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ANTIOXIDANT AND PHYTOCHEMICAL STUDIES IN PEEL EXTRACTS FROM LOCAL VARIETIES OF BANANA

A PROJECT REPORT

Submitted by

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in partial fulfilment for the award of the degree of

BACHELOR OF TECHNOLOGY

in

BIOTECHNOLOGY

KUMARAGURU COLLEGE OF TECHNOLOGY, COIMBATORE

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BONAFIDE CERTIFICATE

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The report of the project work submitted by the above students in partial fulfilment of the degree of Bachelor of Technology in Biotechnology from Anna University, Chennai was confirmed to be the report of the work done by the above students and then evaluated.

(INTERNAL EXAMINER) **(EXTERNAL EXAMINER)**

DECLARATION

We, **NITHYA.R** and **SHYAMPRIYA.R,** hereby declare that the project entitled, **"ANTIOXIDANT AND PHYTOCHEMICAL STUDIES IN PEEL EXTRACTS FROM LOCAL VARIETIES OF BANANA",** submitted to the Anna University, Chennai in partial fulfillment of the requirements for the award of the degree of **Bachelor of Technology** is an original research work done by us under the supervision and guidance of **Dr. R.Baskar,** Associate Professor, Department of Biotechnology, Kumaraguru College of Technology, Coimbatore and that it has not formed the basis for the award of Degree/Diploma/Association /Fellowship or other similar title to any candidate in any University.

NITHYA.R

SHYAMPRIYA.R

Dedicated to our Parents and Respected Guide

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R. Nithya

NITHYA.R

Abstract

ABSTRACT

Organisms undergo metabolic pathways resulting in generation of energy. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are constantly generated *in vivo* for physiological purposes and often over produced in pathological conditions, resulting in oxidative stress. 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 superoxide dismutases (SOD), glutathione peroxidase (GPx) and catalase are the major antioxidant enzymes. The non-enzymatic components consist of an array of small molecules, such as vitamin C, E, reduced glutathione and many others. 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. Banana peel is rich in phytochemical compounds, mainly antioxidants. Though their antioxidant potential has been studied, comparative analysis is yet to be performed. This study aims to evaluate and compare the antioxidant activity of

the peel extracts of nine local varieties of banana, viz., Kadali, Karpooravalli, Monthan, Nendran, Pachainadan, Poovan, Rasthali, Robusta and Sevvazhai. Quantification of enzymatic components such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione s-transferase, glucose-6-phosphate dehydrogenase, polyphenol oxidase and non-enzymatic components such as total reduced glutathione, vitamins A, C and E and phytochemicals such as total phenolics, total flavonoids, carotenoids, lycopenes and alkaloids was done. The data obtained were subjected to statistical analysis and results were compared. Even though, all the banana varieties had remarkable antioxidant and phytochemical potential, Karpooravalli, Robusta and Kadali showed remarkable activity for most of the parameters examined. Thus, the peel extracts obtained from these varieties may be useful to combat free radical- related diseases.

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LIST OF ABBREVIATIONS

µg	microgram
µl	microlitre
µM	micromoles
ALP	Alkaline Phosphatase
ANOVA	Analysis of Variance
Apaf-1	Apoptosome associated factor-1
ATP	Adenosine triphosphate
BHA	Butylated hydroxyanisole
CAT	Catalase
CDNB	1-Chloro-2,4-dinitro phenyl hydrazine
DMRT	Duncun's Multiple Range Test
DNA	Deoxy Nucleic acid
DNPH	2,4 dinitro phenyl hydrazine
EDTA	Ethylene Diamine Tetraacetic acid
FAD	Flavin adenine dinucleotide
FDA	Food and Drug Administration
Fe^{2+}	Ferric ion
Fe^{3+}	Ferrous ion
$FeCl_3$	Ferric chloride
g	gram
G6PD	Glucose-6- phosphate dehydrogenase
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Total Reduced Glutathione

GSSG	Oxidized Glutathione
GST	Glutathione-S-Transferase
H_2O_2	Hydrogen peroxide
HCl	Hydrochloric acid
LDL	Low Density lipoprotein
LPO	Lipid Peroxidation
M	moles
MDA	Malondialdehyde
mg	milligram
mM	millimoles
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NaOH	Sodium hydroxide
O_2	Oxygen
OD	Optical Density
OFR	Oxygen-Free Radicals
$^{\cdot}OH$	Hydroxyl radical
PG	Propyl Gallate
PUFA	Poly Unsaturated Fatty Acids
RBC	Red blood cells
RNS	Reactive Nitrogen Species
ROM	Reactive Oxygen Metabolites
ROOH	Organic hydroperoxides
ROS	Reactive Oxygen Species
SOD	Superoxide Dismutase
TBHQ	Terbutyl hydroquinone
TCA	Trichloro Acetic acid

TFA	Trifluoro Acetic acid
UV	Ultraviolet
XDH	Xanthine dehydrogenase

Introduction

1. INTRODUCTION

Atoms are most stable in the ground state. An atom is considered to be 'ground' when every electron in the outermost shell has a complementary electron that spins in the opposite direction. 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 (Karlsson, 1997). 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 anti-parallel spins from cellular structures or molecules. Thus, the chain reaction continues and can be 'thousands of events long' (Goldfarb, 1999). Free radical formation occurs continuously in the cells as a consequence of both enzymatic and non-enzymatic reactions.

Generation of oxygen radicals, such as superoxide radical, hydroxyl radical and non-free radical species, such as H_2O_2 and singlet oxygen is associated with cellular and metabolic injury, accelerated aging, cancer, cardiovascular diseases, neurodegenerative disorders, and inflammation (Stadtman, 1992; Sun, 1990).

Plant foods, such as fruits, vegetables, and whole grains contain many components that are beneficial to human health. Research supports that some of these foods, as part of an overall healthful diet, have the potential to delay the

research aimed at identifying specific bioactive components in foods, such as antioxidants, which may be responsible for improving and maintaining health.

Antioxidants are substances that help our bodies fight against damage and decay. During normal metabolism, oxygen can create damaging by- products. These by- products or free radicals have a damaging effect on biomolecules. Antioxidants bind themselves to free radicals and transform them into non-damaging compounds. They also repair cellular damage. The highest concentration of antioxidants is found in the deeply or brightly coloured fruits and vegetables. Many phytochemicals and nutrients (vitamin C, vitamin E, carotenoids etc.) act as antioxidants.

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 vegetables.

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 antioxidants in the cell cytoplasm and the blood plasma, while lipid- soluble antioxidants protect cell membranes from lipid peroxidation (Sies, 1997). These compounds may be synthesized in the body or obtained from the diet (Vertuani *et al.*, 2004). The different antioxidants are present at a wide range of concentrations in body fluids and tissues, with some such as glutathione and ubiquinone mostly present within cells, while others such as uric acid are more evenly distributed throughout the body.

The relative importance and interactions between these different antioxidants is a complex area, with the various metabolites and enzyme systems having synergistic and interdependent effects on one other (Sies, 1993). The action of one antioxidant may depend on the proper function of other members of the antioxidant system (Vertuani *et al.*, 2004). The amount of protection provided by any one antioxidant therefore depends on its concentration, its reactivity towards the particular reactive oxygen species being considered, and the status of the antioxidants with which it interacts.

Some compounds contribute to antioxidant defense by chelating transition metals and preventing them from catalysing the production of free radicals in the cell. Particularly important is the ability to sequester iron, which is the function of iron- binding proteins such as transferrin and ferritin. Selenium and zinc are commonly referred to as antioxidant nutrients, but these chemical elements have no antioxidant action themselves and are instead required for the activity of some antioxidant enzymes.

The most important characteristic of antioxidants is that these are molecules that can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Although there are several enzyme systems within the body that scavenge free radicals, the principal micronutrient (vitamin) antioxidants are vitamin E, β- carotene, and vitamin C. Additionally, selenium, a trace metal that is required for proper function of one of the body's antioxidant enzyme systems, is sometimes included in this category. The body cannot manufacture these micronutrients, so they must be supplied in diet.

Humans require external sources of vitamins E, C and β- carotene as the body is unable to produce these nutrients. Hence, efforts should be made to ensure optimum intake of foods rich in antioxidants. Diets need to be improved

and a greater emphasis placed on the consumption of antioxidant rich vegetables and fruits.

It is well known that fruits contain various antioxidants, such as vitamin C, vitamin E, and β- carotene (Kanazawa and Sakakibara, 2000). 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 (Lampe, 1999; Guo & Yang, 2001). Besides classical antioxidants including vitamin C, E and β-carotene, phenolic compounds have been identified as important antioxidants contained in fruits. Some phenolic compounds are even more powerful as antioxidants than vitamin C, E *in vitro* and significantly bioavailable as demonstrated by animal and human studies (Bravo, 1998; Rice Evans *et al.,* 1996; Su *et al.,* 2003; Ader *et al.,* 2000; Cao *et al.*, 1998; Mazza *et al.,* 2002). However, fruits are diverse in antioxidant composition and antioxidant activity and those with high antioxidant activity generally contain more antioxidants (Guo *et al.,* 1997). Interestingly, the peel and seed fractions of some fruits possess higher antioxidant activity than the pulp fractions.

Bananas are one of the most popular fruits on the world market. Banana peel is rich in phytochemical compounds, mainly antioxidants. The total amount of phenolic compounds in banana (*Musa acuminata Colla* AAA) peel ranges from 0.90 to 3.0 g/100 g DW (Nguyen *et al.*, 2003; Someya *et al.,* 2002). The nutritional value of bananas and the lack of awareness of the antioxidant potential of commonly available varieties has provoked us to evaluate and compare the antioxidant potential of the peel extracts from nine local varieties of banana, viz., Sevvazhai, Pachainadan, Robusta, Kadali, Karpooravalli,

The main objective of our study is categorized into two phases:

- To quantify and compare *in vivo* antioxidant activities in the peel extracts of nine varieties of banana and
- To determine the levels of phytochemicals in the peel extracts of nine varieties of banana.

Review of Literature

2. REVIEW OF LITERATURE

2.1. 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 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).

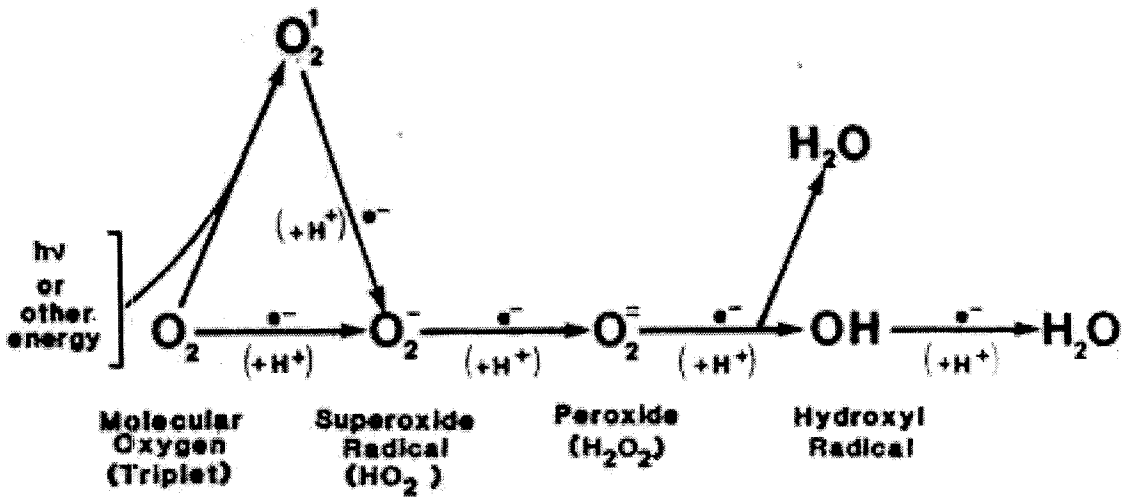
The oxygen and hydrogen peroxide so formed may lead to the formation of the most reactive OH. This hydroxyl radical oxidizes lipids giving rise to lipid peroxidation. Hydrogen peroxide is known to cause DNA breaks in intact cells and purification DNA. Malondialdehyde (MDA) is the major reactive aldehyde 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.,*

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.

2.1.1. Reactive Oxygen Species

Reactive oxygen species (ROS) include oxygen ions, free radicals and peroxides both organic and inorganic. They are generally very small molecules and are highly reactive due to the presence of unpaired valence shell electrons. ROS form as a natural byproduct of the normal metabolism and have important roles in cell signaling. The two of the most important oxygen centered free radicals are the superoxide and the hydroxyl radical.



The molecular oxygen is reduced to water in four single-electron steps. Reduction of non-radical forms of oxygen is a "forbidden" process and thus usually involves spin-orbit coupling by a heavy metal or a halide or excitation to singlet state. An example is the Fenton's reaction, the reduction of peroxide to water and hydroxyl radical by ferrous ion. Hydroxyl radical is one of the most powerful oxidizing agents known. Simply put, reducing agents act as pro-oxidants by reducing non-radical forms of oxygen to radical forms, usually with heavy atom involvement. Similarly, they can act as antioxidants by reducing the radical forms of oxygen, by terminating the radical chain reactions. This dual property can be of great significance (Peter Proctor, 1989).

2.2. FORMATION OF FREE RADICALS

Free radicals are produced due to various reasons. Free radicals are also produced inside organelles, such as the mitochondrion. Mitochondria convert energy for the cell into a usable form, adenosine triphosphate (ATP). The process, in which ATP is produced, called oxidative phosphorylation, involves the transport of protons (hydrogen ions) across the inner mitochondrial membrane by means of the electron transport chain. In the electron transport chain, electrons are passed through a series of proteins via oxidation-reduction reactions, with each acceptor protein along the chain having a greater reduction potential than the last. The last destination of the electron along this chain is an oxygen molecule. Normally this oxygen is reduced to form water; however, in about 0.1-2% of the electrons passing through the chain, oxygen is prematurely and incompletely reduced to give the superoxide radical. Superoxide is not particularly reactive, but can inactivate specific enzymes or initiate lipid peroxidation. If too much damage is caused, a cell undergoes programmed cell death or apoptosis. Thus the most important source of reactive oxygen under normal conditions in aerobic organisms is probably the leakage of activated

Other enzymes capable of producing superoxide are xanthine oxidase, NADPH oxidases and cytochromes P450. Hydrogen peroxide is produced by a wide variety of enzymes including several oxidases.

Metals such as iron, copper, chromium, vanadium and cobalt are capable of redox cycling in which a single electron may be accepted or donated by the metal. This action catalyses reactions that produce reactive oxygen species. The most important reactions are probably Fenton's reaction and Haber-Weiss reaction, in which hydroxyl radical is produced from reduced iron and hydrogen peroxide.

Certain organic compounds can also produce reactive oxygen species. One of the most important classes of these is the quinones. Quinones can redox cycle with their conjugate semiquinones and hydroquinones, in some cases catalyzing the production of superoxide from dioxygen or hydrogen peroxide from superoxide.

The immune system uses the lethal effects of oxidants by making the production of oxidizing species a central mechanism of killing pathogens; with activated phagocytes producing both ROS and reactive nitrogen species. These include superoxide, nitric oxide and their particularly reactive product, peroxynitrite (Nathan and Shiloh, 2000). Although the use of these highly reactive compounds in the cytotoxic response of phagocytes causes damage to the host tissues, the non-specificity of the oxidants is an advantage since they will damage almost every part of the target cell (Rice-Evans and Gopinath, 1995). This prevents a pathogen from escaping this part of the immune response by mutation of a single molecular target.

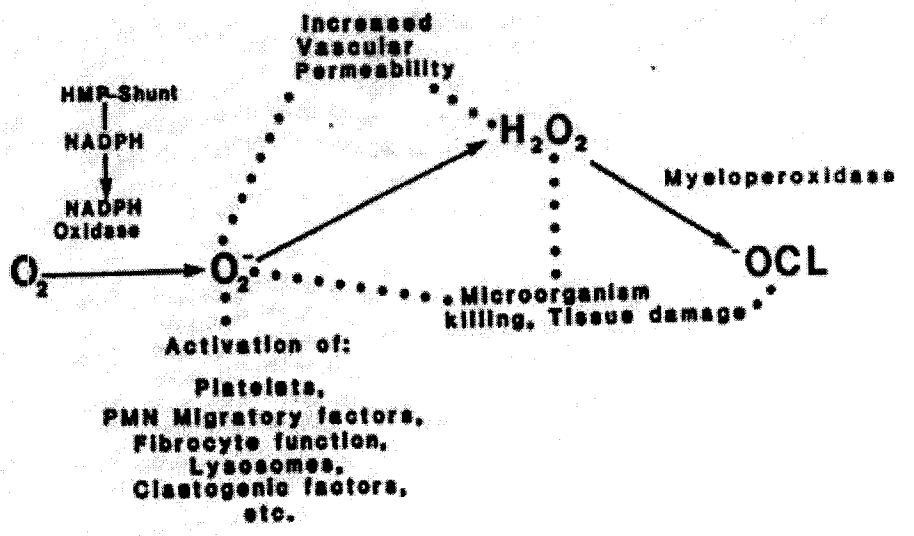


Fig. 2.2. The role of active oxygen species in inflammation

The granulocytes and other phagocytes possess membrane NADPH oxidase, which takes reducing equivalents from the hexose monophosphate shunt and transfers these to molecular oxygen to produce superoxide and other active oxygen species. A further myeloperoxidase converts the peroxide produced in this system to microbicidal products, probably including hypochlorite. Production of activated products by this system probably plays a key role in cell-mediated immunity and microbicidal activity. There is evidence for similar systems in T-lymphocytes, platelets and mucus. (Peter Proctor, 1989).

Another major mechanism for the endogenous production of activated species is by autoxidation- catalyzing charge-transfer agents such as copper, iron, and manganese.

Free radicals are produced in the biological system due to the influence of several external factors as well. Free radicals are produced on exposure to environmental stress, exposure to cigarette smoke, radiation, anti-cancer drugs,

2.2.1. Role of Pro-Oxidants in Free Radical Formation

Pro-oxidants are chemicals that induce the production of free radicals or inhibit the antioxidant system (Puglia and Powell, 1984). The overdose of these chemicals can damage cells and tissues. For example, the overdose of the analgesic paracetamol can cause fatal damage to the liver (Jaeschke *et al.*, 2002), partly through the production of reactive oxygen species (James *et al.*, 2003).

Some substances can act as either antioxidants or pro-oxidants, depending on the specific set of conditions (Herbert, 1996). Some of the conditions that are important include the concentration of the chemical and if oxygen or transition metals are present. While thermodynamically very favored, reduction of molecular oxygen or peroxide to superoxide or hydroxyl radical is fortunately spin forbidden. This greatly reduces the rate of these reactions, thus allowing aerobic organisms to live. As a result, the reduction of molecular oxygen typically involves the initial formation of singlet oxygen or the spim-orbit coupling through the reduction of a transition state metal such as manganese, copper, iron, etc. This reduced metal then transfers the single electron to molecular oxygen or peroxide.

