.17: Banana cv. Kadali [*Musa spp* - Ney Poovan - AB]

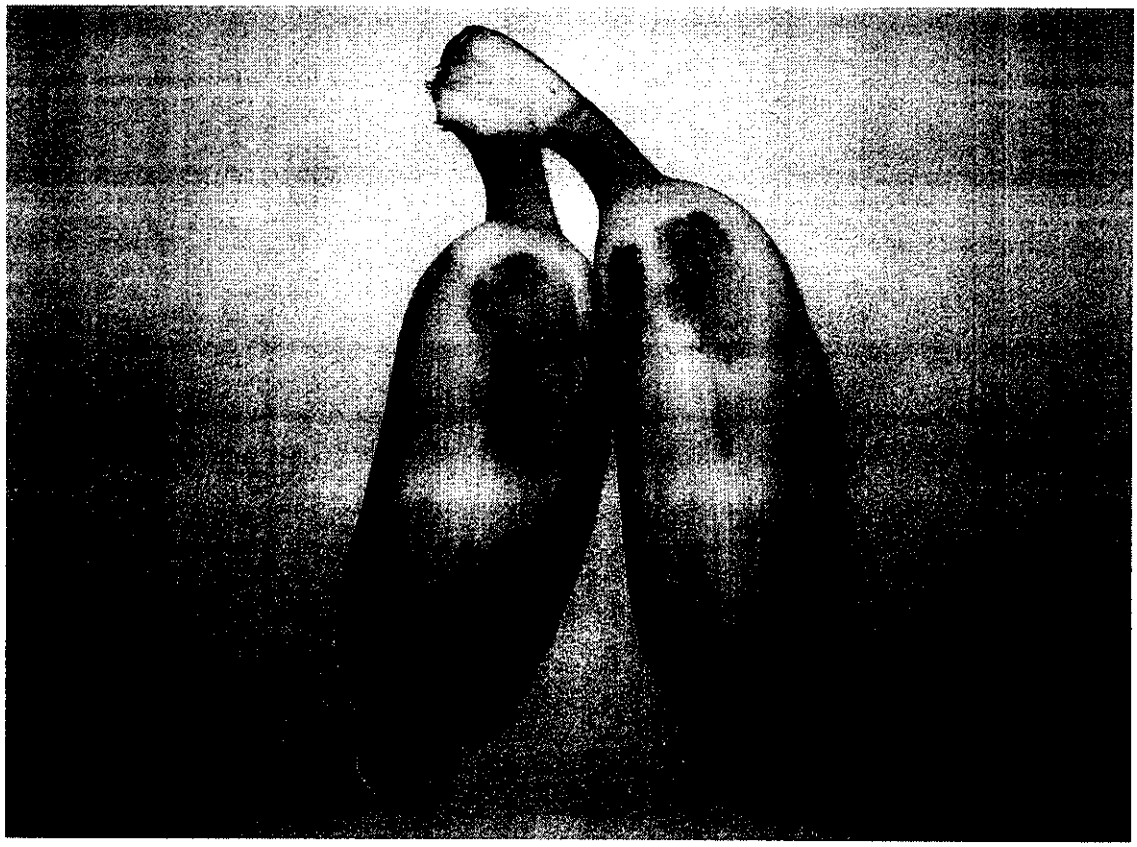

Figure 3.18: Banana cv. Karpooravalli [*Musa spp* –Karpooravalli - ABB]

Figure 3.19: Banana cv. Nendran [*Musa spp* - French Plantaini - AAB]

Figure 3.20: Banana cv. Poovan [*Musa spp* - Mysore - AAB]

Figure 3.21: Banana cv. Pachanadan [*Musa spp* - Pachanadan - AAA]