Transition metals can act as pro-oxidants. Vitamins that are reducing agents can be pro-oxidants. Vitamin C has antioxidant activity when it reduces oxidizing substances such as hydrogen peroxide (Duarte *et al.*, 2005). However it can also reduce metal ions, which leads to the generation of free radicals through the Fenton reaction (Carr *et al.*, 1999).

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

$$2\ Fe^{3+} + \text{Ascorbate} \rightarrow 2\ Fe^{2+} + \text{Dehydroascorbate}$$

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The pro-oxidant properties of reductants can also have important clinical consequences. For example, in humans, uric acid accounts for roughly half the antioxidant ability of plasma. In fact, uric acid may have substituted for ascorbate in human evolution (Jaeschke *et al.*, 2002). Like ascorbate, uric acid can also mediate the production of reactive oxygen species and thus act as a pro-oxidant.

In humans, elevated homocysteine levels are associated with production of free radicals. Several important anticancer drugs both bind DNA and generate reactive oxygen species. These include adriamycin, anthracyclines, bleomycin, cisplatin, etc.

2.3. BIOLOGICAL SIGNIFICANCE OF FREE RADICALS

2.3.1. Positive roles of free radicals

Indirect evidence suggests that free radicals and excited-state species play a key role in both normal biological functions and in the pathogenesis of certain human diseases. For example, generation of activated species by inflammatory cells is a major microbicidal mechanism and may also mediate important components of the inflammatory response. They also have important roles in redox signaling. The free radicals may also be involved in the prevention of aging by the induction of a process known as mitohormesis. They are also involved in the induction of host defense genes and mobilization of ion transport systems. Their roles in signaling are crucial. In particular, platelets involved in wound repair and blood homeostasis release ROS to recruit additional platelets to the sites of injury.

Thus reactive oxygen species play an indispensable role in the normal functioning of biological system.

2.3.2. 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).

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).

2.4. FREE RADICALS AND HUMAN DISEASE

Free radicals are highly reactive they participate in a number of reactions that can result in cell damage. Many forms of cancer are thought to be the result of reactions between free radicals and DNA, resulting in mutations that can adversely affect the cell cycle and potentially lead to malignancy. Some of the symptoms of aging such as atherosclerosis are also attributed to free radical induced oxidation of many chemicals making up the human body. In addition free radicals contribute to the alcohol-induced liver damage, perhaps more than alcohol itself. Radicals in cigarette smoke have been implicated in the inactivation of alpha-1- antitrypsin in the lungs. This process leads to the development of emphysema.

Free radicals may also be involved in Parkinson's disease, senile and drug-induced deafness, schizophrenia, and Alzheimer's disease. The classic free radical syndrome, the iron storage disease hemochromatosis, is typically associated with a constellation of free radical related symptoms including movement disorder, psychosis, skin pigmentary melanin abnormalities, deafness, arthritis and diabetes mellitus (Ames, 1983; Ames, 1998).

Generally the harmful effects of reactive oxygen species on the cell are most often:

- Damage to DNA
- Oxidations of polyunsaturated fatty acids in lipids
- Oxidations of amino acids in proteins
- Oxidatively inactivate specific enzymes by oxidation of co-factors

Oxidative stress due to free radicals contributes to tissue injury following irradiation and hyperoxia. It is linked to cardiovascular disease, since oxidation

Oxidative stress also plays a very important role in ischemic cascade due to oxygen reperfusion injury following hypoxia. This cascade includes roles both in stroke and heart attacks.

Transition metals also produce free radicals and are hence responsible for many diseases. For example, chronic manganism is a classic pro-oxidant disease (Puglia and Powell, 1984). Another disease associated with the chronic presence of a pro-oxidant transition-series metal is hemochromatosis associated with elevated iron levels. Similarly, Wilson's disease is associated with elevated tissue levels of copper. Such syndromes tend to be associated with a common symptomology. This typically includes the various combinations of psychosis, dyskinesia, pigmentary abnormalities, fibrosis, deafness, diabetes and arthritis (James *et al.*, 2003). Wilson's disease and striatal iron have similarly been linked to human Parkinsonism.

Fibrosis is another pro-oxidant free radical symptom. A variety of oxygen-generating agents can produce fibrotic changes. Examples include oxygen itself, nitrofurantoins, and bleomycin, which produce pulmonary fibrosis. Radical generating agents such as iron and copper are also associated with liver fibrosis and fibrotic changes in other organs such as the heart. The induction of vitreous scarring by interocular iron or copper is also well known, as is the association of homocystinuria with fibrotic lesions of the arteries.

Free radicals can also mediate disorders of the purine metabolism. Uric acid and other purines can mediate a Fenton-type reaction with peroxide. Purines also catalyze the autoxidation of epinephrine under certain conditions. The latter may involve a Fenton-type reaction with peroxide produced by adrenaline autoxidation. Uric acid can be involved in the neurological symptoms of Lesch-Nyhan Syndrome. It may also be involved in the gouty

inflammatory disease. High uric acid levels are also involved in atherosclerosis, metabolic syndrome and in stroke.

Excess bilirubin, a product of RBC breakdown can lead to jaundice, which could eventually damage the central nervous system. Elevated levels of homocysteine are also associated with increased incidence of atherosclerosis and may play a role in Alzheimer's. Homocysteine is a powerful reducing agent and can induce oxidative stress. (Valko *et al.*, 2005; Halliwell, 2007; Hao and Maret, 2005 ; Schneider, 2005).

2.5. DEFENSE MECHANISMS

The human body although continuously produces free radicals, it possesses several defense system which includes enzymes and radical scavengers. These are called first line antioxidant defense system. The second line defense system consists of repair system for biomolecules which are damaged by the attack of free radicals. Specific enzymes are known to be involved in the context and several of them have been identified in prokaryotes and in eukaryotes. The function of enzymes are involved in the repairing, directly damaged biomolecules such as lipids, polysaccharides, proteins, nucleic acids etc., or in eliminating oxidized compounds.

2.5.1. Role of apoptosis in defense against free radical damage

Apoptosis or programmed cell death is one of the major protective mechanisms to remove the damaged tissues and cells from the body and thus enables the body to repair or remove the damage caused by free radicals. A brief mechanism of apoptosis for the removal of cells containing damaged mitochondria is described here. Bcl-2 proteins are layered on the surface of mitochondria, detect damage and activate a class of proteins called Bax, which

The Cytochrome C binds to Apaf-1, or apoptotic protease activating factor-1, which is free floating in the cell's cytoplasm. Using energy from the ATPs in the mitochondrion, the Apaf-1 and cytochrome C bind together to form apoptosomes. These apoptosomes bind to and activate caspase-9, another free floating protein. The caspase-9 then cleaves the proteins of the mitochondrial membrane, causing it to break down and start a chain reaction of protein denaturation and eventually phagocytosis of the cell.

2.5.2. 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).

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).

2.5.2.1. Antioxidants 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

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.

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 agmg.

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 formmg stable free radicals that do not initiate or propagate further oxidation of lipids (free radical terminators). Some of the iinportant synthetic antioxidants of this class are butylated hydroxyanisole (BHA), butylated hydroxy toluene, terbutyl hydroquinone (TBHQ), propyl gallate (PG) and tocopherols.

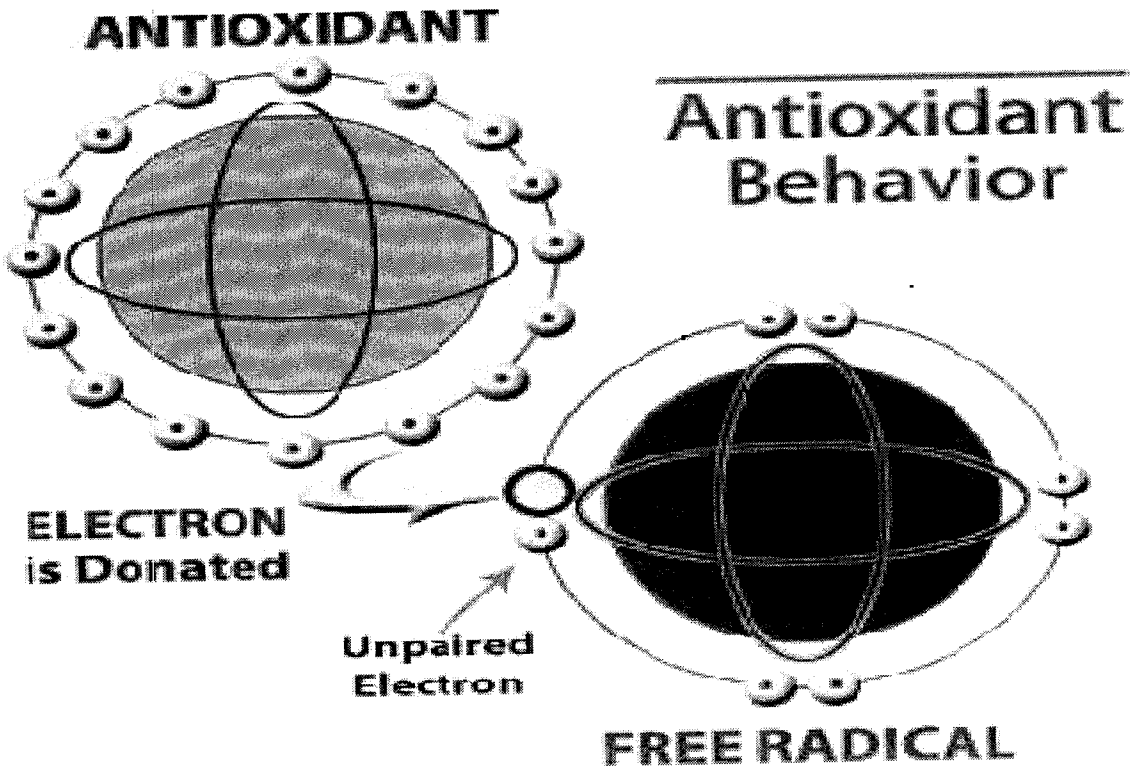


Fig. 2.3. Behaviour of Antioxidants

Antioxidants also referred to as chelators which bind metal ions such as copper and iron that catalyze lipid peroxidation; oxygen scavengers are those compounds that react with oxygen in closed systems and secondary antioxidants which function by breading down the hydroperoxides (Shahidi and Wanasundra, 1992).

In recent years, the use of some synthetic antioxidants has been restricted because of their possible toxic and carcinogenic effects (Frankel, 1991). This concern has resulted in an increased interest in the investigation of the effectiveness of naturally occurring compounds with antioxidant properties. A number of plant and medicinal mushrooms constituents have been recognized to have positive effects when tested against the oxygen reactive compounds. Foods rich in antioxidants have been shown to play an essential role in the prevention

known of which are Parkinson's and Alzheimer's diseases, inflammation and problem caused by cell and cutaneous aging.

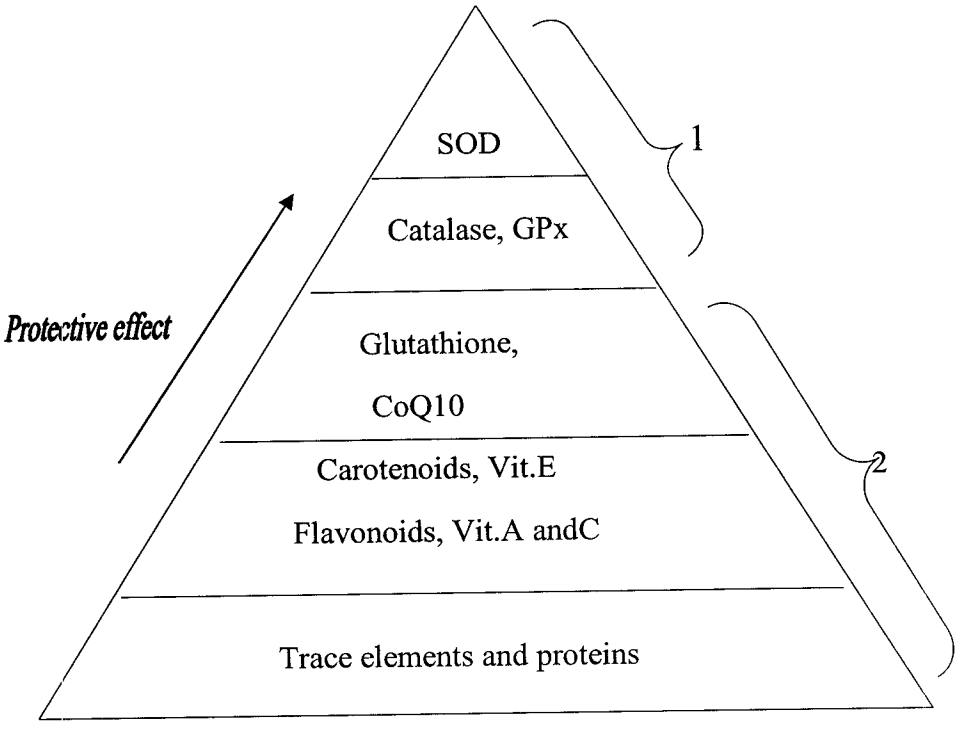


Fig. 2.4. Protective effects of vitamins, flavonoids, trace elements and enzymes

2.5.3. 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.

2.5.3.1. Enzymatic Antioxidants

The first line of defense against O_2 and H_2O_2 mediated injury are

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).

2.5.3.1.1. 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.

2.5.3.1.2. 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.

2.5.3.1.3 Catalase (CAT)

[illegible] that catalyse the conversion of hydrogen peroxide

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).

2.5.3.1.4. 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.

2.5.3.1.5. Glutathione reductase (GR)

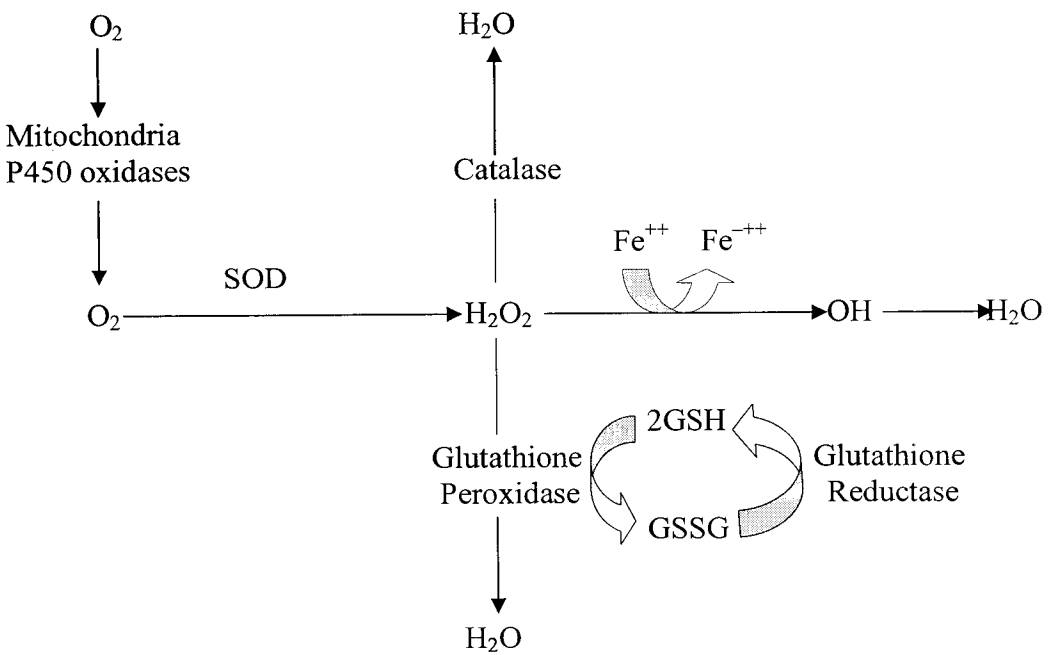
Glutathione reductase, also known as GR, is an enzyme that reduces glutathione disulfide (GSSG) to the sulfhydryl form GSH, which is an important cellular antioxidant. For every mole of oxidized glutathione (GSSG), one mole of NADPH is required to reduce GSSG to GSH. The enzyme forms a FAD bound homodimer.

2.5.3.1.6. 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

2.5.3.1.7. 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 — is 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.



2.5.3.2. Non-Enzymatic Antioxidants

2.5.3.2.1. 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.

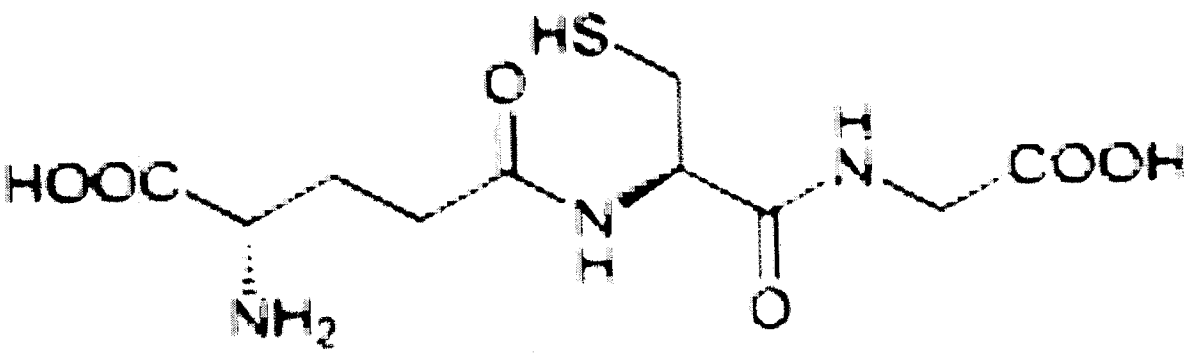


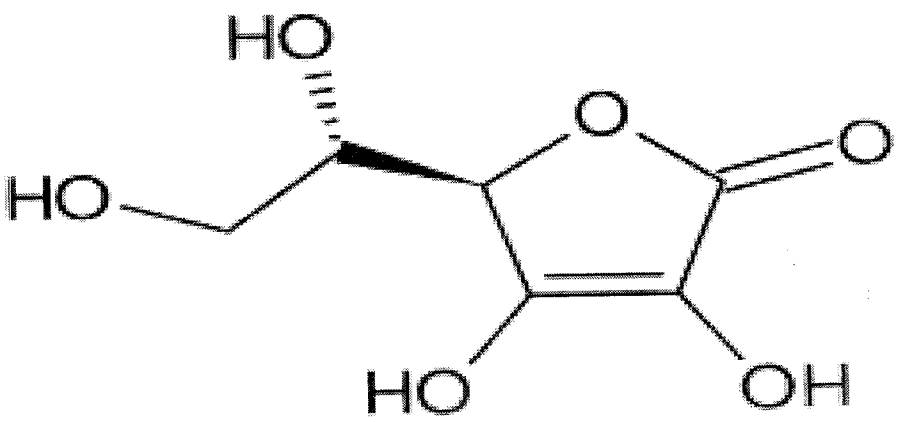
Fig. 2.6. Glutathione

2.5.3.2.2. 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.

2.5.3.2.3. Vitamin C

Ascorbic acid or "vitamin C" is a monosaccharide antioxidant found in both animals and plants. As it cannot be synthesised in humans and must be obtained from the diet, it is a vitamin. In cells, it is maintained in its reduced form by reaction with glutathione, which can be catalysed by protein disulfide isomerase and glutaredoxins (Meister, 1994). Ascorbic acid is a reducing agent and can reduce and thereby neutralize reactive oxygen species such as hydrogen peroxide (Padayatty *et al.*, 2003). In addition to its direct antioxidant effects, ascorbic acid is also a substrate for the antioxidant enzyme ascorbate peroxidase, a function that is particularly important in stress resistance in plants.



2.5.3.2.4. Vitamin E

Vitamin E is a generic term for tocopherols and tocotrienols. Vitamin E is a family of α-, β-, γ-, and δ-tocopherols and corresponding four tocotrienols. Vitamin E prevents the oxidation of lipids (fats). It prevents the cell's protective coatings from becoming rancid as a result of the attack by free radicals. It also plays a role in the prevention of cataracts caused by free radical damage.

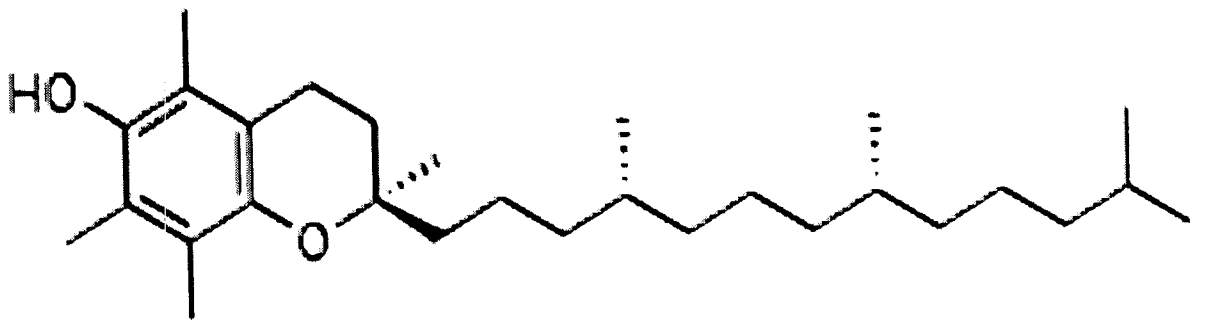


Fig. 2.8. α- tocopherol

2.5.3.2.5. Carotenoids

Carotenoids are a group of red, orange and yellow pigments found in plant foods, particularly fruits and vegetables. Some carotenoids like β-carotene act as a precursor of vitamin A; others do not. β- carotene is an effective antioxidant as it is one of the most powerful singlet oxygen quenchers. 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.

2.5.3.2.6. Lycopenes

Lycopene is a bright red carotene and carotenoid pigment and

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 than vitamin E.

Table 2.1. Non-Enzymatic Antioxidants

ANTIOXIDANT METABOLITE	SOLUBILITY	CONCENTRATION IN HUMAN SERUM (μM)	CONCENTRATION IN LIVER TISSUE (μmol/kg)
Ascorbic acid (Vitamin C)	Water	50-60	260 (Human)
Glutathione	Water	325-650	6400 (Human)
Lipoic acid	Water	0.1-0.7	4-5 (Rat)
Uric acid	Water	200-400	1600 (Human)
Carotenes	Lipid	β-carotene: 0.5-1.0 Vitamin A: 1-3	5 (Human, total carotenoids)
α-Tocopherol (Vitamin E)	Lipid	10-40	50 (Human)
Ubiquinol (Coenzyme Q)	Lipid	5	200 (Human)

2.6. Antioxidants from dietary sources

2.6.1. Importance of Antioxidant rich dietary sources

The body naturally creates some antioxidants, but it relies heavily on a proper diet to get the rest of its natural antioxidants. Spinach as well as blueberries, apples and several other plant foods are rich in antioxidants. Flaxseed contains lignans, which may have antioxidant effects. Flaxseeds are said to fight cancer due to the fact that the omega-3 fatty acids found within it act as antioxidants in the body. The antioxidants in dark chocolate have shown to make a difference in cardiovascular health. Research shows that anti-aging creams that do not have antioxidants do not work as effectively as those that contain it. Free radicals are the forerunners of devastating diseases like cancer, osteoporosis and heart disease: antioxidants can help get rid of these free radicals from our body. Garlic also serves as an antioxidant and some studies even indicate that it can help to protect against cancer. Thus fruits and vegetables contain essential antioxidants for the body. The effect of *Aloe vera* is interesting because it makes Vitamin C, Vitamin E and other antioxidants work well.

2.6.2. Natural Sources of antioxidants

Many people these days take antioxidants to counter the detrimental effect of free radicals. In fact, artichokes are among the best sources of antioxidants. Berries in general are among the foods, which are the most antioxidant-dense. Carotenoids are antioxidants that are in carrots, apricots and all major citrus fruits including oranges. Beta-carotene is one popular natural antioxidant and is present in various natural food products. Strawberries are high in antioxidants. Apart from natural sources supplements can go a long way

2.7. Evaluation and Comparison of Antioxidant potential in peel extracts from local varieties of Banana

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. Bananas are one among them. The nutritional value of bananas and the lack of awareness of the antioxidant potential of commonly available varieties have provoked us to evaluate and compare the antioxidant potential of the peel extracts from nine local varieties of banana. Bananas have a great nutritive value and their consumption has been recommended for the prevention of free radical induced diseases like arthritis, diabetes, cancer, etc. This project therefore aims to bring to limelight the *in vivo* antioxidant and phytochemical activities in the peel extracts from nine local varieties of banana, viz.,

1. Banana cv. Sevvazhai- *Musa spp* - Sevvazhai AAA
2. Banana cv. Pachinadan- *Musa spp* - Pachainadan AAB
3. Banana cv. Robusta-
   Cavendish sub group- *Musa spp* - Robusta AAA
4. Banana cv. Karpooravalli- *Musa spp* - Karpooravalli ABB
5. Banana cv. Kadali- *Musa* spp - Ney Poovan AB
6. Banana cv. Monthan- *Musa* spp - Bluggoe ABB
7. Banana cv. Rasthali- *Musa* spp - Rasthali AAB
8. Banana cv. Poovan- *Musa* spp - Mysore AAB
9. Banana cv. Nendran- *Musa* spp - French Plantain AAB

2.8. BANANAS

Banana is a tree – like plant of the genus *Musa* in the family Musaceae. It is one of the most popular fruits on the world market. Banana is a climacteric fruit made up of peel and edible pulp that has a high nutritional value. Edible bananas are vegetatively parthenocarpic berries; *i.e.*, they develop a mass of edible pulp without pollination. The fruit develops from the inferior ovary of the female flower. The ovules shrivel early but may be recognised in the mature fruit as minute brown flecks in the central part of the edible pulp. The extensive literature reviews showed that botany, cytology, breeding, horticulture, physiology, biochemistry, nutritional and therapeutic value of banana had been already studied in depth. The respected herbalist, Maud Grieve noted in her book published in 1931 that the banana family is more of interest for its nutrient than for its medicinal properties. It contains 74% water, 23% carbohydrates, 2.6% fiber, 1% proteins and 0.5% fat (these values vary between different banana cultivars, degree of ripeness and growing conditions).

2.8.1. Banana, *Musa* spp.

2.8.1.1. Taxonomy and classification of banana

Bananas are large, monocotyledonous herbs belonging to the Musaceae family of the order Zingiberales. The genus *Musa* is comprised of all edible cultivars that are further divided into four sections, *Eumusa, Rhodochlamys, Australimusa* and *Callimusa.*

The edible banana cultivars are mostly derived from two wild species of genus *Musa,* namely, *Musa acuminata* and *Musa balbisiana. Musa acuminata* is a diverse species and consists of at least nine subspecies while *Musa balbisiana* is less diverse and no subspecies has been suggested so far. All the edible cultivars originated from these two species belong to various genome groups. They are differed from each other depending on whether the clones are pure

diploid, triploid or tetraploid hybrids of two wild species. Hence, a classification system was developed by Simmonds and Shepherd (1955) to classify all the edible banana cultivars systematically. On the basis of 15 vegetative and reproductive morphological characters, the differences between *Musa acuminate* and *Musa balbisiana* could clearly be discerned (Table 2.2). It is also necessary to determine the ploidy of a clone before it can be satisfactorily classified. Ploidy and relative contribution of the two species to a given banana cultivar is specified with a shorthand lettering system. Haploid contribution of *Musa acuminata* and *Musa balbisiana* are designated as A and B, respectively. Basically, all edible banana cultivars can be classified into six groups which are AA, BB, AAA, AAB, ABB, and ABBB. They are respectively diploid, triploid and tetraploid. However, most of them are triploid.

Table 2.2. Characters used in distinguishing banana cultivars

Character	*M. acuminata*	*M. balbisiana*
Pseudostem colour	More or less heavily marked with brown or black blotches	Blotches slight or absent
Petiolar canal	Margin erect or spreading, with scarious wings below, not clasping pseudostem	Margin enclosed, not winged below, clasping pseudostem
Peduncle	Usually downy or hairy	Glabrous
Pedicels	Short	Long
Ovules	Two regular rows in each locules	Four irregular rows in each locules
Bract shoulder	Usually high (ratio < 0.28)	Usually low (ratio > 0.30)
Bract curling	Bracts reflex and roll back after Opening	Bracts lift but do not roll
Bract shape	Lanceolate or narrowly ovate, tapering sharply from the shoulder	Broadly ovate not tapering sharply
Bract apex	Acute	Obtuse
Bract colour	Red, dull purple or yellow outside; pink, dull purple or yellow outside	Distinctive brownish - purple outside; bright crimson inside
Colour fading	Inside bract colour fades to yellow towards the base	Inside bract colour continuous to base
Bract scars	Prominent	Scarcely prominent
Free tepal or male flower	Variably corrugated below tip	Rarely corrugated
Male flower Colour	Creamy white	Variably flushed with pink

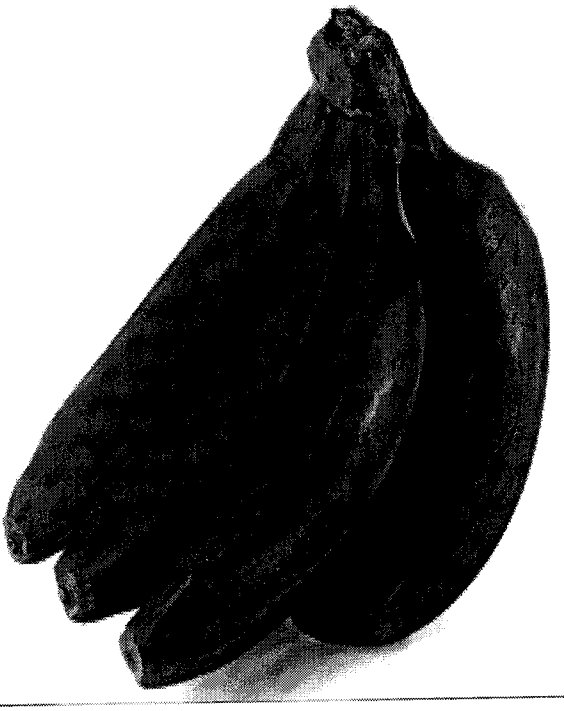


Fig. 2.9. *Musa spp* - Sevvazhai AAA

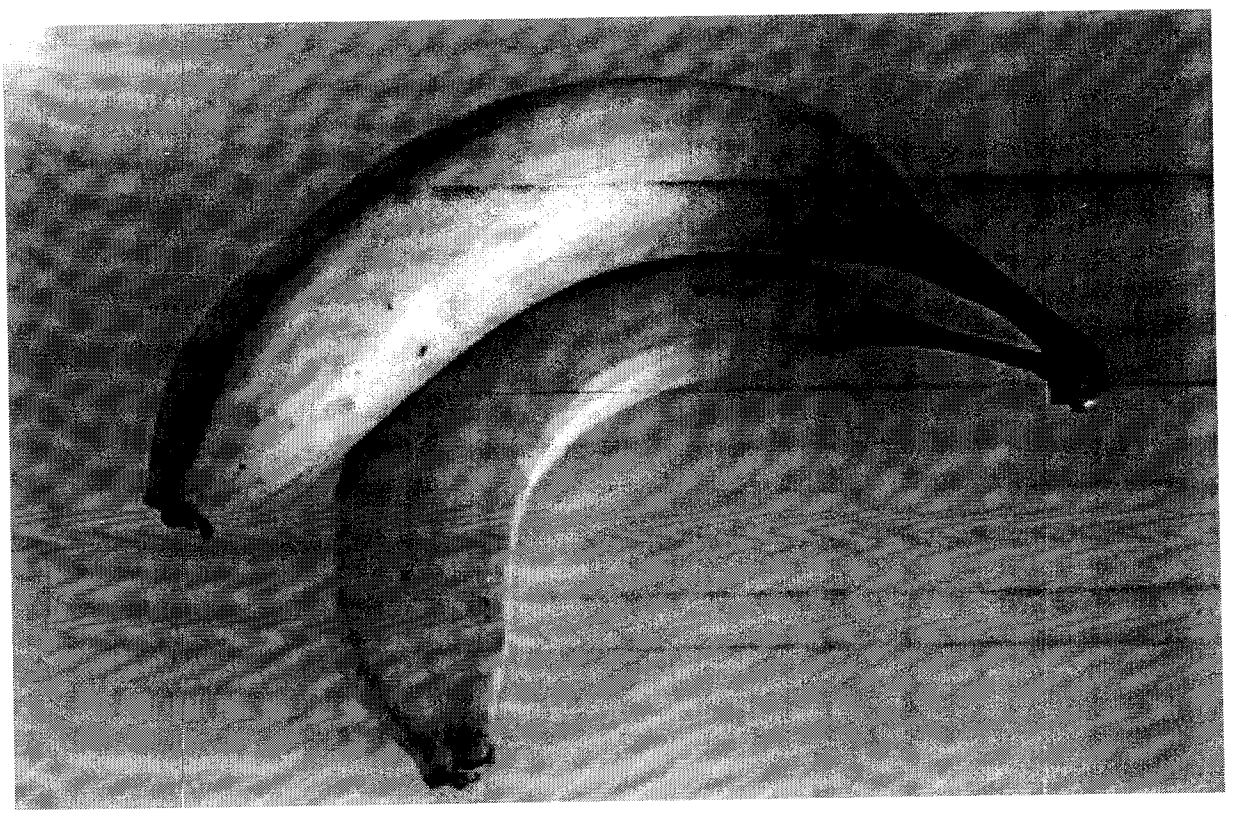


Fig. 2.10. *Musa spp* - Pachainadan AAB

Fig. 2.11. *Musa spp* - Robusta AAA

Fig. 2.13. *Musa* spp - Ney Poovan AB

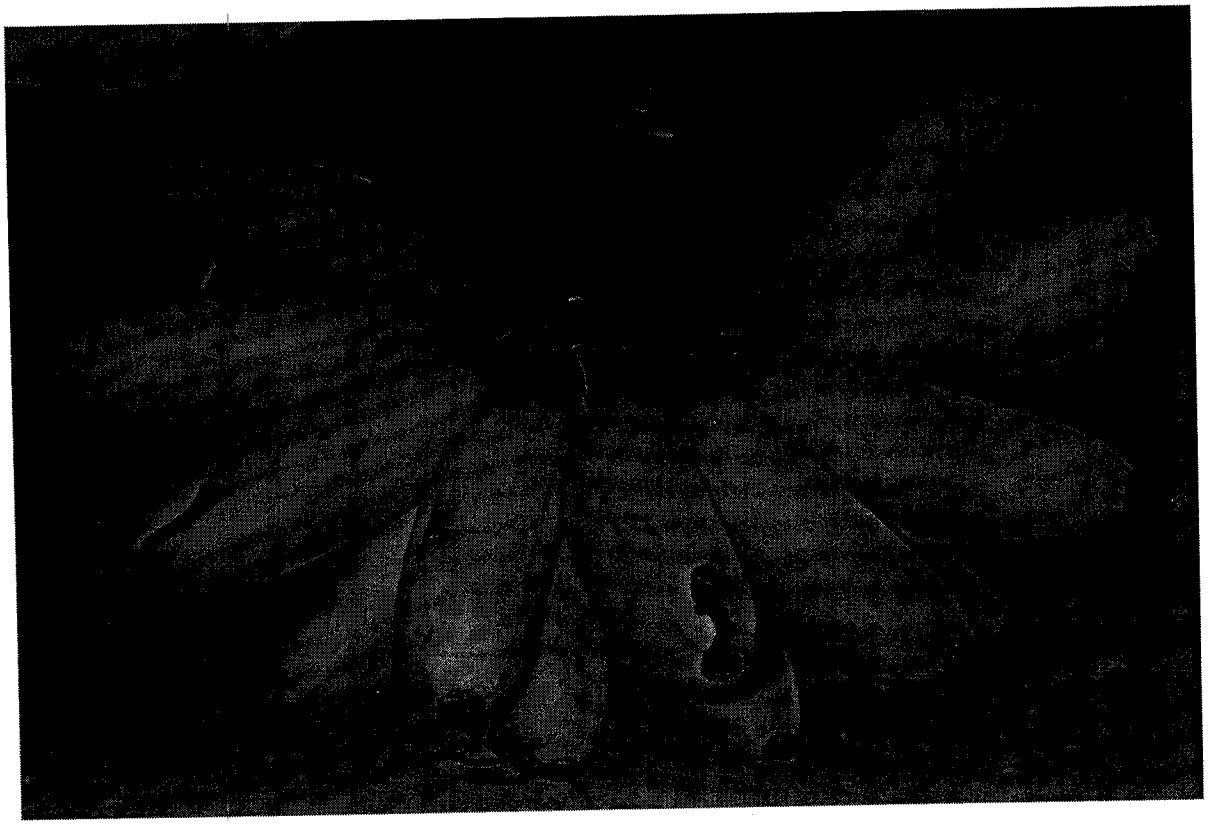
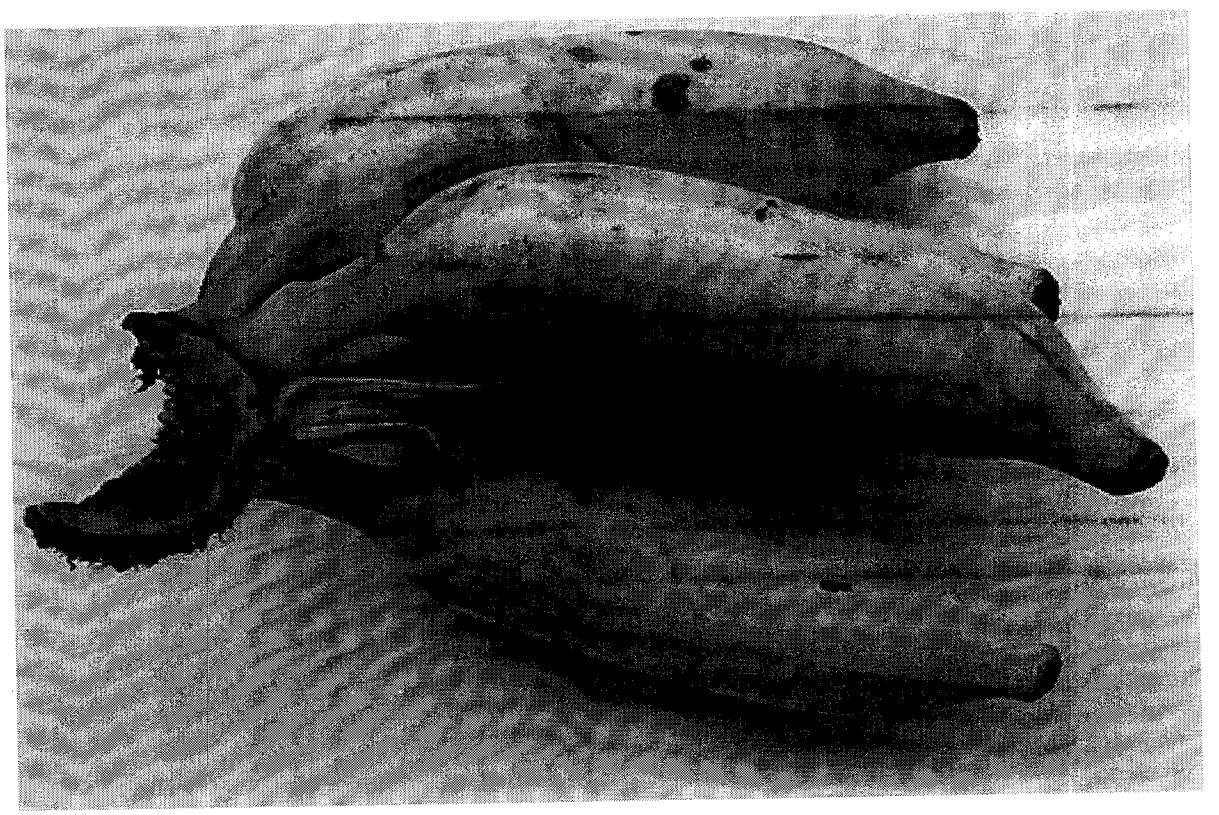