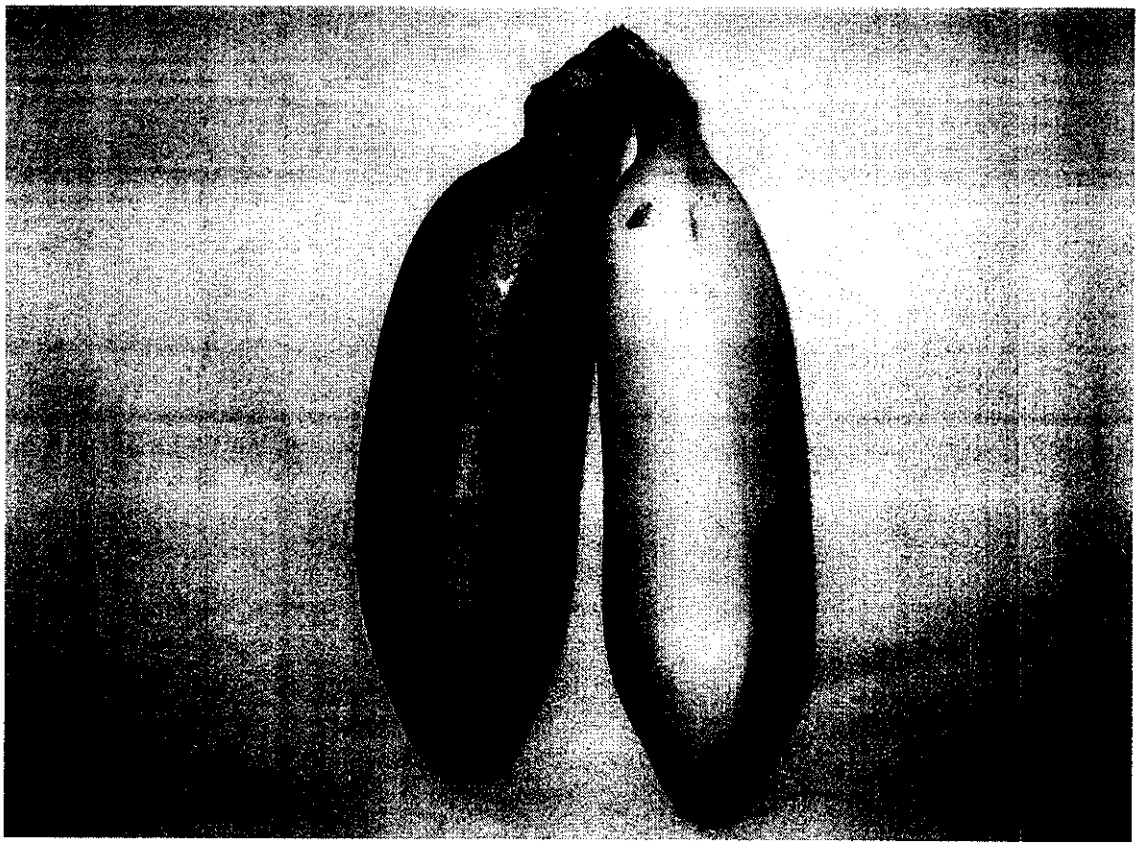

Figure 3.22: Banana cv. Rasthali [*Musa spp* - Rasthali - AAB]

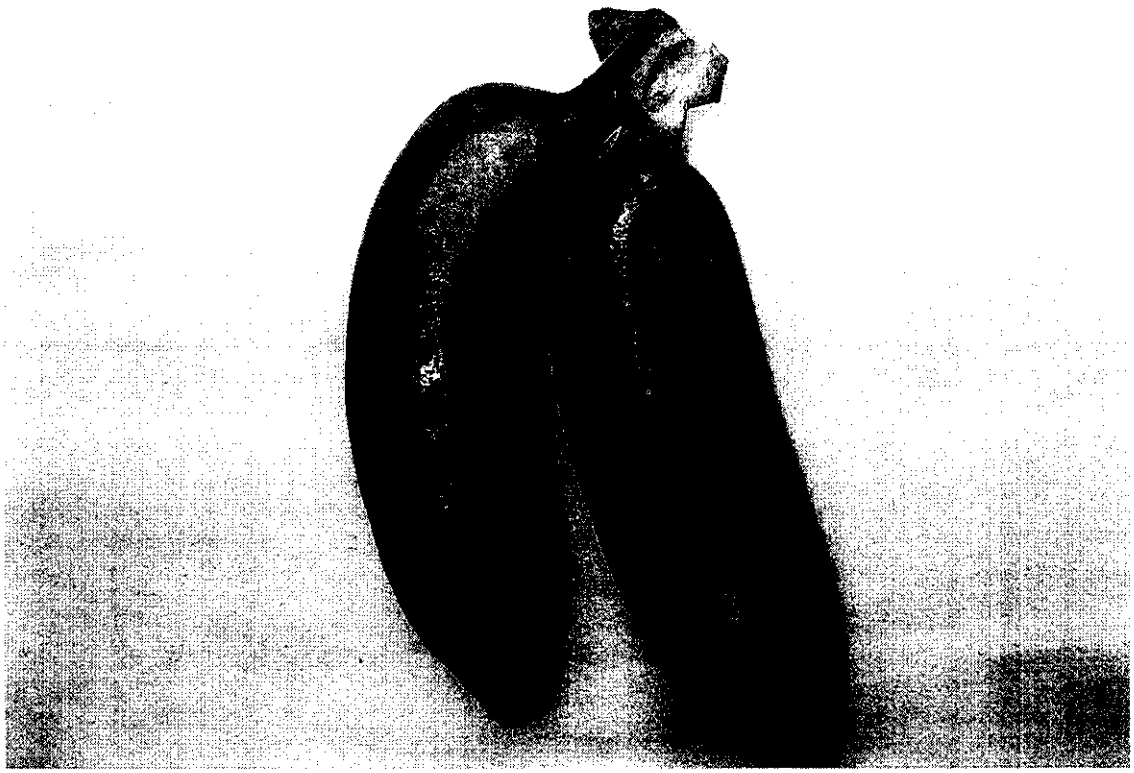

Figure 3.23: Banana cv. Red banana - [*Musa spp* – Red banana – AAA]

MATERIALS AND METHODS

4. MATERIALS AND METHODS

4.1 Chemicals used

Petroleum ether, hexane, dichloro methane, methanol, acetone, Sulphuric acid, ammonium molybdate, disodium hydrogen phosphate, 1,1-Diphenyl-2-Picryl hydrazyl (DPPH), sodiumnitroprusside, sulphanilamide, napthyl ethylene diamine dihydrochloride, o-phosphoric acid, potassium dihydrogen phosphate, Ethylene diamine tetraacteic acid (EDTA), ferric chloride, trichloroaceticacid (TCA), potassium ferricyanide, ascorbic acid, riboflavin, nitro blue tetrazolium (NBT). All reagents were used for analytical grade.

4.2 Banana varieties

1. Banana cv. Kadali-[*Musa spp* - Ney Poovan - AB].
2. Banana cv. Karpooravalli-[*Musa spp* - Karpooravalli - ABB].
3. Banana cv. Nendran-[*Musa spp* - French Plantaini - AAB].
4. Banana cv. Poovan-[*Musa spp* - Mysore - AAB].
5. Banana cv. Pachanadan-[*Musa spp* - Pachanadan - AABS].
6. Banana cv. Rasthali - [*Musa spp* - Rasthali - AAB].
7. Banana cv. Red banana - [*Musa spp* – Red banana - AAA].