Fig. 2.15. *Musa* spp - Rasthali AAB



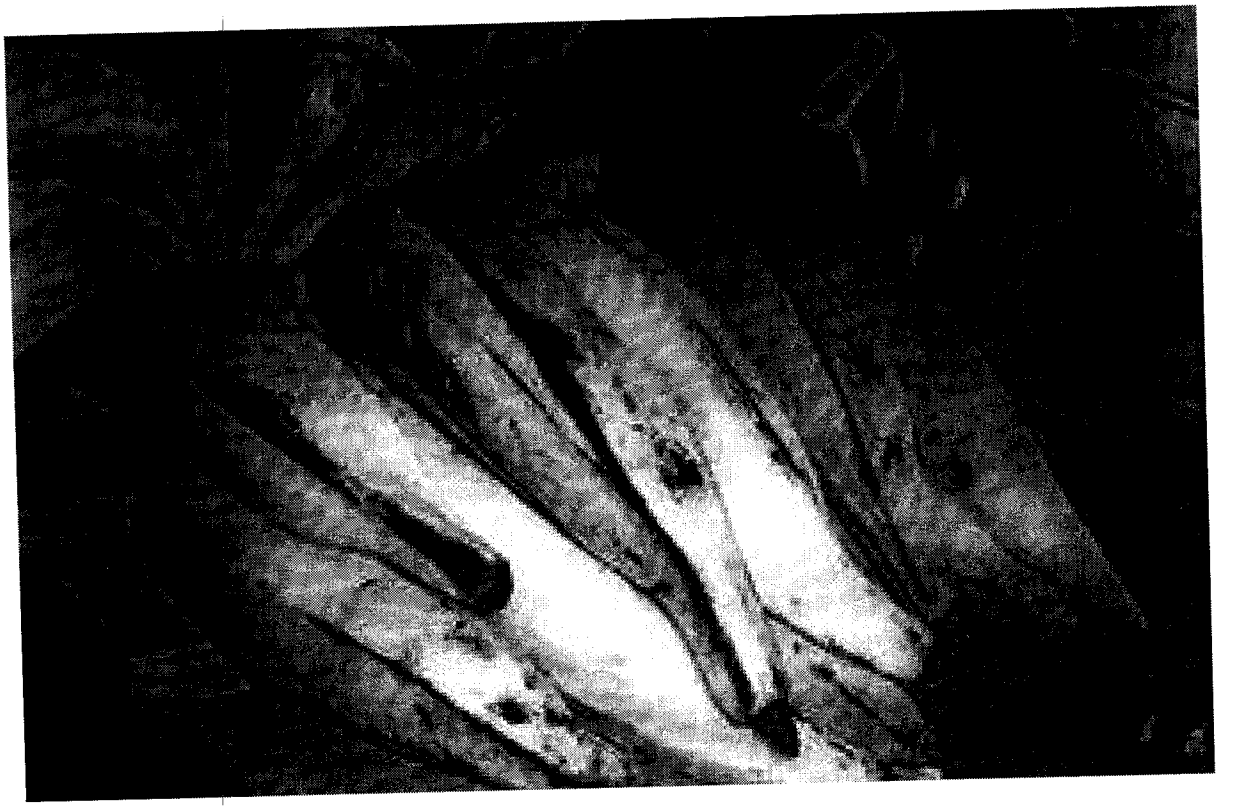


Fig. 2.17. *Musa* spp - French Plantain AAB

Materials & Methods

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Chemicals

Methanol, Sulphanilamide, Napthyl ethylene diamine dihydrochloride, Ortho phosphoric acid, Deoxyribose, Ethylene diamine tetraacteic acid (EDTA), Ferric chloride, Trichloro acetic acid (TCA), Glacial acetic acid, Sulfuric acid, Disodium hydrogen phosphate, Sodium dihydrogen phosphate, Hydrogen peroxide, Sodium nitrite, Aluminium chloride, Sodium hydroxide, Potassium hydroxide, Calcium carbonate, Ascorbic acid, 2,4-dinitrophenyl hydrazine (DNPH), Methionine, Triton-X-100, Hydroxylamine hydrochloride, Riboflavin, Potassium dichromate, Sodium azide, Reduced glutathione, Ethanol, Folin-Ciocalteau reagent, Sodium carbonate, Petroleum ether, Chloroform, Trifluoroacetic acid (TFA), Oxalic acid, Activated charcoal, Sodium sulphite, DTNB, Sodium citrate, 1- chloro 2, 4 dinitrobenzene (CDNB), Potassium sodium tartarate, Copper sulphate, Oxidized glutathione, NADPH, Magnesium chloride, Hydrochloric acid, Glucose- 6- phosphate, 3-methyl 2-benzothiazilinone hydrazine hydrochloride, Sodium meta periodate, Tris base, Thiourea, Xylene, 2,2' dipyridyl, Tocopherol, Catechol.

All reagents used were of analytical grade.

3.1.2. Samples

The following banana varieties obtained commercially from the stores were used for the study:

1. Banana cv. Sevvazhai- *Musa spp* - Sevvazhai AAA
2. Banana cv. Pachinadan- *Musa spp* - Pachainadan AAB

Cavendish sub group- *Musa spp* - Robusta AAA

4. Banana cv. Karpooravalli- *Musa spp* - Karpooravalli ABB

5. Banana cv. Kadali- *Musa* spp - Ney Poovan AB

6. Banana cv. Monthan- *Musa* spp - Bluggoe ABB

7. Banana cv. Rasthali- *Musa* spp - Rasthali AAB

8. Banana cv. Poovan- *Musa* spp - Mysore AAB

9. Banana cv. Nendran- *Musa* spp - French Plantain AAB

The afore mentioned banana varieties were authenticated by Dr.T.N.Balamohan, Professor and Head, Faculty of Horticulture, Tamil Nadu Agricultural University, Coimbatore.

3.2. Methods

3.2.1. Phase I- Estimation of Enzymatic and Non- enzymatic antioxidants

3.2.I.1. Estimation of Enzymatic antioxidants

3.2.1.1.1. Estimation of Superoxide Dismutase (Das *et al.*, 2000)

Principle:

The method involves generation of superoxide radical of riboflavin and its detection by nitrite formation from hydroxylamine hydrochloride. The nitrite reacts with sulphanilic acid to produce a diazonium compound which subsequently reacts with naphthylamine to produce a red azo compound whose absorbance is measured at 540nm.

Reagents:

1) 50mM phosphate buffer, pH 7.4 (Refer Appendix 1).

3) 1% (v/v) Triton X-100.

4) 10mM Hydroxylamine hydrochloride.

5) 50 µM EDTA.

6) 50 µM Riboflavin.

7) Griess reagent: 1% sulphanilamide, 2% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride.

Procedure:

1.39 ml aliquot of reaction mixture was pipetted (1.1 ml of phosphate buffer, 75 µl of methionine, 40 µl of Triton X-100, 75 µl of hydroxylamine hydrochloride and 100 µl of EDTA) into a test tube. 100 µl of the sample was added followed by preincubation at 37°C for 5 min. 80 µl of Riboflavin was added and the tubes were exposed to UV lamp for 10 min. The control tube contained equal amount of buffer instead of sample. The sample and its respective control were run together. At the end of the exposure time, 1.0 ml 0f Griess reagent was added to each tube and the absorbance of the colour formed was measured at 540nm.

One unit of enzyme activity was defined as the amount of SOD capable of inhibiting 50% of nitrite formation under assay conditions.

3.2.1.1.2. Estimation of Catalase (Sinha, 1972)

Principle:

Catalase causes rapid decomposition of hydrogen peroxide to water.

$$H_2O_2 \longrightarrow H_2O + O_2$$

[illegible] that dichromate in acetic acid reduces to

formation of perchloric acid as an unstable intermediate. The chromic acetate thus produced is measured colorimetrically at 610nm. Simce dichromate has no absorbency in this region, the presence of the compound in the assay mixture does not interfere with the colorimetric determination of chromic acetate. The catalase preparation is allowed to split Hydrogen peroxide for different periods of time. The reaction is stopped at specific time intervals by the addition of dichromate/acetic acid mixture and the remaining Hydrogen peroxide is determined by measuring chromic acetate colorimetrically after heating the reaction mixture.

Reagents:

1) 0.01 M phosphate buffer, pH 7.0 (Refer Appendix 2).

2) 0.2 M Hydrogen peroxide.

3) Stock dichromate/acetic acid solution: Mixed 5% potassium dichromate with glacial acetic acid.

4) Working dichromate/acetic acid solution: The stock was diluted to 1:5 with water to make the working dichromate/acetic acid solution.

Procedure:

The assay mixture contained 1.0 ml of buffer, 0.5 ml of hydrogen peroxide and 0.4 ml of water. 0.2 ml of sample was added to initiate the reaction. 2.0 ml of the dichromate/acetic acid reagent was added after 0,30,60,90 seconds of incubation. To the control tube the sample was added after the addition of the acid reagent. The tubes were then kept in boiling water bath for 10 min and the colour developed was read at 610nm. The activity of catalase was expressed as mole of hydrogen peroxide decomposed/min/mg

3.2.1.1.3. Estimation of Glutathione Peroxidase (Ellman, 1959)

Reagents:

1) 0.4 M sodium phosphate buffer, pH 7.0 (Refer Appendix 3).

2) 10mM sodium azide.

3) 2.5mM Hydrogen peroxide.

4) 4mM reduced glutathione.

5) 10% TCA.

6) 0.3M phosphate solution.

7) 0.04% DTNB in 1% sodium citrate.

8) Reduced glutathione: 20 mg reduced glutathione was dissolved in 100ml of distilled water.

Procedure:

0.4 ml of buffer, 0.1 ml of sodium azide, 0.2 ml of reduced glutathione, 0.1 ml of hydrogen peroxide, 0.2 ml of sample and 1.0 ml of water were added to a final incubation volume of 2.0 ml. The tubes were incubated for 0, 30, 60, 90 seconds. The reaction was then terminated by the addition of 0.5 ml of TCA. To determine the glutathione content, 2.0 ml of the supernatant was removed by centrifugation and 3.0 ml of disodium hydrogen phosphate solution and 1.0 ml of DTNB reagent was added. The colour developed was read at 412nm. Standards in the range of 40-200 µg were taken and treated in the similar manner.

The activity was expressed in terms of n moles of glutathione utilized min^{-1} mg $protein^{-1}$

3.2.1.1.4. Estimation of Glutathione S- transferase (Habig *et al.*, 1973)

Principle:

Glutathione S- transferase catalyses the reaction of 1- chloro 2, 4 dinitrobenzene (CDNB) with the sulfhydryl group of glutathione.

$$\text{CDNB} + \text{GSH} \longrightarrow \text{CDNB-S-glutathione.}$$

The conjugate, CDNB-glutathione, absorbs light at 340nm and the activity of the enzyme can therefore be estimated by measuring the increase in absorbance.

Reagents:

1) 0.5 M phosphate buffer, pH 6.5(Refer Appendix 4).

2) 30mM CDNB in 95% ethanol.

3) 30mM reduced glutathione.

Procedure:

To 1.0 ml of buffer, 1.7 ml of water, 0.1 ml of CDNB and 0.1 ml of sample were added and incubated at 37°C for 5 min. After incubation, 0.1 ml of reduced glutathione was added. The increase in OD was measured against that of the blank at 340nm.

The enzyme activity was calculated in terms of µmoles of GSH - CDNB conjugate formed min^{-1} mg $protein^{-1}$.

3.2.1.1.5. Estimation of Glutathione Reductase (Beutler, 1984)

Principle:

Glutathione reductase catalyses the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH) and is assayed by measuring the decrease in absorbance at 340 nm.

$NADPH\ (NADH) + H^+ + GSSG \text{ ----------} \rightarrow NADP^+\ (NAD)^+ + 2GSH$

Reagents:

1) 0.3 M Phosphate buffer, pH 6.8 (Refer Appendix 5).

2) 25 mM EDTA (93 mg/10 ml H_2O)

3) 12.5 mM Oxidised glutathione (11.5 mg/ 1.5 ml H_2O)

4) 3 mM NADPH (2.5 mg/ 1 ml H_2O)

Procedure:

0.2 ml of sample, 1.5 ml of buffer, 0.5 ml EDTA, 0.2 ml GSSG and 0.1 ml NADPH were added. The decrease in optical density of the enzyme was measured against that of the blank at 340 nm.

The enzyme activity is calculated in terms of µmoles of NADPH oxidized min^{-1} mg $protein^{-1}$.

3.2.1.1.6. Estimation of Glucose 6 Phosphate Dehydrogenase (Balinsky and Bernstein, 1963)

Principle:

Glucose 6 phosphate dehydrogenase is assayed by measuring the increase in absorbance which occurs at 340 nm when NADP reduces to

6 phosphate to NADP in the reaction catalysed by Glucose 6 phosphate dehydrogenase.

Reagents:

1) 0.1 M Tris HCl buffer, pH 8.2 (Refer Appendix 6).
2) 0.2 mM NADP
3) 0.1 M Mangnesium chloride
4) 6 mM Glucose 6 phosphate

Procedure:

0.4 ml of Tris-HCl buffer, 0.2 ml of NADP, 0.2 ml of magnesium chloride, 1.0 ml water and 0.2 ml of enzyme were taken in cuvette. The reaction was started by the addition of 0.2 ml of glucose 6 phosphate and the increase in OD was measured at 340 nm.

The activity was expressed in terms of units/mg protein, in which one unit is equal to the amount of enzyme that brought about a change in OD of $0.01 min^{-1}$.

3.2.1.1.7. Estimation of Polyphenol Oxidase (Rocha and Morais, 2001; Yemenicioglu, 2002)

Reagents:

1) 0.1M phosphate buffer, pH 6.0.

2) 0.001M Catechol dissolved in phosphate buffer.

Procedure:

1g of the sample was taken and ground in a mortar and pestle using 10ml of 0.1M phosphate buffer, pH 6.0. This was centrifuged at 6000 rpm for 10

3.0ml of phosphate buffer and 1ml of catechol was added and mixed well. The absorbance was read at 495nm at an interval of 30 seconds for 3 minutes.

The activity was expressed in terms of units mg protein^{-1}, in which one unit is equal to the amount of enzyme that brought about a change in OD of 0.01min^{-1}.

3.2.1.2. Estimation of Non- Enzymatic antioxidants

3.2.1.2.1. Estimation of Total Reduced Glutathione

Principle:

Glutathione (GSH) was measured by its reaction with DTNB to give a compound that absorbs at 412nm.

Reagents:

1) 0.2M phosphate buffer, pH 8.0 (Refer Appendix 7).

2) 0.4M Disodium hydrogen phosphate.

3) 0.04% DTNB in 1% sodium citrate.

4) 5% TCA.

5) Standard glutathione: 20 mg reduced glutathione was dissolved in 100 ml of distilled water.

Procedure:

1.0 ml of 10% homogenate was mixed with 1.0 ml of TCA. The solution was centrifuged at 1000 rpm for 10 min. To 1.0 ml of supernatant, 1.0 ml of disodium hydrogen phosphate and 2.0 ml of DTNB reagent was added. The absorbance was read within 2 min at 412nm against a reagent blank. A set of

standards was also treated in the above manner. The amount of glutathione was expressed as mg g^{-1} fresh tissue.

3.2.1.2.2. Estimation of Vitamin C (Ascorbic acid) (Sadasivam and Manickam, 1997)

Principle:

Ascorbic acid is oxidized by copper to form dehydroascorbic acid and diketoglutaric acid. These products are treated with 2, 4 dinitrophenyl hydrazine to form the derivative of bis 2,4 dinitrophenyl hydrazine. This compound in strong sulphuric acid undergoes a rearrangement to form a product with an absorption that is measured at 540nm. The reaction is run in the presence of thiourea to provide a mildly reducing medium, which helps to prevent interference from non-ascorbic acid chromogens.

Reagents:

1) 4% oxalic acid.

2) 0.5N sulphuric acid.

3) 2% DNPH in 0.5N sulphuric acid.

4) 10% thiourea.

5) 80% sulphuric acid.

6) Standard solution: 100 mg of ascorbic acid was dissolved in 100 ml of 4% oxalic acid.

7) Working standard: The stock was diluted to 1:10 with 4% oxalic acid.

Procedure:

5.0 g of sample was ground in 5-10 ml of 4% oxalic acid in pestle and mortar and centrifuged at 10000 rpm for 20 min. 1.0 ml of supernatant was transferred to test tube and activated charcoal was added in drop wise. Then the solution was made upto known volume of 5-10 ml with 4% oxalic acid.

1.0 ml of sample (from the above), 3.0 ml of water, 1.0 ml of DNPH and 1 to 2 drops of thiourea was added and incubated at 37°C for 3 hours. Then, 7.0 ml of ice-cold 80% sulphuric acid was added mixed well and the solutions were allowed to stand at room temperature for an additional 30 min. The absorbance was read at 540nm. The results were expressed as mg g^{-1} fresh tissue.