All the above seven banana varieties were identified and authenticated for their scientific names by Dr.T.N.Balamohan, The Professor & Head, Department of Fruits & Crops, Horticultural College & Research Institute, TNAU, Coimbatore.

4.3 Methods

4.3.1 Phase 1

4.3.1.1 Estimation of total carotenoids in banana varieties

Principle

The total carotenoids in the sample are extracted with petroleum ether. The total carotenoids are estimated spectrometrically at 450nm.

Reagents

1) 12% KOH.

2) Ethanol.

3) Petroleum ether.

4) Sodium sulphite.

Procedure

5-10g of sample was saponified using 12% KOH in ethanol and kept for 30 minutes in a shaker. The sample was transferred to a separating funnel containing 10-15ml of petroleum ether and mixed gently. This was allowed to stand till the layers were separated completely. The pigments were collected in the petroleum layer. This was then transferred to the separating funnel and extracted using petroleum ether homogenous phase. The extraction of the aqueous phase was repeated with petroleum ether until it became colourless. The aqueous layer was discarded. To the extract sodium sulphite was added to remove turbidity. The final volume of extract was noted to a known volume. The absorbance was read at 450nm.

4.3.2 Phase 2

4.3.2.1 Extraction of carotenoids

Reagents

1.2% ethanolic KOH

2. Hexane

Procedure

Extraction of carotenoid was carried out by taking 10 g of the each fresh samples with 100 ml of the 2% ethanolic KOH. The mixture ground well for 5 minutes using pestle and mortar at room temperature in dim light. The extraction was repeated till the resultant extract was

colourless. Total volume of the ethanol extract was 500ml and which was concentrated to 50ml. Hexane was added to the ethanol extract in the ratio of 1:2 in a separating funnel, shaken well for 5 minutes and kept in the dark for 15 minutes. Two phases were separated and the solvent partitioning was repeated till the hexane extract was colourless. All the hexane phases were pooled and flash evaporated at 300C and redissolved in known volume of hexane (Thammanna Gowda *et al.*, 2010).

4.3.2.2 Separation and isolation of lutein by Thin layer chromatography

Principle

Separation of compounds based on the competition of the solute and the mobile phase for binding places on the stationary phase. For instance, if normal phase silica gel is used as a stationary phase it can be considered as polar. The components present in the sample which differ in polarity, the more polar compounds has a strong interaction with silica and is therefore more capable to dispel the mobile phase from the binding places. Consequently the less polar compound higher up the plate (resulting in a higher R_f value). If the mobile phase is changed to polar solvent or a mixture of solvents, it is more capable of dispelling solutes from the silica binding places and all compounds on the TLC plate will move higher up the plate.

Reagents

1. Silica gel
2. Hexane: Acetone (7:3)

Procedure

Silica gel is used as adsorbent material for Thin layer chromatography. The mobile phase solvent comprises of hexane and acetone (7:3) (Thammanna Gowda *et al.*, 2010).The sample of volume 100 µl is loaded on the TLC plate and it moves up the plate due to capillary action. The R_f is calculated and it is compared to R_f value of standards.

4.3.2.3 Isolation of lutein using column chromatography

Principle

Column chromatography is one of the most useful methods for the separation and purification of both solids and liquids in carrying out small scale experiments. Column chromatography is another solid-liquid technique in which the two phases are a solid (stationary phase) and the liquid (mobile phase). The theory of column chromatography is analogous to that

of TLC. The most common adsorbents are silica gel and neutral alumina. The eluent focus down to the column filled with the adsorbent. There is an equilibrium established between the solute adsorbed on the silica/ alumina, eluting solvent following down to the column.

Reagents

1. Neutral alumina
2. Hexane
3. Dichloro methane: methanol(1:1)

Procedure

Carotenoids such as β-carotene and lutein were separated by open column chromatography (Rangaswamy *et al.*, 2005) (25x 2.0 cm). Neutral alumina was used as an adsorbent (70-230 mesh). A known amount of aliquot of hexane extract was applied to the column. The β-carotene was eluted with hexane and the lutein was eluted with methanol: dichloro methane (1:1 v/v). Collect the fractions of volume 2ml at the rate of 0.5ml/min. Measure the absorbance of the eluted samples at 450 nm.

4.3.3 *IN VITRO* ANTIOXIDANT CAPACITY ASSAYS

4.3.3.1 Total Antioxidant Capacity Assay (Prieto, 1999)

Principle

This assay is based on the principle of reduction of Molybdenum (VI) to Molybdenum (V) by the extract and the subsequent formation of green phosphate /molybdenum complex at acid pH.

Reagents

1. Reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate)
2. Ascorbic acid

Procedure

The working solutions (1-10 mg/ml) of the samples were prepared by dissolving the extracts in water. 0.2 ml of the extracts were mixed with 2ml of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate).The tubes were capped with

silver foil and incubated at 95°C for 90 minutes. The tubes were then cooled to room temperature and the absorbance was measured at 695nm against a blank. Ascorbic acid was used as the standard. The total antioxidant capacity was expressed as ascorbic acid equivalent (Raghavan Govindarajan *et al.*, 2003 and Umamaheshwari *et al.*, 2008)

4.3.3.2 Determination of DPPH Radical Scavenging Activity (Shimada *et al.*, 1992)

Principle

DPPH scavenging activity was measured by the slightly modified spectrophotometric method. 1,1-diphenyl-2-picryl-hydrazyl radical is scavenged by antioxidants through the donation of protons forming reduced DPPH. The electrons become paired off and the solution loses color depending on the number of electrons taken up. The color change from purple to yellow after reduction is quantified by the decrease of absorbance at 517nm (Ajay Sharma *et al.*, 2007).