3.2.1.2.3. Estimation of Vitamin E

Principle:

Tocopherols can be estimated using Emmerie-Engel reaction of ferric to ferrous ions by tocopherols, which then forms a red colour with 2,2' dipyridyl. Tocopherols are carotenes are first extracted with xylene and the extinction read at 460 nm to measure carotenes. A correlation is made for these after adding ferric chloride and reading at 520 nm.

Reagents:

1) Absolute ethanol

2) Xylene

3) 2,2' dipyridyl: 1.2 g/L n-propanol

4) Ferric chloride: 1.2g of $FeCl_3$.6H2O or 720 mg of anhydrous ferric chloride in one litre of ethanol.

5) Standard D, L-α-tocopherol: 10 mg/ L in absolute ethanol. 91 mg of α-tocopherol is equivalent to 100 mg of tocopherol acetate.

6) Sample extraction: Weighed 1.0 g of the tissue and were homogenized in a blender and transferred to a conical flask. Added 50 ml of 0.1 N sulphuric acid slowly without shaking. Stoppered and allowed to stand overnight. The next day, the contents of flask were shaken vigorously and filtered through Whatmann.1 filter paper, discarding the initial 10-15 ml of the filtrate. Aliquots of the filtrate were used for the estimation.

Procedure:

Into 3 stoppered centrifuge tubes (test, standard and blank) 1.5ml of each tissue extract, 1.5ml of the standard and 1.5ml of water respectively was pipetted out. To the test and blank 1.5ml ethanol was added and to the standard 1.5ml of water was added. Then, 1.5ml of xylene was added to all the tubes, stoppered, mixed well and centrifuged.

1.0ml of xylene layer was transferred into another stoppered tube, taking care not to include any ethanol or protein. 1.0ml of 2,2'dipyridyl reagent was added to each tube, stoppered and mixed. 1.5ml of the mixtures were pipetted out into spectrophotometer cuvettes and the absorbance of test and standard against the blank was read at 460 nm. Then in turn beginning with the blank, 0.33ml of ferric chloride solution was added. This was mixed well and exactly after 1.5 minutes test and standard were read against the blank at 520 nm. The amount of vitamin E was expressed as μg α- tocopherol equivalents g^{-1} fresh tissue.

3.2.1.2.4. Estimation of Vitamin A

Reagents:

1) 2N KOH.

2) 90% alcohol.

4) Sodium Sulphate (anhydrous).

5) Chloroform.

6) TFA

7) 50mM Phosphate buffer, pH 7.4 (Refer Appendix 8).

Procedure:

1.0g of the sample was taken. It was ground to a fine paste with phosphate buffer, pH 7.4. 1ml of this homogenate was taken and to this 1ml of 2N KOH in 90% alcohol was added. The mixture was refluxed for 20 minutes at 60°C. The tubes were cooled to room temperature. 25ml of distilled water was added and mixed well. β- carotene was extracted with 25, 15, 10ml portions of petroleum ether in a separating funnel. The extract (organic layer) was pooled and sodium sulphite (anhydrous) was added to remove the moisture and left for 30- 60 minutes. Aliquots of the ether extract were taken and evaporated to dryness at 60°C. The dried residue was dissolved in 2ml of TFA reagent (1ml of TFA in 2ml of chloroform).

Estimation was carried out as follows. Aliquots of the standard vitamin A acetate were pipetted out to a series of clean, dry test tubes at varying concentrations. The volume in each test tube was made upto 1ml with chloroform. 2ml of TCA was added to this. The contents were mixed well. The absorbance was recorded immediately at 520nm. The amount of vitamin A in mg vitamin A acetate equivalents g^{-1} fresh tissue was calculated.

3.2.2. Phase II- Estimation of Phytochemicals

3.2.2.1. Estimation of Carotenoids and Lycopene

Principle:

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

Lycopene has absorption maxima at 473 and 503nm. A rapid method for the estimation of lycopene in plant products is based on the measurements of absorption of the petroleum ether extract of the total carotenoids at 450nm. After measuring the total carotenoids at 450nm, the same extract can be used for estimating lycopene at 503nm.

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.

remove turbidity. The final volume of extract was noted to a known volume. The absorbance was read at 450nm and 503nm.

3.2.2.2. Estimation of Flavonoids

Reagents:

1) Methanol.

2) Sodium nitrite.

3) Aluminium chloride.

4) 1M NaOH.

Procedure:

1g of the sample was weighed and ground with 10ml of 80% aqueous methanol in a mortar and pestle. The ground sample was filtered with the help of a Whatman filter paper no. 42 and a clear sample solution was obtained. 0.5 ml of this sample was taken in a test tube to which 1.25ml of distilled water was added. 0.075ml of 5% sodium nitrite was then added to the test tube, mixed well and the mixture was allowed to stand for 5 minutes. Then 0.15ml of 10% aluminium chloride was added. After 6 minutes, 0.5ml of 1M NaOH was introduced into the test tube. The solution in the test tube was then diluted with 0.275ml of distilled water. The absorbance was read at 510nm.

3.2.2.3. Estimation of Total Phenols

Principle:

Phenols react with phosphomolybdic acid in Folin- Ciocalteau reagent in alkaline medium and produce a blue coloured complex (molybdenum blue) that can be estimated colorimetrically at 650nm. This assay is preferred because the

Reagents:

1) Ethanol.

2) Folin- Ciocalteau reagent.

3) Sodium carbonate.

Procedure:

1g of the sample was weighed and ground with 10ml of 80% aqueous ethanol in a mortar and pestle. The ground sample was then centrifuged at 6000 rpm for 10 minutes. The supernatant obtained was taken in a separate tube. The pellet was re-extracted in 5ml ethanol. This sample was then centrifuged at 6000rpm for 10mins. The supernatant was cooled and then pooled together. The supernatants were then allowed to boil to evaporate the ethanol. The residue obtained was then dissolved in 10ml of distilled water. The estimation of total phenols was carried out by taking 0.1ml of the prepared sample and making it up to 3ml with distilled water.0.5ml of Folin- Ciocalteau reagent was added to the sample. The mixture was incubated for 3 minutes. 2ml of 20% sodium carbonate was then added. The sample was then boiled in a water bath for a minute and then it was read at 650nm.

3.2.2.4. Estimation of Alkaloids

Reagents:

1) 0.1M acetic acid.

2) 0.01M Sodium meta periodate

3) 0.01M 3-methyl 2-benzothiazilinone hydrazine hydrochloride

4) Sample Preparation: 0.5 g of the sample was taken in a clean, dry conical flask and to this 50ml of 10% acetic acid in ethanol was added. This was kept in an orbital shaker for 4 hours.

Procedure:

1.5 ml of the prepared sample was taken in a 25ml standard flask. To this 1ml of 0.01M SPI, 0.5ml of 0.1M acetic acid and 10 ml of distilled water were added and kept in a boiling water bath for 10 minutes. To this reaction mixture 2ml of 0.01 MBTH was added and kept in a boiling water bath for 2 minutes. Then the flasks were cooled and made upto the mark with distilled water. The blue colour formed was measured at 630nm.

3.2.3. Estimation of Protein (Lowry *et al.*, 1951)

Principle:

The blue colour developed by the reduction of phosphomolybdic-phosphotungstic components in the Folin-Ciocalteau reagent by the aminoacids tyrosine and tryptophan present in the protein plus the c lor developed by the biuret reaction of the protein with the alkaline cupric tartar re measured at 660nm.

Reagents:

1) 2% sodium carbonate in 0.1% NaOH (Reagent A).

2) 0.5% copper sulphate in 1% potassium sodium tartarate

(Reagent B).

3) Alkaline copper reagent: Mixed 50ml of A and 1.0 ml of B prior to use.

4) Folin-Ciocalteau reagent: Mixed 1 part of reagent with 2 parts of water.

5) Stock standard: Weighed 50 mg of bovine serum albumin and made upto 50 ml in a standard flask with saline.

6) Working standard: Diluted 10 ml of the stock to 50 ml with distilled water. 1.0 ml of this solution contains 200 μg of protein.

Procedure:

0.2 ml to 1.0 ml of working standard solution was pipetted out into a series of test tubes. 0.1 ml of the sample was taken. The volumes in all the tubes were made upto 1.0 ml with distilled water. 5.0 ml of alkaline copper reagent was added to each tube. The contents were mixed well and allowed to stand for 10 min. Then, 0.5 ml of Folin-Ciocalteau reagent was added. This was again mixed well and incubated at room temperature for 30 min. A reagent blank was also prepared. After 30 min, the blue colour developed was read at 660nm. The results were expressed as mg protein.

3.2.4. Statistical Analysis

The experimental results are expressed as mean ± SD of three replicates. The data were subjected to two- way ANOVA and significance of difference between the sample means were calculated by DMRT using IRRISTAT version 3.1. p values<0.05 were regarded as significant.

Results&Discussion

4. RESULTS AND DISCUSSION

Free radicals are atoms, molecules, or ions with unpaired electrons on an open shell configuration. The unpaired electrons cause them to be highly chemically reactive. Radicals play an important role in combustion, atmospheric chemistry, polymerization, plasma chemistry, biochemistry, and many other chemical processes, including human physiology.

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols.

A paradox in metabolism is that while the vast majority of complex life on Earth requires oxygen for its existence, oxygen is a highly reactive molecule that damages living organisms by producing reactive oxygen species. Consequently, organisms contain a complex network of antioxidant metabolites such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases, that work together to prevent oxidative damage to cellular components such as DNA, proteins and lipids. In general, antioxidant systems either prevent these reactive species from being formed, or remove them before they can damage vital components of the cell. However, since reactive oxygen species do have useful functions in cells, such as redox signaling, the function of antioxidant systems is not to remove oxidants entirely, but instead to keep them at an optimum level.

Even though the body is equipped with such a complex system of antioxidant compounds, sometimes, low levels of antioxidants, or inhibition of the antioxidant enzymes, causes oxidative stress and may damage or kill cells. Oxidative stress is thought to contribute to the development of a wide range of diseases including Alzheimer's disease, Parkinson's disease, the pathologies caused by diabetes, rheumatoid arthritis, and neurodegeneration in motor neuron diseases. These adverse conditions can however be kept at bay by consumption of natural food stuffs that have a commendable antioxidant potential.

The antioxidant activity in fruits is notable, since fruits are rich in compounds that have an important role in free radical-scavenging activity. Those compounds are polyphenols, such as flavonoids, tannins, and catechins (Macheix *et al.,* 1990). Moreover, fruits contain many vitamins, which express antioxidant activity, e.g. vitamin C, vitamin E, and β-carotene (Paul and Southgate, 1978; Hernandez *et al.*, 2006). The antioxidant activity in fruits varies among species and cultivars (Award *et al.*, 2001; Kondo *et al.*, 2004). The diverse antioxidant compounds present in fruits are responsible for the high antioxidant capacity. Many experiments have reported the antioxidant activity of fruit juice and fruit pulp from edible fruits (Mokbel and Hashinaga, 2006; Valcheva-Kuzmanova *et al.,* 2004; Bub *et al.,* 2003). However, there is a little information on the antioxidant activity in fruit peels. Interestingly, the peel and seed fractions of some fruits have higher antioxidant activity than the pulp fractions (Jayaprakasha *et al.*, 2001). For example, pomegranate peel has a higher antioxidant activity than its pulp (Li *et al.,* 2006).

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

diseases (Someya *et al.,* 2002). Therefore, attention in recent times has been focused on the isolation, characterization and utilization of natural antioxidants.

Since the banana fruits are widely available, they been used as food without apparent toxic effect. The peel could be a potential source of antioxidant and phytochemical compounds. The present investigation was undertaken to evaluate and compare the antioxidant and phytochemical potential in peel extracts from nine local varieties of banana.

4.1. Enzymatic antioxidants

SOD catalyses the conversion of superoxide anion radical into hydrogen peroxide, removes singlet oxygen as well as prevents formation of $OH^{\cdot}$ (Fridovich, 1973), and has been implicated as an essential defense against the potential toxicity of oxygen.

Catalase is an iron containing enzyme catalysing the dismutation of H_2O_2 into O_2 and H_2O. It is a major antioxidant enzyme in curtailing the peroxidative damage in biological systems. CAT, one of the most important enzymes scavenging the active oxygen species and is responsible for scavenging the active oxygen species and is responsible for scavenging H_2O_2 formed by G-POD. The enzyme is found in all aerobic eukaryotes and is important in the removal of hydrogen peroxide generated in peroxisomes by oxidases (Redinbaugh *et al.,* 1988).

The SOD activity in banana peels varied from 3.140 to 27.290 units mg^{-1} protein. The highest activity was recorded in Sevvazhai and the lowest in Karpooravalli. Hence, Sevvazhai has the highest potential to scavenge superoxide radicals. The peel extract obtained from Robusta variety has the greatest potential to decompose hydrogen peroxide as it showed highest catalase activity.

Table 4.1. Activities of Hydrogen peroxide metabolizing enzymes in the peel extracts from local varieties of Banana

Varieties	Superoxide Dismutase	Catalase
Sevvazhai	27.290^{e} ± 1.000	1.210^{c} ± 0.200
Pachainadan	5.860^{b} ± 0.335	0.550^{ab} ± 0.127
Robusta	3.880^{a} ± 0.798	2.520^{e} ± 0.319
Kadali	9.710^{d} ± 0.528	0.400^{a} ± 0.086
Karpooravalli	3.140^{a} ± 0.242	1.690^{d} ± 0.193
Monthan	8.500^{c} ± 0.570	1.990^{d} ± 0.536
Rasthali	5.130^{b} ± 0.615	0.770^{abc} ± 0.104
Poovan	6.030^{b} ± [illegible]75	1.190^{c} ± 0.120
Nendran	10.800^{d} ± 1.196	0.980^{bc} ± 0.181

Values represent mean ± SD of 3 replicates.

Means ranked by a common letter are not significantly [illegible] at the 5% level by DMRT.

Units: SOD - 50% inhibition of nitrate.

CAT - n moles of H_2O_2 decomposed min^{-1} mg $protein^{-1}$.

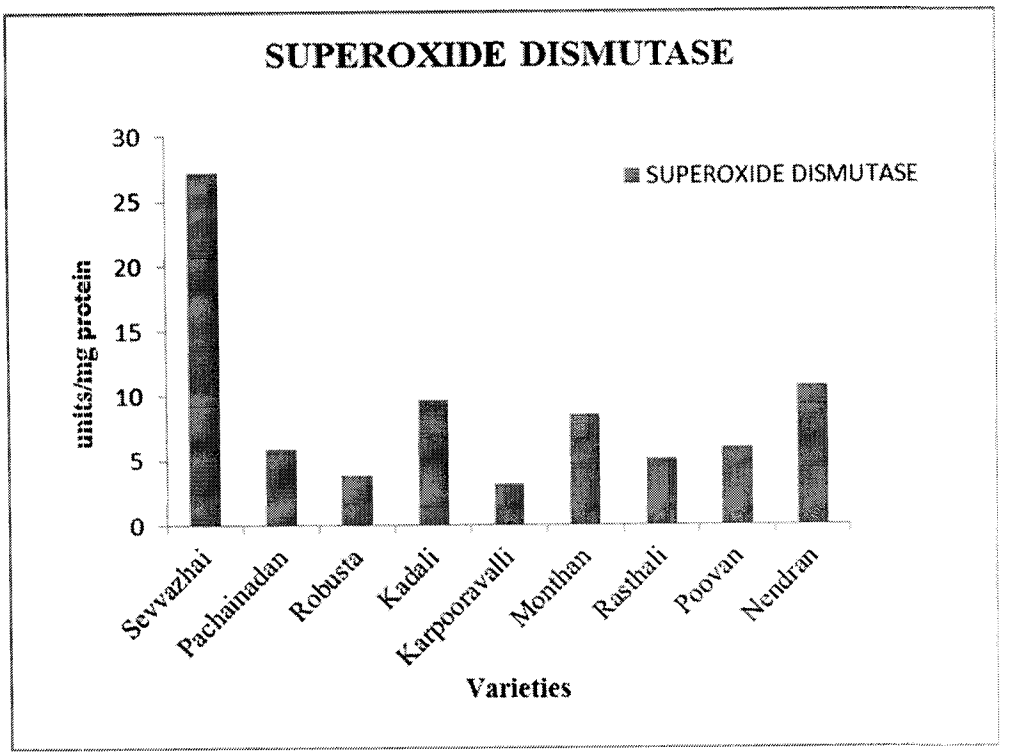


Fig.4.1. Superoxide Dismutase

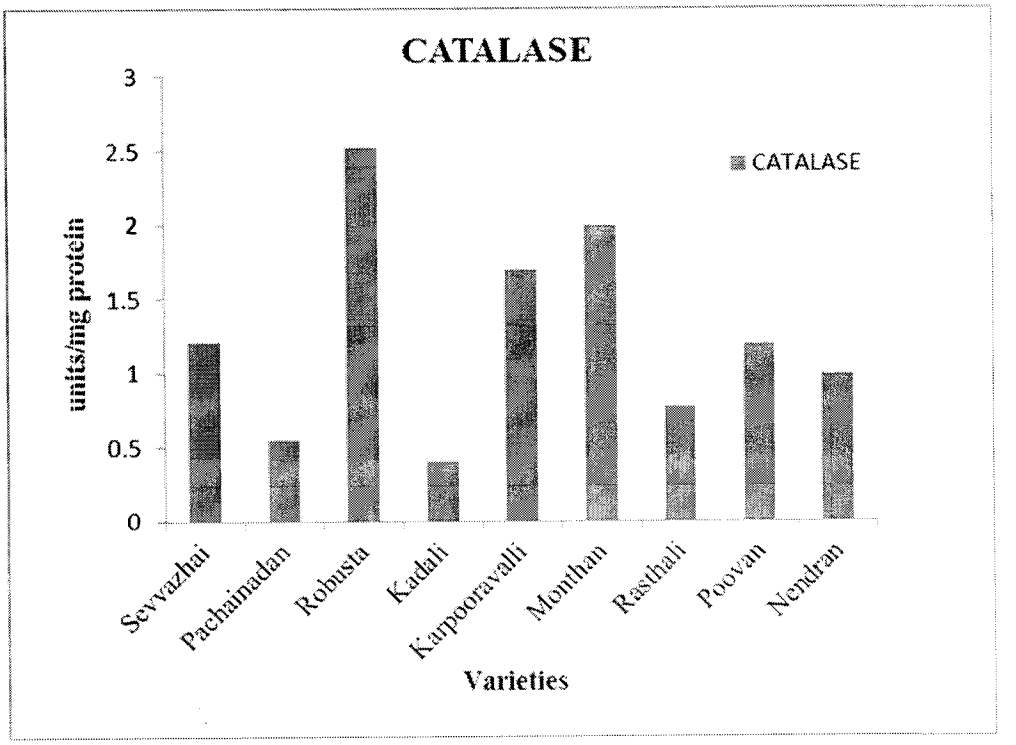


Fig.4.2. Catalase

Glutathione peroxidase, a selenium enzyme, plays a major role in regulating the concentration of H_2O_2 and a wide variety of organic peroxides. GST offers protection against lipid peroxidation by promoting the conjugation of toxic electrophiles with GSH (Jakoby, 1988). GR maintains the cellular levels of GSH (by the reduction of GSSG), which protects the cellular membranes from peroxides (Zarida, 1993).