Reagents

1. Methanol
2. DPPH in methanol (0.004%)

Procedure

The banana extracts were dissolved in ethanol. A solution of DPPH in methanol (0.6mM) was prepared freshly. 3ml of this solution was mixed with 1ml of the samples of varying concentrations (1-10 mg/ml).The solution in the test tubes were vortexed and incubated in the dark for 30 min at room temperature. The decrease in absorbance was measured at 517nm.The control had equal volume of DPPH in methanol instead of extract. 5ml of methanol was taken as blank. The percentage inhibition of the radicals due to the antioxidant property of the extract was calculated using the formula:

$$\%\text{inhibition} = [(A_{\text{control}} - A\ \text{sample})/ A_{\text{control}}] * 100$$

4.3.3.3 Determination of Nitric Oxide Radical Scavenging Activity

(Raghavan Govindarajan *et al.*, 2003).

Principle

Nitric oxide scavenging was measured spectrophotometrically. The nitric oxide generated using sodium nitroprusside is converted into nitrite ions. The chromospheres are formed due to the diazotization of nitrite ions with sulfanilamide and subsequent coupling with naphthyl ethylene diamine. This is measured at 546 nm (Raghavan Govindarajan *et al.*, 2003).

Reagents

1. Sodium nitroprusside (5mM)
2. Phosphate buffer saline
3. Griess reagent

Procedure

Sodium nitroprusside (5mM) was prepared in Phosphate buffer saline.1 ml of this was mixed with 1 ml of extracts of different concentrations (1-10 mg/ml) in methanol. The mixture was incubated at 25°C for 30 min. After 30 min, an equal volume of Griess reagent was added to the incubated solution. The absorbance of the chromophore formed due to diazotization of nitrite with sulfanilamide and subsequent coupling with naphthylethylene diamine was measured at 546 nm. Control was a solution of reagents devoid of extracts.

4.3.3.4 Determination of Superoxide radical Scavenging Activity

Reagents

1. Phosphate buffer
2. Riboflavin
3. EDTA
4. NBT
5. Sodium cyanide

Procedure

The scavenging activity towards the superoxide radical ($O_2^{\cdot-}$) was measured in terms of inhibition of generation of $O_2^{\cdot-}$ (Sanchez-Moreno, 2002). The reaction mixture consisted of 2.0 ml of phosphate buffer (50 mM, pH 7.6), 0.2 ml of riboflavin (20 µg / 0.2 ml), 0.2 ml of EDTA (12 mM), 0.2 ml of NBT (0.1 mg / 3ml) and 0.2 ml of sodium cyanide (3µg / 0.2 ml) Test compounds of various concentrations of (1- 10 mg / ml) were added to make a total volume of 3.0 ml. The absorbance was read at 530 nm before and after illumination under UV lamp for 15 minutes against a control with buffer instead of sample and 3.0 ml of buffer as blank.

4.3.3.5 Determination of Ferric Reducing Antioxidant Potential Activity

(Oyaizu, 1986)

Reagents

1.Phosphate buffer (0.2M, pH 6.6)

2.1% Potassium ferricyanide

3.10% TCA

4. 0.1% Ferric chloride

Procedure

Different concentrations of the samples (1-10 mg/ml) were prepared by dissolving the extracts in water. 2.5ml of the samples were mixed with 2.5ml of phosphate buffer (0.2M, pH 6.6) and 2.5ml of 1% potassium ferricyanide. The tubes were incubated at 50°C for 20 min. To the incubated solutions, 2.5ml of 10% TCA was added. The solutions were centrifuged at 650g for 10 min. About 5ml of the supernatant was withdrawn from each tube. To this, 1ml of 0.1% ferric chloride was added. The absorbance was measured at 700nm. A higher absorbance indicated a higher reducing power. The blank was chosen as 5ml of buffer with 1ml of ferric chloride.

RESULTS AND DISCUSSIONS

5. Results and Discussion

A free radical is nothing more than a molecular structure which contains an unpaired electron. Its random and wild molecular movements within cellular material can create cellular damage, which can eventually result in degeneration or mutation.

A free radical can destroy a protein, an enzyme or even a complete cell. To make matters worse, free radicals can multiply through a chain reaction mechanism resulting in the release of thousands of these cellular oxidants. When this happens, cells can become so badly damaged that DNA codes can be altered and immunity can be compromised. Contact with a free radical or oxidant on this scale can create cellular deterioration, resulting in diseases like cancer. Tissue breakdown from this oxidative stress can also occur, which contributes to aging, arthritis and a whole host of other degenerative conditions.

Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Bananas are one of the most popular fruits on the world and it well be known that fruits contain various antioxidants compounds such as gallocatechin and dopamine. Since the banana fruits are widely available, they been used as food without apparent toxic effect. Lutein a xanthophyll and naturally-occurring carotenoids is employed by organisms as an antioxidant and for blue light absorption. Lutein provides nutritional support to our eyes and skin – the only organs of the body directly exposed to the outside environment. Lutein has been linked to promoting healthy eyes through reducing the risk of macular degeneration.

5.1 Phase 1

5.1.1 Estimation of total Carotenoids

Caroetnoids are one of the most important classes of plant pigments and play a crucial role in defining the quality parameters of fruits and vegetables(Wang *et al.*,2003) .Pachainadan showed highest content of carotenoidsThe data obtained are displayed in theTable 5.1

Banana varieties	Total Carotenoids
Kadali	9.23±0.31
Karpooravalli	16.30±0.30
Nendran	5.66±0.73
Pachanadan	**52.43±0.19**
Poovan	32.10±0.57
Rasthali	6.23±0.31
Sevvazhai	13.05±0.12

Table 5.1 Total Carotenoid contents in the peel extracts of seven varieties of Banana.