Glutathione reductase is an NADPH flavoprotein which reduces GSSG to GSH at the expense of oxidizing NADPH. Glutathione reductase contains two protein subunits each with a flavin, FAD at its active site. The NADPH reduces the FAD which then passes its electron onto a disulfide bridge, S-S, between the two cysteine residues in the protein. The two SH formed then interact with GSSG and reduce it to GSH.

Table 4.2 shows the activities of the three glutathione [illegible]lising enzymes. The values range from 0.134 units mg protein^{-1} to [illegible]g protein^{-1} for glutathione peroxidase, the highest activity being seen [illegible]Karpooravalli and least in Poovan. Karpooravalli again leads all other varieties in GR activity, the value being 2.799 units mg protein^{-1}. Hence, Karpooravalli peel extract has the highest potential to decompose hydrogen peroxide to water as well to maintain reduced glutathione levels in the body, which in turn quenches reactive oxygen species, thereby preventing many free radical related diseases. While Robusta showed least GR activity, it has the highest potential to link toxic electrophiles to GSH and thereby suppress their activity, because its peel extract is found to show highest GST activity. The least GST activity was seen in Poovan.

Table 4.2. Activities of Glutathione utilizing enzymes from local varieties of Banana:

Varieties	Glutathione Peroxidase	Glutathione Reductase	Glutathione S-Transferase
Sevvazhai	$0.505^{cd} \pm 0.030$	$0.281^{a} \pm 0.002$	$1.170^{ab} \pm 0.045$
Pachainadan	$1.027^{e} \pm 0.108$	$0.833^{b} \pm 0.047$	$6.810^{d} \pm 0.676$
Robusta	$1.093^{ef} \pm 0.160$	$0.224^{a} \pm 0.053$	**12.070**$^{e} \pm 1.160$
Kadali	$0.589^{d} \pm 0.075$	$1.909^{c} \pm 0.152$	$3.700^{bc} \pm 0.209$
Karpooravalli	**1.281**$^{f} \pm 0.244$	**2.799**$^{d} \pm 0.390$	$6.530^{d} \pm 0.415$
Monthan	$0.288^{ab} \pm 0.075$	$0.336^{a} \pm 0.053$	$5.030^{cd} \pm 0.707$
Rasthali	$0.381^{bc} \pm 0.010$	$0.325^{a} \pm 0.042$	$4.860^{cd} \pm 0.512$
Poovan	$0.134^{a} \pm 0.010$	$0.344^{a} \pm 0.049$	$0.940^{a} \pm 0.012$
Nendran	$0.585^{d} \pm 0.014$	$0.274^{a} \pm 0.066$	$4.620^{cd} \pm 0.484$

Values represent mean ± SD of 3 replicates.

Means ranked by a common letter are not significantly different at the 5% level by DMRT.

Units: GPx - n moles of GSH consumed min^{-1} mg $protein^{-1}$.

GR - µmoles of GSH utilized min^{-1} mg $protein^{-1}$.

GST - µmoles of CDNB-GSH conjugate formed min^{-1} mg $protein^{-1}$.

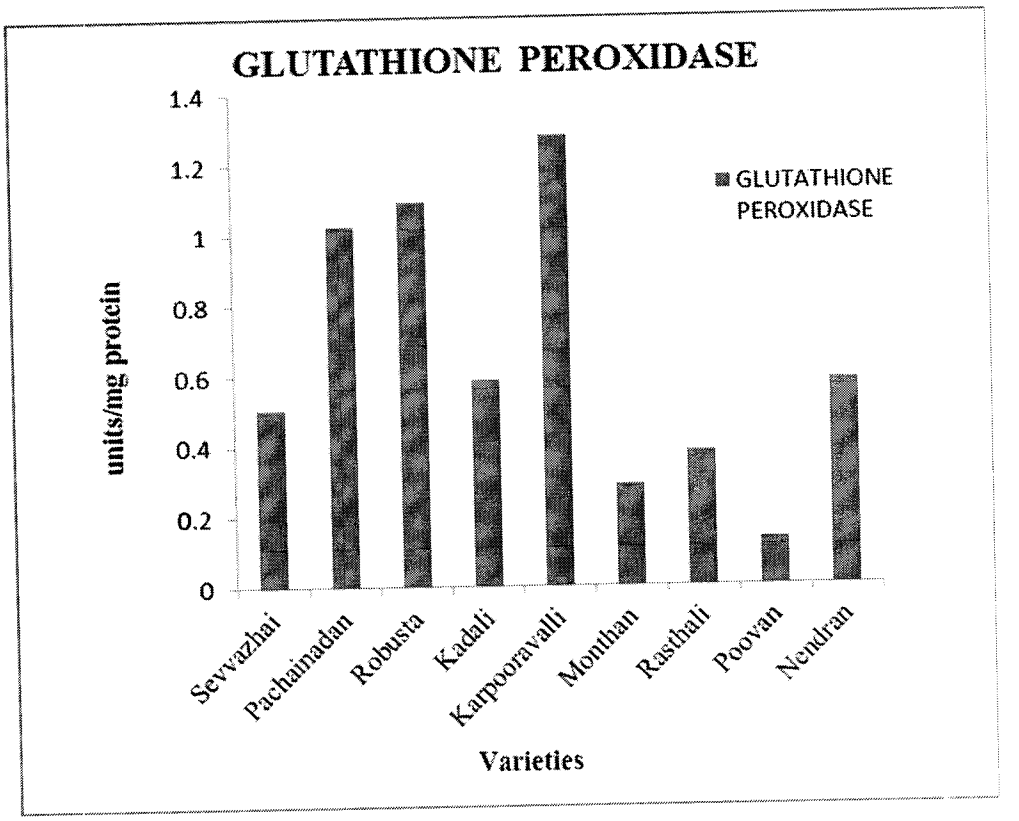
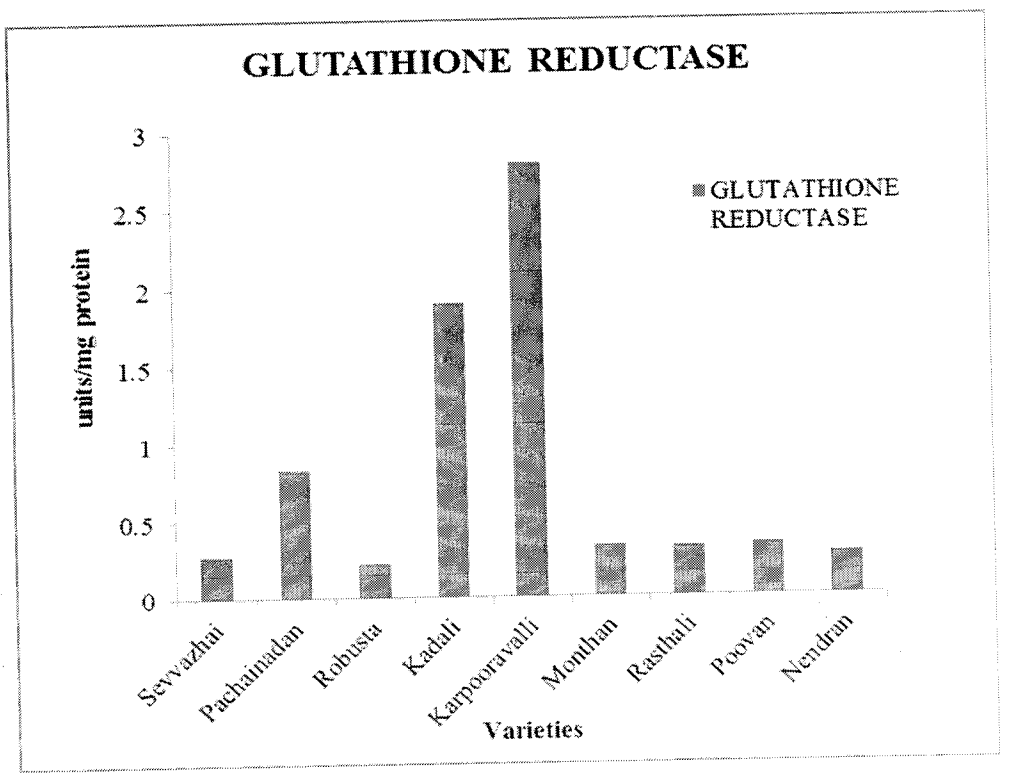


Fig.4.3. Glutathione peroxidase



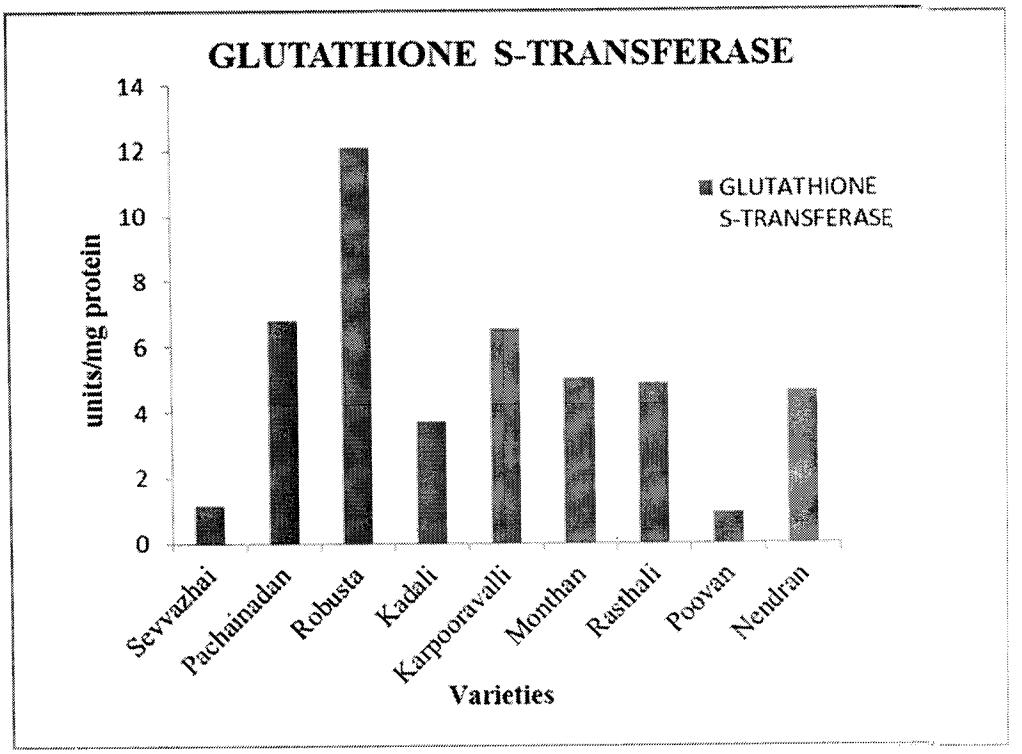


Fig.4.5. Glutathione-S-transferase

G6PD is a cytosolic NADP dependent enzyme. This generates NADPH which is necessary for the regeneration of reduced glutathione from oxidized GSH. Maintenance of GSH in the reduced state is an important function of G6PD.

Polyphenol oxidase is an oxygen transferring enzyme. PPO in the presence of oxygen, catalyses the oxidation of phenolic compounds to form corresponding quinine intermediates which polymerise to form undesirable pigments. The enzyme catalyses two types of oxidative reactions: the hydroxylation of monophenols to o- diphenols, and the oxidation of o-diphenols to o- quinines.

G6PD activity in banana varieties ranged between 0.099 to 0.696 units mg protein^{-1}. Karpooravalli again topped the list among all varieties. Hence, it

levels. Reduced glutathione in turn forms conjugate with free radicals thereby combating several diseases. On the contrary, least G6PD activity was observed in Pachainadan. PPO activity was highest in Monthan peel extract, the least being in Rasthali.

Table 4.3. Activities of Glucose-6-phosphate dehydrogenase and Polyphenol Oxidase in peel extracts from local varieties of Banana:

Varieties	Glucose-6-Phosphate Dehydrogenase	Polyphenol Oxidase
Sevvazhai	$0.177^{a} \pm 0.011$	$0.044^{b} \pm 0.007$
Pachainadan	$0.099^{a} \pm 0.007$	$0.040^{ab} \pm 0.005$
Robusta	$0.244^{a} \pm 0.025$	$0.157^{d} \pm 0.010$
Kadali	$0.582^{b} \pm 0.040$	$0.032^{ab} \pm 0.006$
Karpooravalli	$0.696^{b} \pm 0.468$	$0.031^{ab} \pm 0.009$
Monthan	$0.148^{a} \pm 0.014$	$0.469^{e} \pm 0.040$
Rasthali	$0.132^{a} \pm 0.056$	$0.010^{a} \pm 0.003$
Poovan	$0.179^{a} \pm 0.059$	$0.170^{d} \pm 0.017$
Nendran	$0.304^{a} \pm 0.028$	$0.098^{c} \pm 0.004$

Values represent mean ± SD of 3 replicates.

Means ranked by a common letter are not significantly different at the 5% level by DMRT.

Units: G6PD, PPO - 0.01 OD min^{-1} mg $protein^{-1}$.

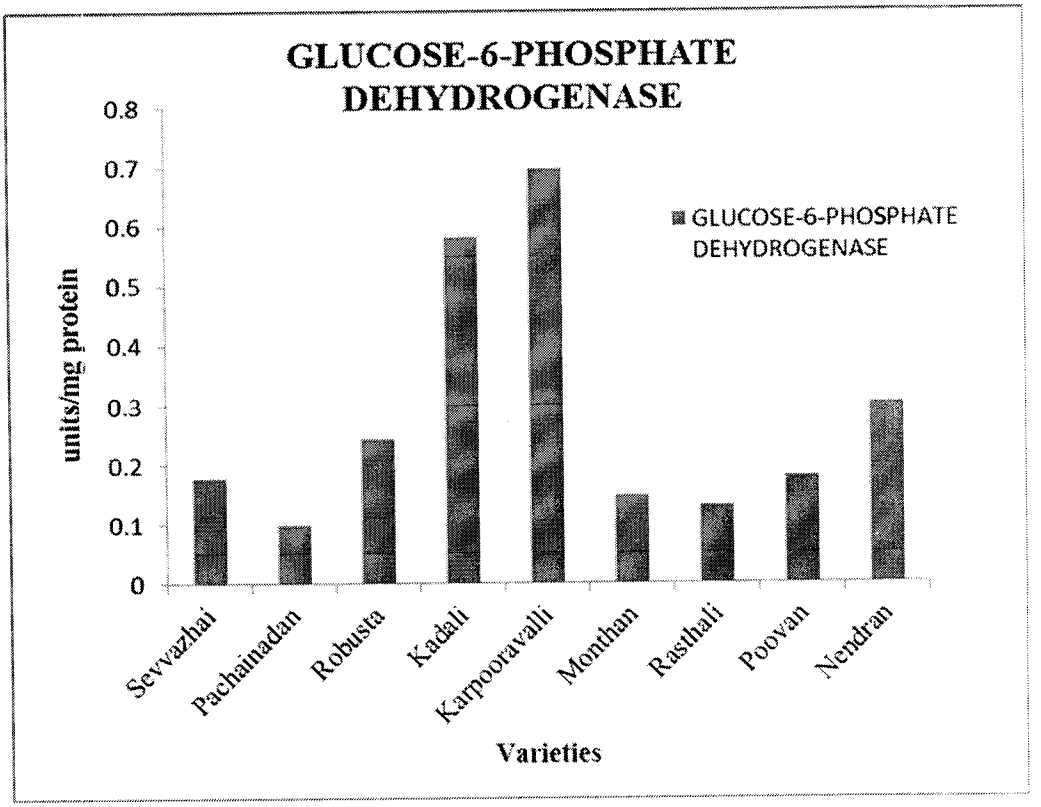


Fig.4.6. Glucose-6-phosphate dehydrogenase

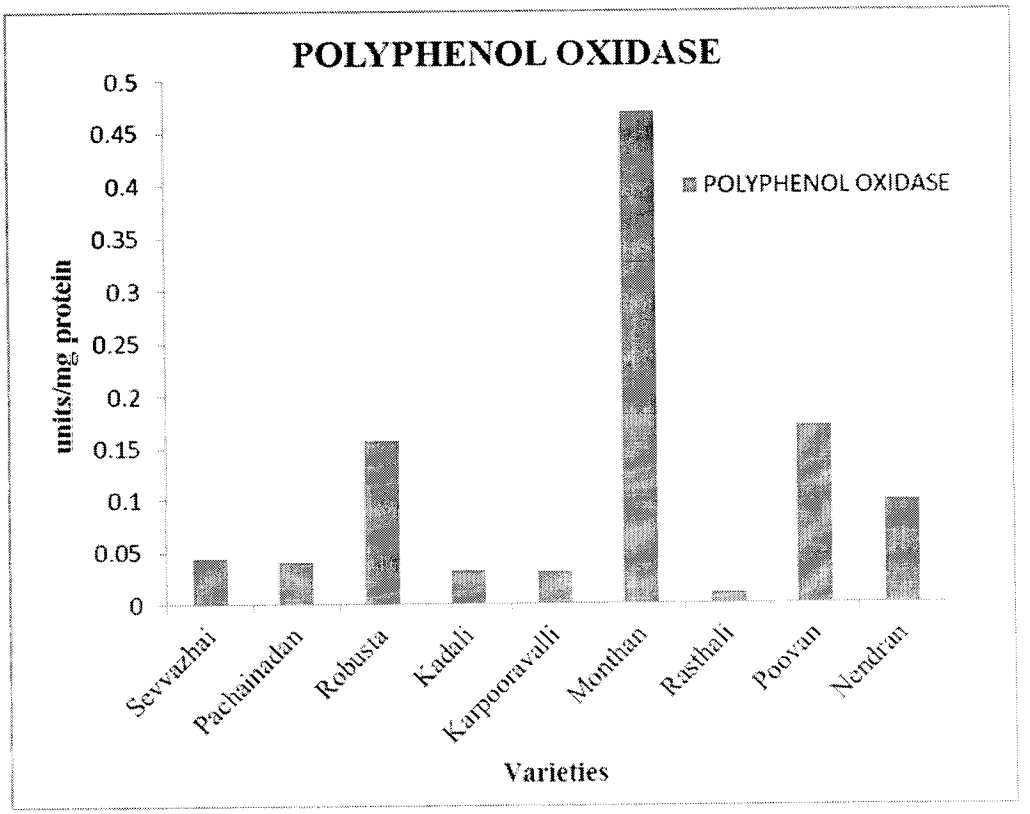


Fig.4.7. Polyphenol oxidase

4.2. Non- Enzymatic antioxidants

Ascorbic acid functions as the main water-soluble antioxidant protecting tissue from oxidative damage. It acts as a direct scavenger of free radicals and acts as a reductant in enzymatic reactions (Nagel *et al.,* 1997).

GSH, an important protein thiol in living organisms plays a central role in coordinating the body's antioxidant defence process (Boyd *et al.,* 1981). Reducing GSH constitutes the first line of defence against free radicals (Raja *et al.,* 2006).Glutathione can function as an antioxidant in many ways. It can react chemically with singlet oxygen, superoxides and hydroxyl radicals and therefore, function directly as free radical scavenger. GSH may stabilise membrane structure by removing acyl peroxides formed by lipid peroxidation reaction (Price *et al.,* 1990). GSH is the reducing agent that recycles ascorbic acid from its oxidised to its reduced form by the enzyme dehydroascorbate reductase (Loewus, 1988). It also participates in the detoxification of xenobiotics, as a substrate for the enzyme glutathione-S-transferase. GSH is also the precursor of the phytochelatins that act as heavy metal binding peptides in plants (Ruegsegger *et al.,* 1990).

Ascorbic acid content was examined in the peel extract obtained from the nine varieties of banana. The peel extract obtained from Kadali showed highest ascorbic acid content. Kadali, Robusta and Monthan peel extracts showed highest GSH content. Since, GSH converts ascorbic acid from its oxidized to its reduced state,by the enzyme dehydroascorbate reductase, Kadali banana can serve the purpose of both ascorbic acid and GSH in the body. Unlike Kadali, Rasthali showed lowest ascorbic acid and GSH contents. Karpooravalli was on par with Rasthali in ascorbic acid content.

Table 4.4. Ascorbic acid and Total Reduced Glutathione contents in peel extracts from local varieties of Banana:

Varieties	Ascorbic Acid	Total Reduced Glutathione
Sevvazhai	$0.128^{c} \pm 0.012$	$0.027^{bc} \pm 0.004$
Pachainadan	$0.072^{ab} \pm 0.014$	$0.017^{b} \pm 0.001$
Robusta	$0.062^{ab} \pm 0.005$	$\mathbf{0.031}^{c} \pm 0.007$
Kadali	$\mathbf{0.265}^{d} \pm 0.064$	$\mathbf{0.031}^{c} \pm 0.005$
Karpooravalli	$0.045^{a} \pm 0.009$	$0.019^{ab} \pm 0.002$
Monthan	$0.095^{abc} \pm 0.012$	$\mathbf{0.031}^{c} \pm 0.007$
Rasthali	$0.045^{ab} \pm 0.012$	$0.014^{bc} \pm 0.001$
Poovan	$0.062^{a} \pm 0.007$	$0.026^{a} \pm 0.003$
Nendran	$0.114^{bc} \pm 0.026$	$0.025^{bc} \pm 0.011$

Values represent mean ± SD of 3 replicates.

Means ranked by a common letter are not significantly different at the 5% level by DMRT.

Units: Ascorbic acid, Total GSH – mg g^{-1} fresh tissue

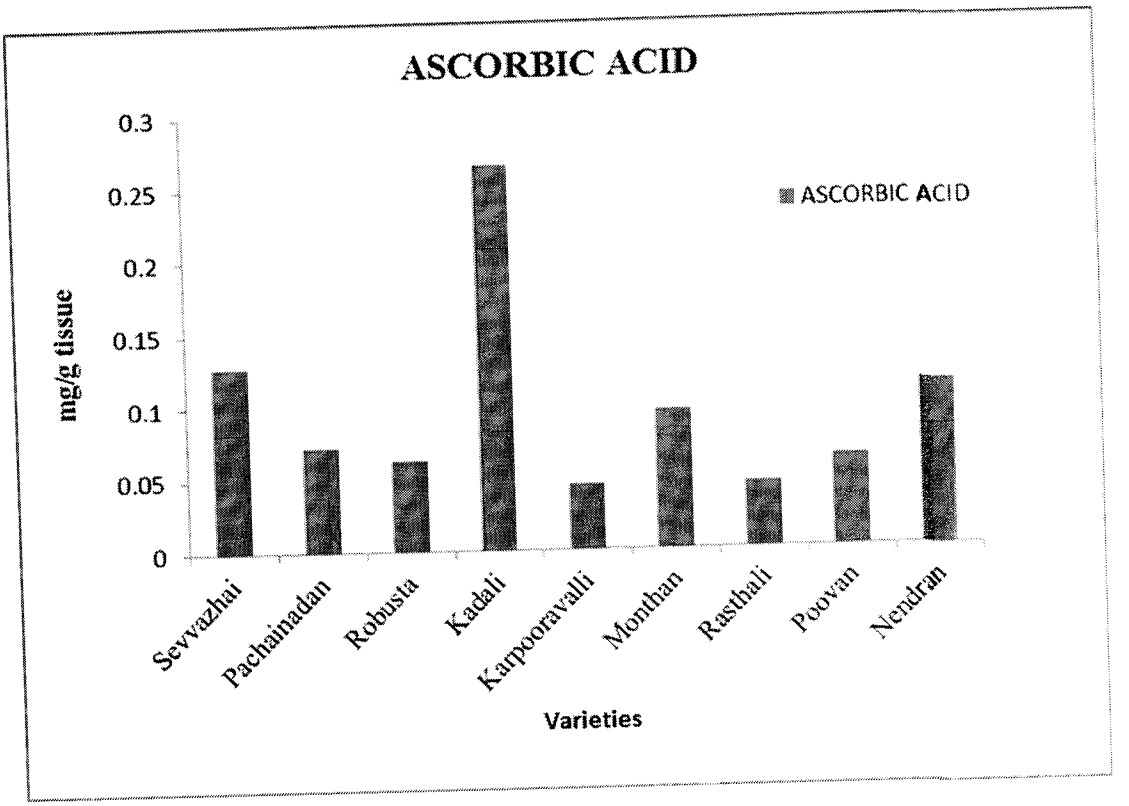


Fig.4.8. Ascorbic acid

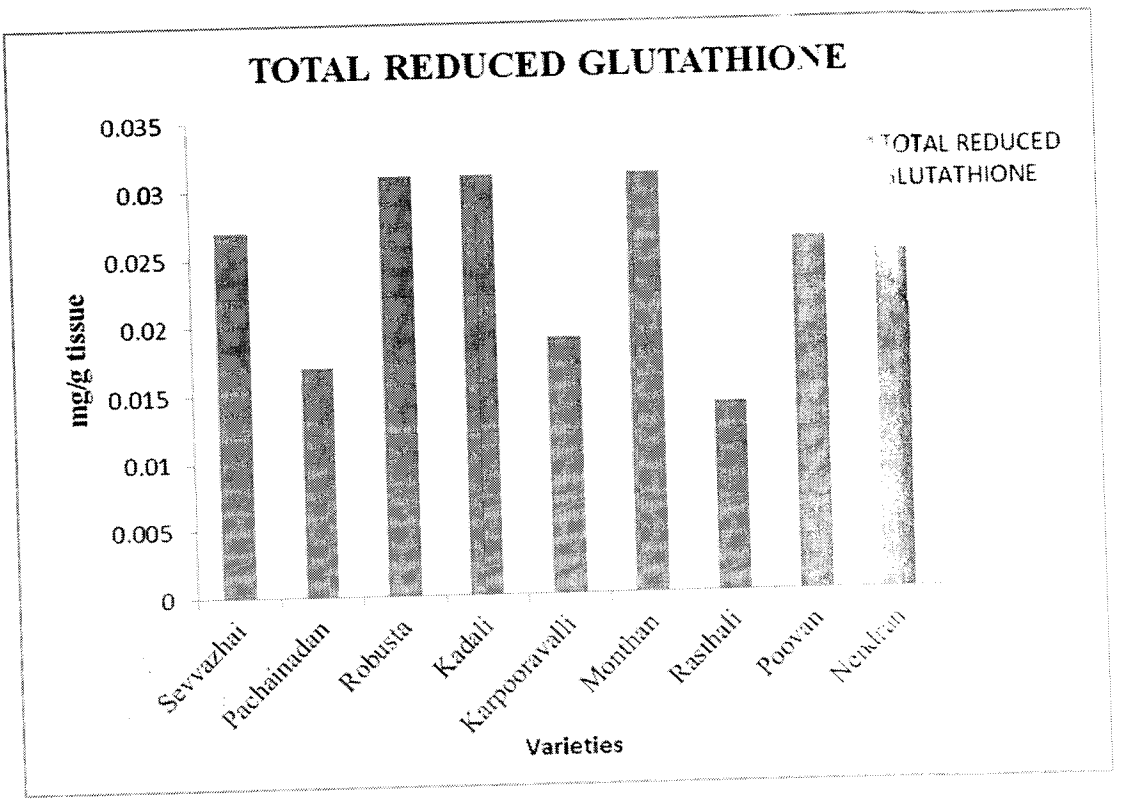


Fig.4.9. Total Reduced Glutathione

Vitamin A cannot be synthesized in the body and has to be taken through diet. It is an important antioxidative vitamin and plays an important role in counteracting free radicals. Vitamin E is a group of compounds with well known antioxidant functions. Among vitamin E compounds, tocopherol and especially alphtocopherol possesses the strongest biological activity. Tocopherol is prevalently found in mammalian tissue.

The quantification procedure was carried out for all the nine banana varieties using the peel extracts obtained from them. Vitamin A and vitamin E contents in the different varieties are shown in Table 4.5. Karpooravalli and Rasthali topped the list for vitamin A and vitamin E respectively. On the other hand Kadali and Karpooravalli showed lowest vitamin A and vitamin E content.

Table 4.5. Vitamin A and Vitamin E contents in peel extracts from local varieties of Banana:

Varieties	Vitamin A	Vitamin E
Sevvazhai	$0.422^{d} \pm 0.003$	1.097 ± 0.005
Pachainadan	$0.359^{c} \pm 0.039$	$1.040^{d} \pm 0.044$
Robusta	$0.263^{b} \pm 0.014$	$0.724^{bc} \pm 0.016$
Kadali	$0.050^{a} \pm 0.004$	$0.697^{b} \pm 0.056$
Karpooravalli	$\mathbf{1.276}^{f} \pm 0.051$	$0.561^{a} \pm 0.055$
Monthan	$0.067^{a} \pm 0.011$	$0.775^{bc} \pm 0.007$
Rasthali	$0.849^{a} \pm 0.090$	$\mathbf{1.130}^{d} \pm 0.014$
Poovan	$0.063^{e} \pm 0.008$	$1.035^{e} \pm 0.073$
Nendran	$0.279^{b} \pm 0.008$	$0.805^{c} \pm 0.073$

Values represent mean ± SD of 3 replicates.

Means ranked by a common letter are not significantly different at the 5% level by DMRT.

Units: Vitamin A - mg vitamin A acetate equivalents g^{-1} fresh tissue.

Vitamin E - µg α- tocopherol equivalents g^{-1} fresh tissue.

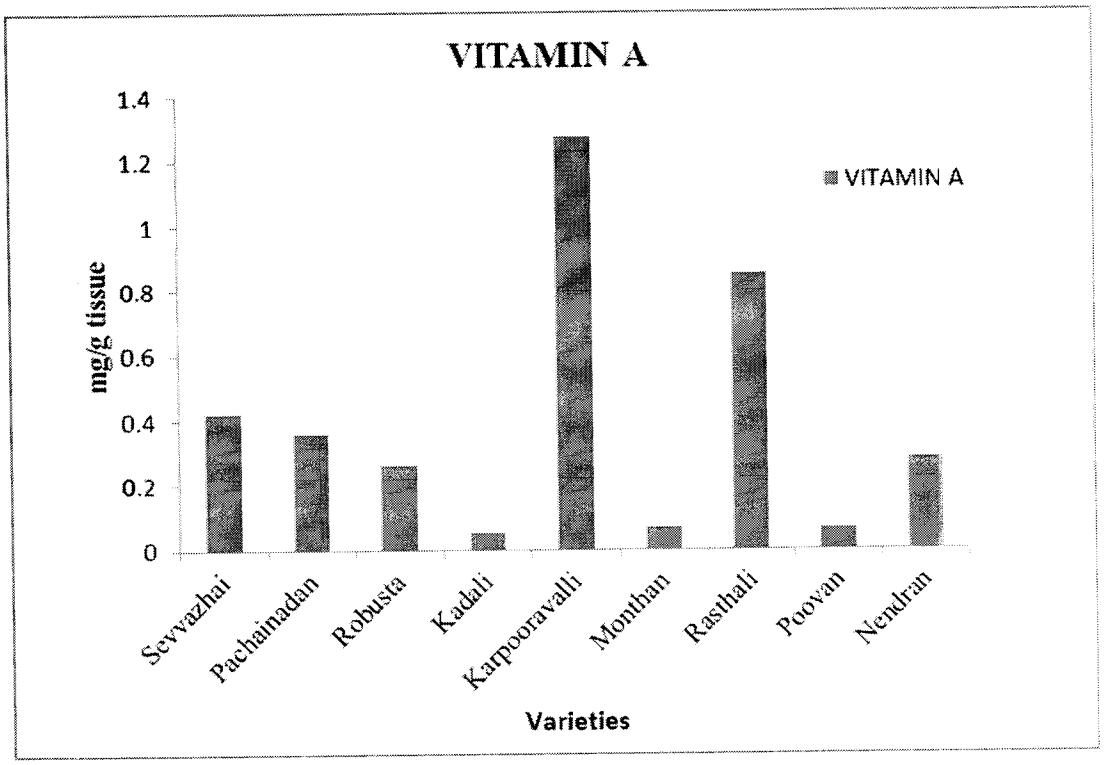


Fig.4.10. Vitamin A

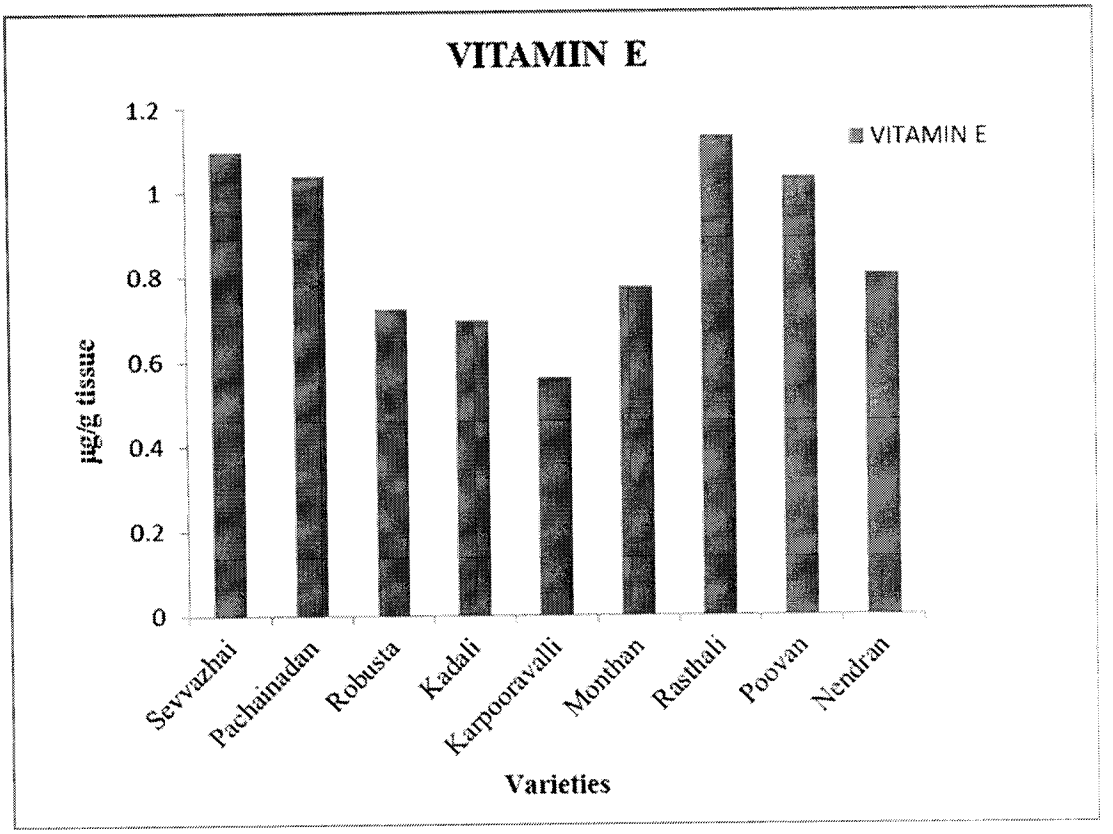


Fig.4.11. Vitamin E

4.3. Phytochemicals

Phenolic compounds, among others flavonoids and phenolic acids, show antioxidant and antiradical activities *in vitro*. There is evidence that phenolic compounds can also act as antioxidants and scavengers of free radicals *in vivo*. The antiradical and antioxidant effects of phenolic compounds *in vivo* may slow down the ageing processes as well as protect the human body against diseases such as atherosclerosis, coronary heart disease and cancer

Flavonoids, a group of polyphenolic compounds, can widely be found in fruits and vegetables. Numerous positive health effects of flavonoids have been described. They have been reported to exhibit anti-cancer , anti-viral and anti-inflammatory effects, and to reduce the risk of cardiovascular diseases . These activities are generally associated with antioxidant or free radical scavenging

and their antioxidant properties are very different. Alkaloids are a class of non-nutritive phytochemical compounds that are synthesized as secondary metablites by the plant cells. They fight against free radicals and are capable of quenching their activity.

Phenols, flavonoids and alkaloids were found in different quantities in the peel extracts from the bananas. The values obtained are depicted in the Table 4.6. Rasthali, Poovan and Robusta showed greatest phenol, flavonoid and alkaloid contents respectively. Thus, these varieties have the capability to slow down the ageing process, fight against cancer and can reduce the risk of cardiovascular diseases. However, Kadali and Monthan were not rich in these phytochemicals.

Table 4.6. Total Phenolic, Flavonoid and Alkaloid contents in peel extracts from local varieties of Banana:

Varieties	Total Phenols	Flavonoids	Alkaloids
Sevvazhai	$0.459^{cd} \pm 0.066$	$12.960^{ab} \pm 1.040$	$0.120^{ef} \pm 0.004$
Pachainadan	$0.298^{b} \pm 0.015$	$18.790^{d} \pm 1.352$	$0.125^{g} \pm 0.003$
Robusta	$0.199^{a} \pm 0.025$	$17.930^{cd} \pm 0.525$	$\mathbf{0.134}^{f} \pm 0.002$
Kadali	$0.153^{a} \pm 0.005$	$13.840^{b} \pm 0.504$	$0.054^{e} \pm 0.001$
Karpooravalli	$0.191^{a} \pm 0.007$	$16.910^{c} \pm 0.229$	$0.117^{ab} \pm 0.003$
Monthan	$0.317^{b} \pm 0.027$	$11.910^{a} \pm 0.228$	$0.059^{d} \pm 0.004$
Rasthali	$\mathbf{0.600}^{c} \pm 0.060$	$21.330^{e} \pm 6.446$	$0.063^{c} \pm 0.002$
Poovan	$0.399^{e} \pm 0.086$	$\mathbf{22.830}^{e} \pm 1.536$	$0.075^{b} \pm 0.003$
Nendran	$0.490^{d} \pm 0.059$	$21.720^{e} \pm 0.709$	$0.103^{a} \pm 0.006$

Values represent mean ± SD of 3 replicates.