Values represent mean ± SD of 3 replicates

Hence, the Pachainadan variety was taken to identify lutein using chromatographic techniques and to carry out further assays to determine its antioxidant potential.

5.2 Phase 2

5.2.1 Identification of Lutein using thin layer chromatography

The thin layer chromatography was performed for the extract obtained using mobile phase hexane: acetone (7:3) and the no. of spots obtained was 6. The R_f obtained for the lutein component is 0.15 when hexane: acetone is used as the mobile phase (Thammanna Gowda *et al.*, 2010).

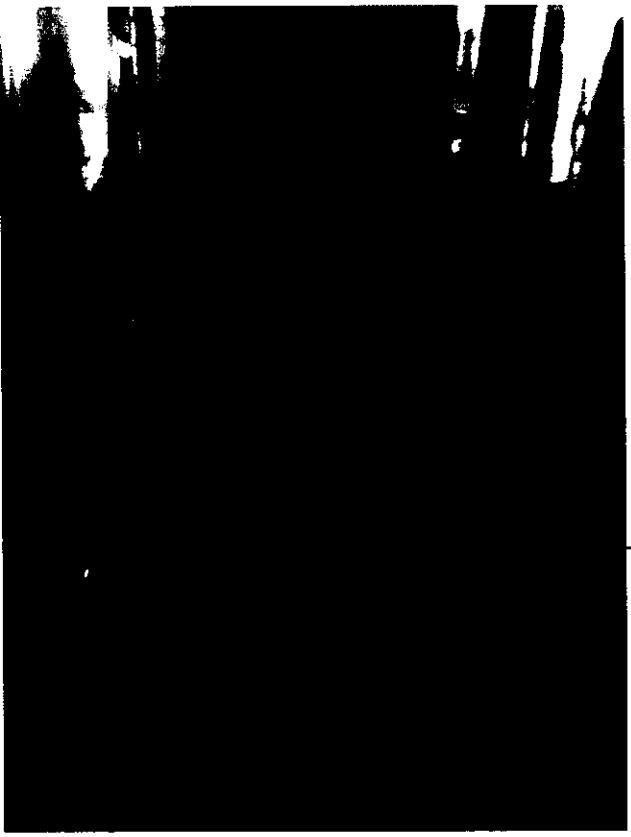

Figure 5.1 TLC plate showing the distribution of various components.

5.2.2 Isolation of lutein using column chromatography

The solvents like hexane and methanol: dichloromethane (1:1) were used to elute β-carotene and lutein. The various fractions were collected at the rate of 0.5ml/min. The absorbances of the various fractions collected were measured and the sample with high absorbance was loaded on the TLC plate and the R_f value obtained is compared with crude lutein's R_f value (Buddhika Priyadarshani, 2001).Tha following graph shows the absorbance values of the eluted fractions of β-carotene and lutein.

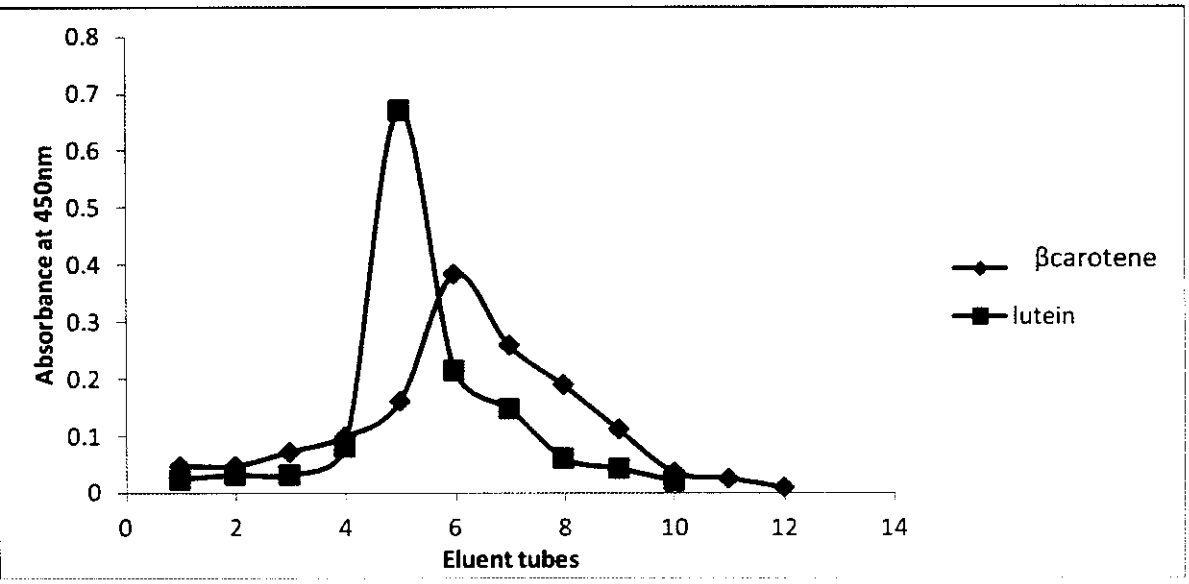

Figure 5.2 Comparision of the absorbance of eluted β-carotene and lutein.

5.3 Phase 3

5.3.1 *IN VITRO* ANTIOXIDANT CAPACITY ASSAYS

5.3.1.1 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 lutein as a scavenger of free radicals (Madan *et al.*, 2005).

Concentration of the sample (mg)	Ascorbic acid equivalent (AAE) µM/g
1	16.88±0.06
2	27.37±0.10
3	33.91±0.17
4	42.92±0.08
5	49.05±0.27
6	49.71±0.39
7	56.71±0.19
8	66.47±0.26
9	73.83±0.17
10	78.63±0.46

Table 5.2 Total antioxidant activity in Ascorbic acid equivalents of the crude lutein.

Values represent mean ± SD of 3 replicates.

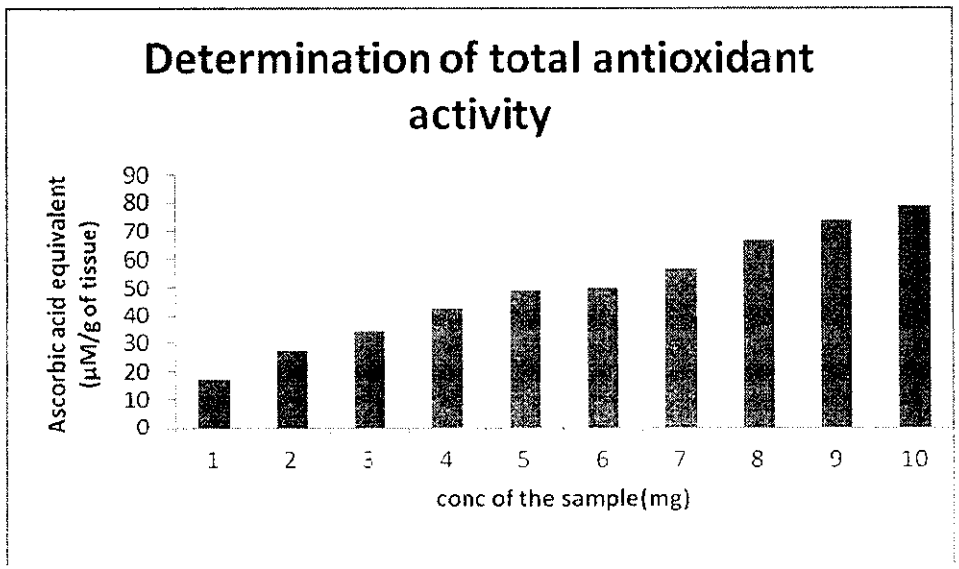

Figure 5.3 Total antioxidant activity in Ascorbic acid equivalents of lutein.

The significant activity was found with the highest concentration of the sample as depicted in table 5.2

5.3.1.2 DPPH radical scavenging activity

A rapid simple inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH). DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of foods. A simple method that has been developed to determine the antioxidant activity the antioxidant activity utilizes the stable DPPH radical. The odd electron in the DPPH free radical gives a strong absorption maximum at 517nm and is purple in colour. The colour turns from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with a hydrogen from a free radical scavenging antioxidant to form a the reduced DPPH-H. The resulting decolorization is stoichiometric with respect to number of electrons captured (Baskar *et al.*,2007).

Extent of DPPH radical scavenged was determined by the decrease in intensity of violet colour in the form of OD values. A descrease in OD values with increasing concentration represents higher antioxidant activity.

Concentration of the sample (mg/ml)	% inhibition of the sample	Concentration of the standard (µg/ml)	Ascorbic acid
1	18.00±0.360	10	49.66±2.08
2	23.00±2.149	20	55.59±2.43
3	23.80±0.544	30	65.41±1.71
4	24.20±0.586	40	73.98±2.59
5	24.98±0.049	50	79.30±1.80
6	25.69±0.127	60	84.38±2.06
7	26.68±0.289	70	87.99±0.99
8	28.00±0.318	80	90.03±0.92
9	32.98±0.148	90	93.33±0.88
10	33.70±0.197	100	96.37±1.31

Table 5.3 Scavenging activity (%) on DPPH radical by the crude lutein

Values represent mean ± SD of 3 replicates

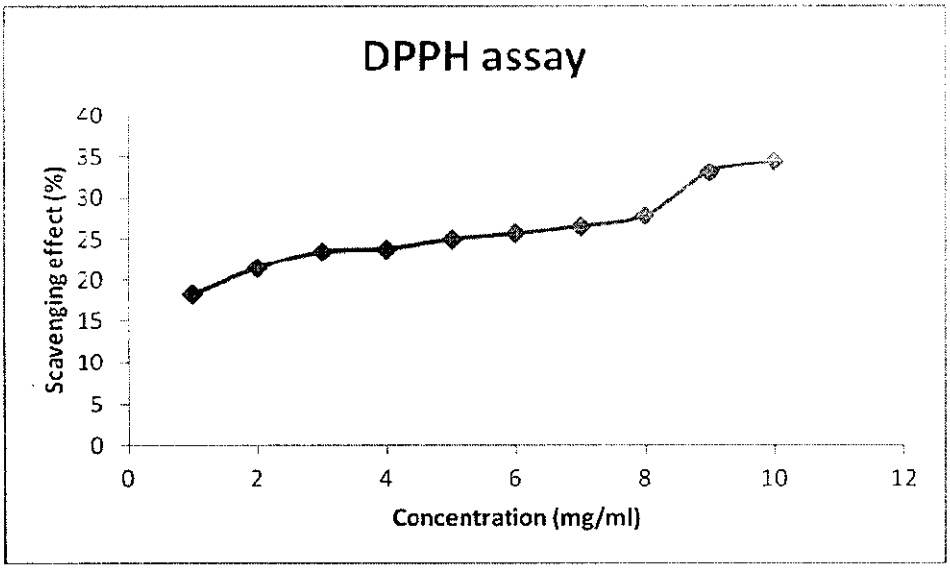

Figure 5.4 Scavenging activity (%) on DPPH radical by the crude lutein

Free radical scavenging potential of the crude lutein from the peel of Pachainadan is furnished in Table 5.3 which increases with the increase in concentration. At 1-10mg ml^{-1}, scavenging ability of the crude lutein from pachainadan on DPPH radicals exhibited 18.51%-34.98%.