Means ranked by a common letter are not significantly different at the 5% level by DMRT.

Units: Total Phenols – mg catechol equivalents g^{-1} fresh tissue.

Flavonoids – mg rutin equivalents g^{-1} fresh tissue.

Alkaloids – mg theophylline equivalents g^{-1} fresh tissue.

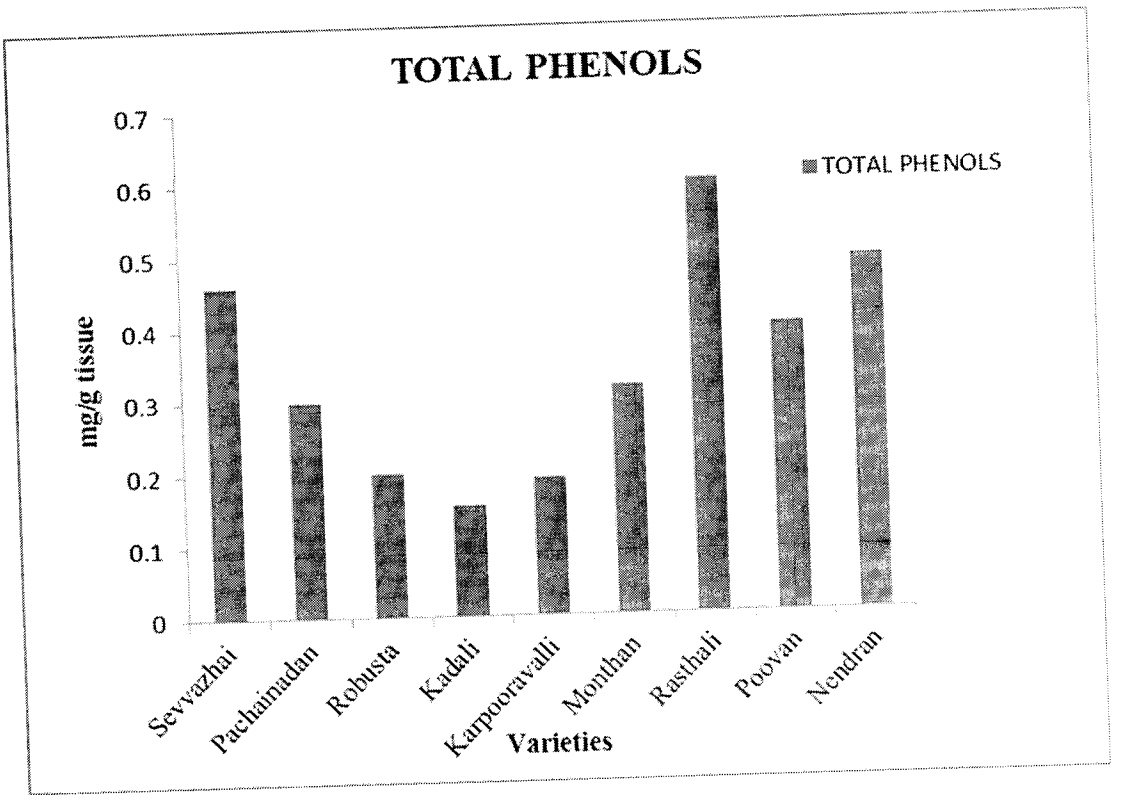


Fig.4.12. Total phenols

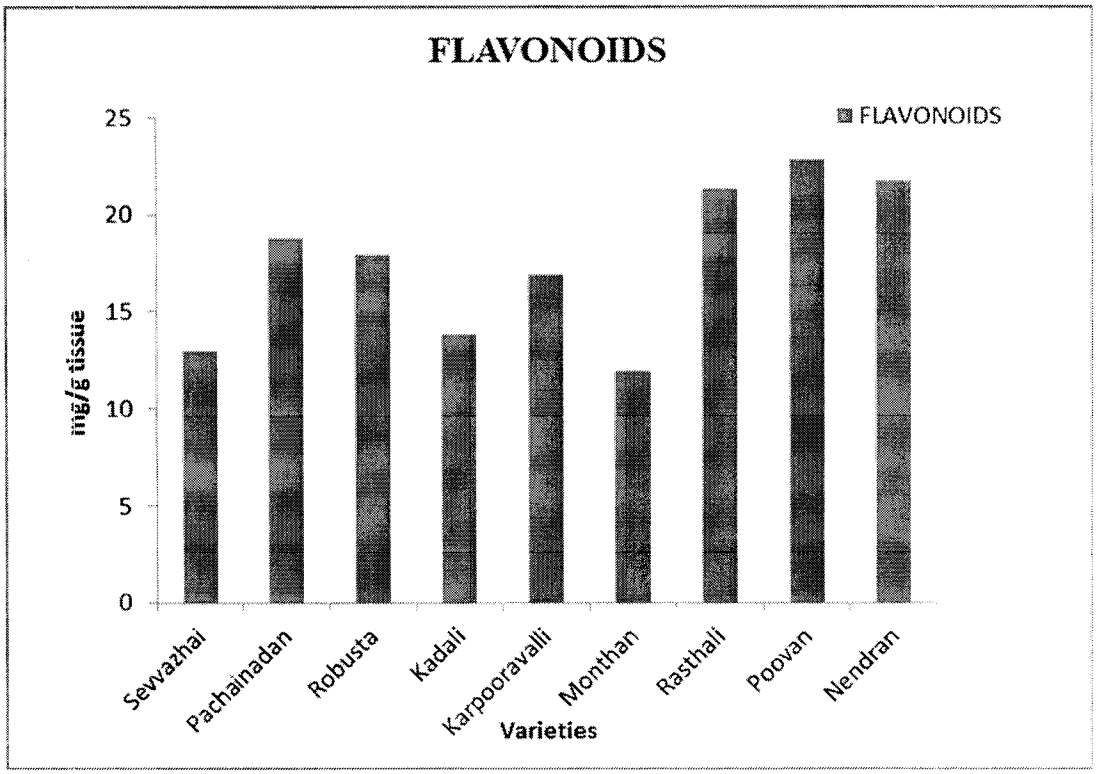


Fig.4.13. Flavonoids

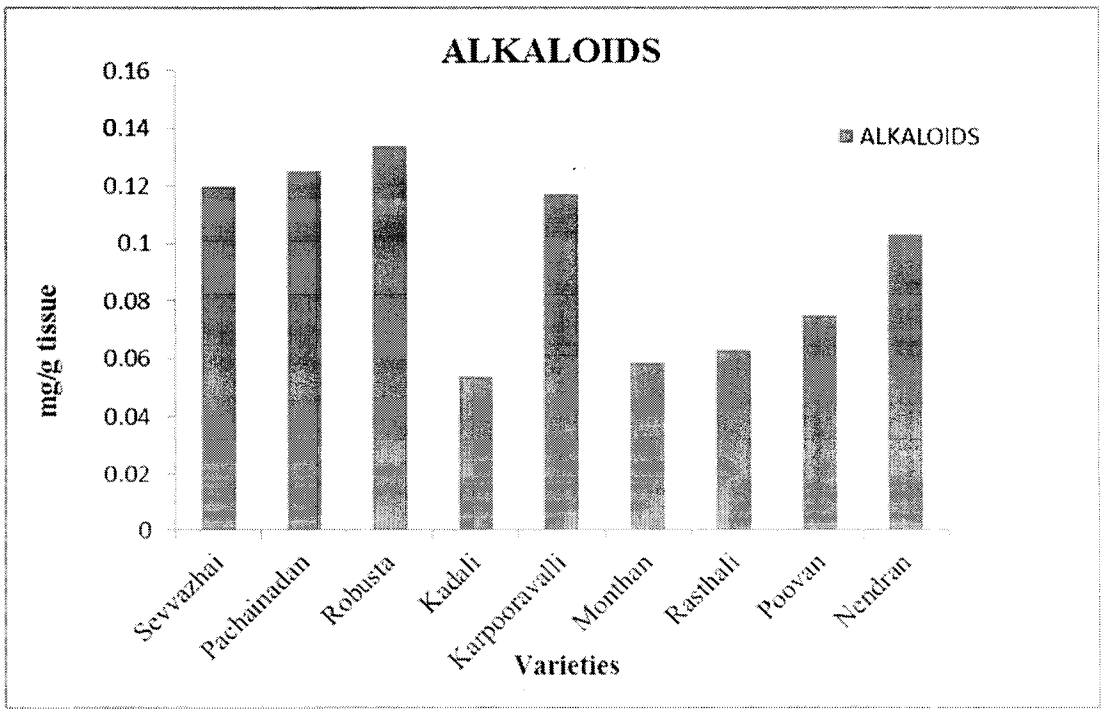


Fig.4.14. Alkaloids

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. Lycopenes are efficient quenchers of singlet oxygen. Kadali showed highest content of both carotenoids and lycopenes. Pachainadan and Monthan showed, on the other hand, showed lowest content of carotenoids and lycopenes respectively. The data obtained are displayed in theTable -

Table 4.7. Carotenoid and Lycopene contents in peel extracts from local varieties of Banana:

Varieties	Carotenoids	Lycopenes
Sevvazhai	$0.187^{d} \pm 0.009$	$2^{d} \pm 0.001$
Pachainadan	$0.091^{a} \pm 0.006$	$3^{bc} \pm 0.002$
Robusta	$0.109^{b} \pm 0.0$	$0.010^{cd} \pm 0.002$
Kadali	$\mathbf{0.416}^{} \pm 0.008$	$\mathbf{0.041}^{f} \pm 0.001$
Karpooravalli	$0.263^{e} \pm 0.008$	$0.027^{e} \pm 0.001$
Monthan	$0.096^{a} \pm 0.004$	$0.005^{a} \pm 0.002$
Rasthali	$0.153^{f} \pm 0.005$	$0.009^{e} \pm 0.001$
Poovan	$0.393^{c} \pm 0.004$	$0.027^{bc} \pm 0.001$
Nendran	$0.091^{a} \pm 0.008$	$0.007^{ab} \pm 0.002$

Values represent mean ± SD of 3 replicates.

Means ranked by a common letter are not significantly different at the 5% level by DMRT.

Units: Carotenoids, Lycopenes – mg g^{-1} fresh tissue.

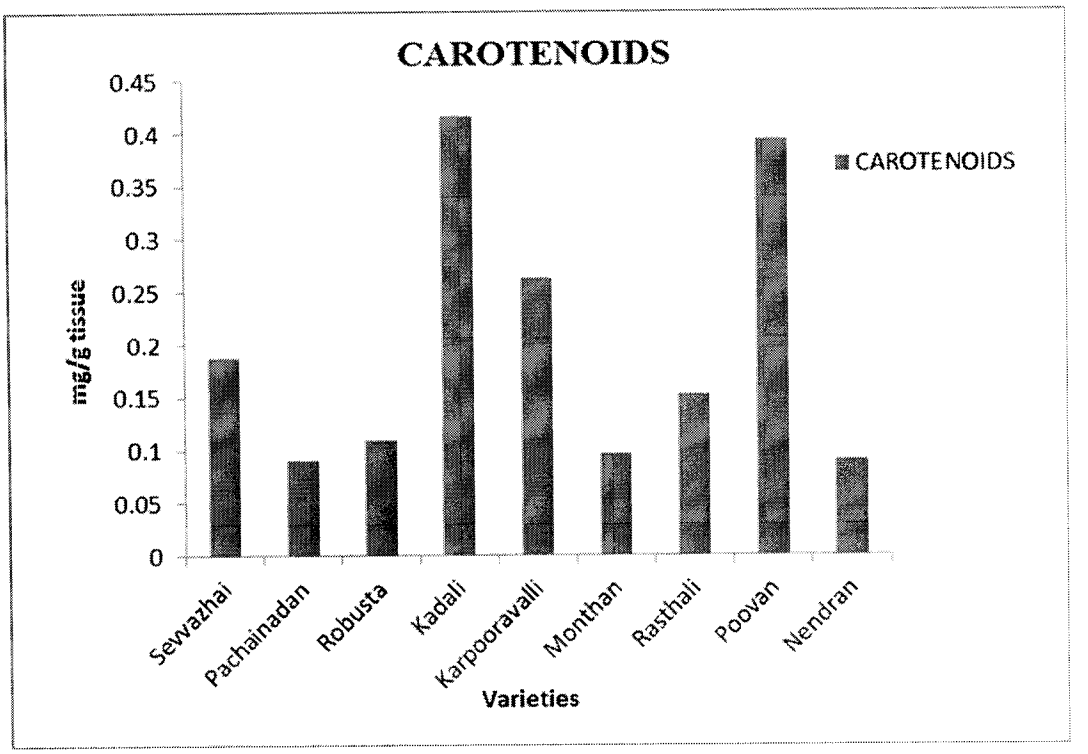


Fig.4.15. Carotenoids

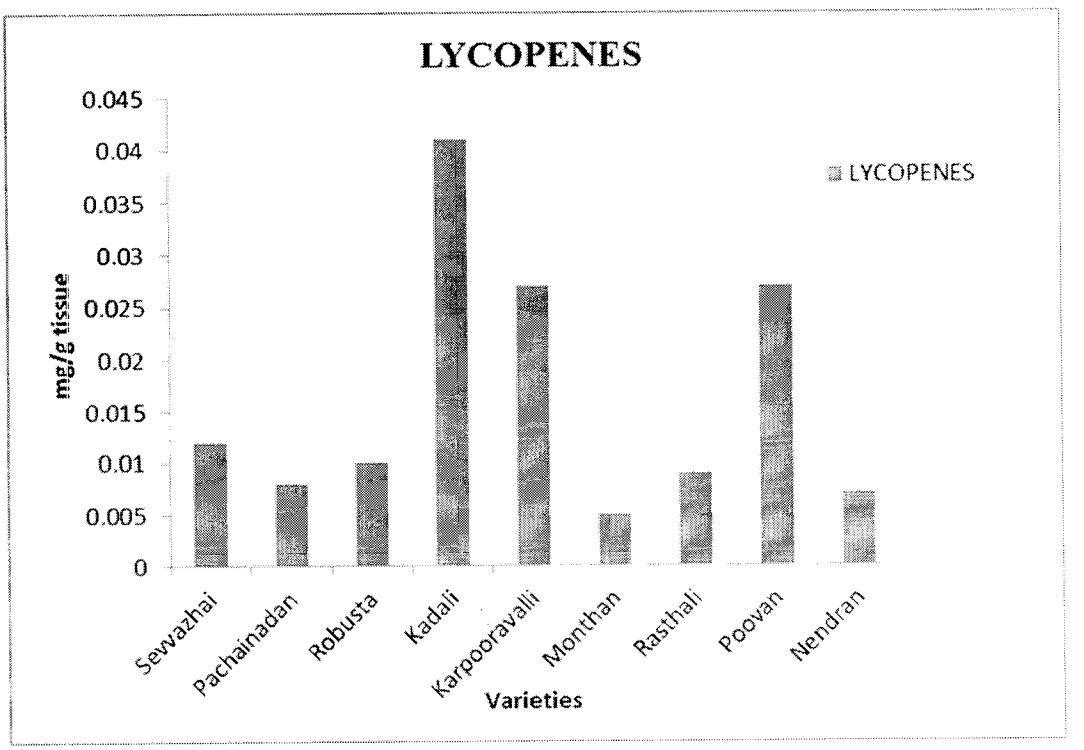


Fig.4.16. Lycopenes

Conclusion

5. 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 evaluate and compare the antioxidant potentials in the peel extracts obtained from nine local varieties of banana which includes, Sevvazhai, Pachainadan, Robusta, Kadali, Karpooravalli, Monthan, Rasthali, Poovan and Nendran. The peel extracts were assayed for different enzymatic, non- enzymatic and phytochemical compounds like SOD, GPx, GR, Vitamin C, Vitamin A, Vitamin E, Carotenoids, Total phenols, flavonoids etc. The results obtained were subjected to two- way ANOVA and the varieties were ranked according to their antioxidant and phytochemical content. Even though all the varieties showed significant antioxidant and phytochemical potential, Karpooravalli, Robusta and Kadali showed highest antioxidant and phytochemical potential.

The main objective of this study was to evaluate and compare the antioxidant and phytochemical potentials in the peel extracts of nine local bananas. The future goals of the study would be to carry out studies in *in vivo* and use the peel extracts in functional foods.

Appendices

APPENDICES

Appendix 1- Buffer preparation for SOD

50mM Phosphate Buffer, pH 7.4:

A – 890mg of Na_2HPO_4 dissolved in 100ml of distilled water.

B – 692mg of NaH_2PO_4 dissolved in 100ml of distilled water.

Mix 19ml of A and 81ml of B and dilute to 200ml with distilled water.

Appendix 2- Buffer preparation for CAT

0.01M Phosphate Buffer, pH 7.0:

A – 890mg of Na_2HPO_4 dissolved in 500ml of distilled water.

B – 692mg of NaH_2PO_4 dissolved in 500ml of distilled water.

Mix 39ml of A and 61ml of B and dilute to 200ml with distilled water.

Appendix 3- Buffer preparation for GPx

0.4M Phosphate Buffer, pH 7.0:

A – 35.61g of Na_2HPO_4 dissolved in 500ml of distilled

B – 27.67mg of NaH_2PO_4 dissolved in 500ml of distilled wat

Mix 39ml of A and 61ml of B and dilute to 200ml with distilled water.

Appendix 4- Buffer preparation for GST

0.5M Phosphate Buffer, pH 6.5:

A – 44.5g of Na_2HPO_4 dissolved in 500ml of distilled water.

B – 34.6g of NaH_2PO_4 dissolved in 500ml of distilled water.

Mix 68.5ml of A and 31.5ml of B and dilute to 200ml with distilled water.

Appendix 5- Buffer preparation for GR

0.3M Phosphate Buffer, pH6.8:

A – 26.70g of Na_2HPO_4 dissolved in 500ml of distilled water.

B – 20.75g of NaH_2PO_4 dissolved in 500ml of distilled water.

Mix 51ml of A and 49ml of B and dilute to 200ml with distilled water.

Appendix 6- Buffer preparation for G6PD

0.1M Tris HCl Buffer:

A – 0.1M solution of Tris (12.1g dissolved in 1000ml of distilled water).

B – 0.1M HCl.

Mix 50ml of A and 21.9ml of B and dilute to 200ml with distilled water.

Appendix 7- Buffer preparation for Total GSH

0.2M Phosphate Buffer:

A – 17.80g of Na_2HPO_4 dissolved in 500ml of distilled water.

B – 13.83g of NaH_2PO_4 dissolved in 500ml of distilled water.

Mix 5.3ml of A and 94.7ml of B and dilute to 200ml with distilled water.

Appendix 8- Buffer preparation for Vitamin A

50mM Phosphate Buffer, pH 7.4:

A – 890mg of Na_2HPO_4 dissolved in 100ml of distilled water.

B – 692mg of NaH_2PO_4 dissolved in 100ml of distilled water.

Mix 19ml of A and 81ml of B and dilute to 200ml with distilled water.

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