5.3.1.3 Nitric oxide radical scavenging activity

Nitric oxide is a free radical produced in mammalian cells, and is involved in the regulation of various physiological processes. However, excess production of NO is associated with several diseases (Ross, 1993 and Ialenti *et al.*, 1993). Oxygen reacts with excess NO to generate nitrite and peroxynitrite anions, which act as free radicals (Cotran *et al.*, 1999).

Extent of nitric oxide radical scavenged was determined by the decrease in intensity of pink coloured chromophore at 546nm. represents higher antioxidant activity.

Concentration of the sample(mg/ml)	% inhibition	Concentration of the standard(µg/ml)	Ascorbic acid
1	21.13±0.57	10	43.81±2.42
2	24.49±0.72	20	44.64±1.45
3	26.36±0.07	30	55.35±1.33
4	28.48±0.64	40	58.28±1.87
5	30.28±0.90	50	61.00±1.12
6	32.54±0.85	60	66.29±1.57
7	33.15±0.84	70	71.00±1.14
8	35.49±0.33	80	76.39±2.60
9	41.48±0.60	90	80.54±1.54
10	45.73±0.07	100	85.65±1.97

Table 5.4 Scavenging activity (%) on nitric oxide radical by the crude lutein

Values represent mean ± SD of 3 replicates

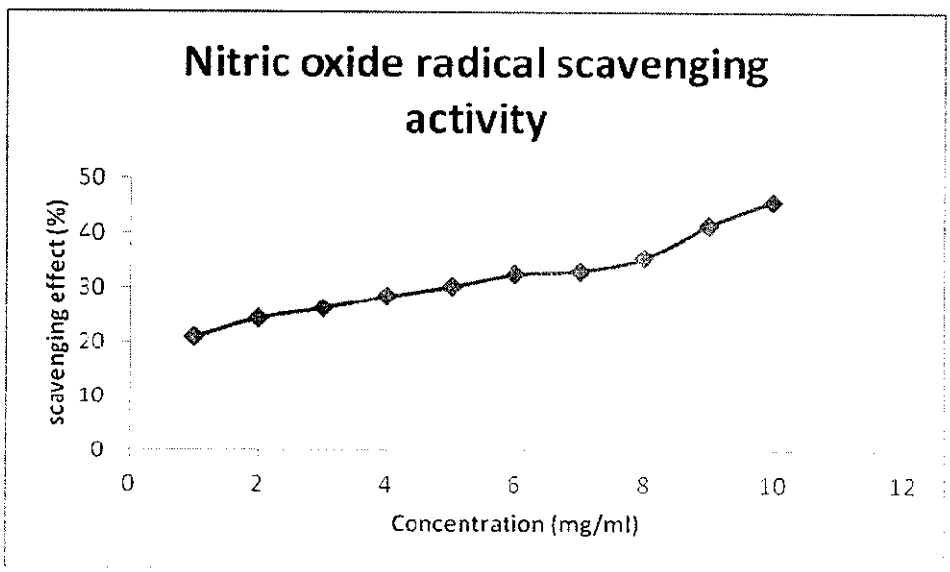

Figure 5.5 Scavenging activity (%) on nitric oxide radical by the crude lutein

Free radical scavenging potential of the crude lutein from the peel of Pachainadan is furnished in Table 5.4 which increases with the increase in concentration.

At 1-10mg ml^{-1}, scavenging ability of crude lutein obtained from the peel of Pachainadan on nitric oxide radical is 22.6%-45.78%.

5.3.1.4 Superoxide radical scavenging activity

The superoxide radical scavenging assay is carried out to determine the ability of the banana extracts to scavenge free radicals by donating electrons. The greater the increase in percentage inhibition of superoxide radical, the greater the scavenging activity by the banana extract (Sakanaka and Tachibana, 2006).

Concentration of the sample(mg/ml)	% inhibition	Concentration of the standard(µg/ml)	Ascorbic acid
1	5.67±1.06	10	35.25±1.58
2	10.92±1.73	20	42.63±2.06
3	14.48±0.96	30	51.42±1.36
4	17.01±1.14	40	55.75±1.65
5	19.75±0.56	50	62.66±2.14
6	23.81±1.92	60	69.18±1.39
7	28.89±1.55	70	75.32±1.81
8	33.71±0.29	80	82.73±1.96
9	37.57±1.13	90	86.52±1.42
10	44.17±0.66	100	95.95±2.16

Table 5.5 Scavenging activity (%) on super oxide radical by the crude lutein

Values represent mean ± SD of 3 replicates

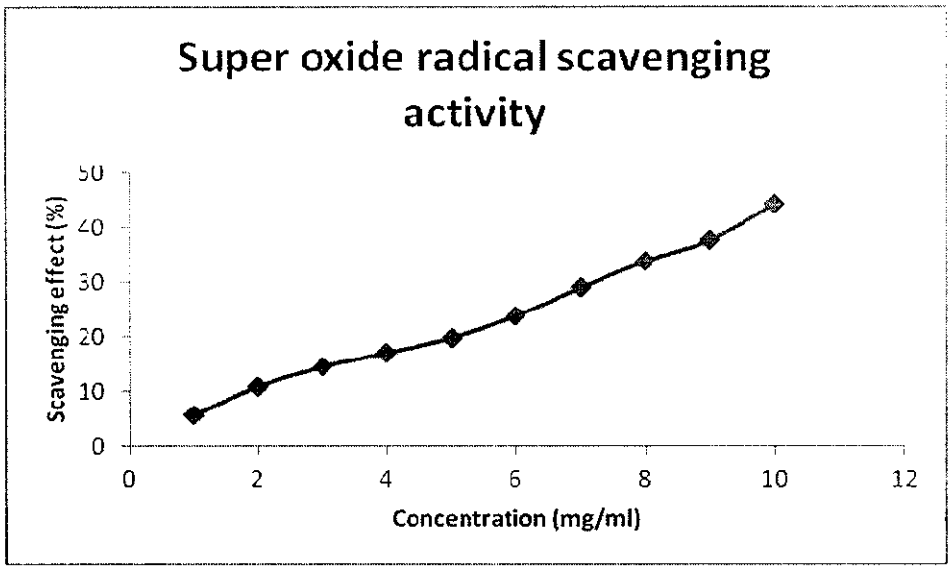

Figure 5.6 Scavenging activity (%) on superoxide radical by the crude lutein

Free radical scavenging potential of the crude lutein from the peel of Pachainadan is furnished in Table 5.5 which increases with the increase in concentration.

At 1-10mg ml^{-1}, scavenging ability of crude lutein obtained from the Pachainadan peel on super oxide radical is 5.67%-44.17%.

5.3.1.5 Ferric Reducing Antioxidant Potential activity

In this method, antioxidant compounds form a colored complex with potassium ferricyanide, trichloro acetic acid and ferric chloride that was measured at 700 nm. Increase in absorbance of the reaction mixture indicates the increase in the reducing power of the sample. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Suter *et al.*, 2000).

Concentration of the sample(mg/ml)	Absorbance at 700nm	Concentration of the standard(µg/ml)	Ascorbic acid
1	0.211±0.06	10	0.15±0.01
2	0.354±0.02	20	0.24±0.02
3	0.480±0.08	30	0.30±0.03
4	0.548±0.11	40	0.38±0.02
5	0.580±0.01	50	0.48±0.03
6	0.603±0.08	60	0.59±0.02
7	0.670±0.13	70	0.69±0.03
8	0.705±0.07	80	0.75±0.03
9	0.733±0.02	90	0.85±0.02
10	0.940±0.05	100	0.95±0.03

Table 5.6 Reducing power of the crude lutein

Values represent mean ± SD of 3 replicates

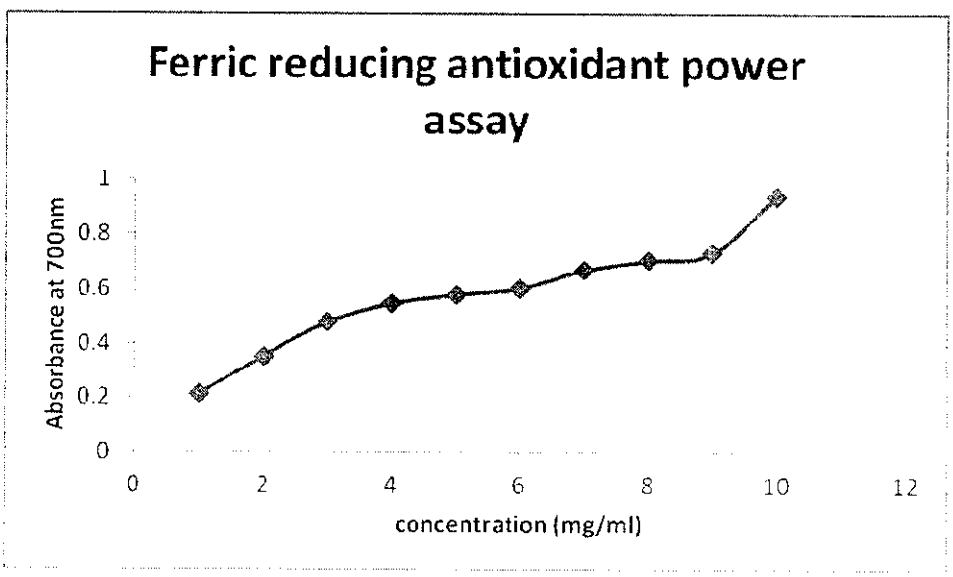

Figure 5.7 Reducing activity of the crude lutein on ferric ion.

The reducing power of the crude lutein from the peel of Pachainadan increases with the increase in concentration as depicted in Table 5.6. At 1-10mg ml^{-1}, reducing power of the crude lutein from the Pachainadan is 0.211-0.940.

CONCLUSION

6. CONCLUSION

The human body has been naturally blessed with a number of disease combating compounds that are sensibly programmed to act instantaneously. It is during the deficit of these substances that our body becomes afflicted with various ailments that may subsequently turn chronic. To prevent this, it is often recommended that people should intake natural supplements of these substances and antioxidants take the priority lead considering its valuable functions in the body. The present work has been undertaken to screen for the total carotenoid content in the peel extracts obtained from seven local varieties of banana which includes, Sevvazhai, Pachainadan, Kadali, Karpooravalli, Rasthali, Poovan and Nendran. The preliminary study carried out in our work has confirmed the significant carotenoid content in Pachainadan.Also the lutein was isolated from the crude peel extract of the Pachainadan variety. The free radical scavenging ability of the lutein present in banana peel extract was tested against various free radicals generated *in vitro*.

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7. REFERENCES

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