P-3092

ANTIOXIDANT, PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES IN PULP EXTRACTS OF NINE LOCAL VARIETIES OF BANANA (*Musa* sp.)

PROJECT REPORT

Submitted by

P.POONGODI

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In partial fulfillment for the award of the degree

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KUMARAGURU COLLEGE OF TECHNOLOGY

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Department of Biotechnology

PROJECT WORK -PHASE II

MAY 2010

This is to certify that the project entitled **ANTIOXIDANT, PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES IN PULP EXTRACTS OF NINE LOCAL VARIETIES OF BANANA (*Musa* sp.)** is the bonafide record of project work done by **P.POONGODI Register No: 0820203008** of M.Tech during the year 2009-2010.

Dr. R.BASKAR

Associate Professor

Department of Biotechnology

Kumaraguru College of Technology

Coimbatore - 641 006

Dr. S.SADASIVAM

Dean - Biotechnology

Department of Biotechnology

Kumaraguru College of Technology

Coimbatore - 641 006

Submitted for the Project Viva-Voce examination held on 17.5.10.

Internal Examiner

External Examiner

Declaration

DECLARATION

I affirm that the project work titled **Antioxidant, Phytochemical and Antimicrobial studies in pulp extracts of nine local varieties of Banana (*Musa* sp.)** being submitted in partial fulfillment for the award of **M.Tech(Biotechnology)** is the original work carried out by me. It has not formed the part of any other project work submitted for award of any degree or diploma, either in this or any other University.

P.Poongodi

0820203008

I certify that the declaration made by the above candidate is true.

Signature of the Guide,

04/05/10

Dr.R.Baskar

Associate Professor

Department of Biotechnology

Kumaraguru College of Technology

Coimbatore

Acknowledgement

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P. Poongodi

P.POONGODI

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ABSTRACT

Role of antioxidants in keeping a check on the free radical causing health complications has become inevitable with the rise in diseases like cancer, age related macular degeneration, arthritis, cardiovascular disorder and so on. To prevent their possible damages to biological molecules, especially to DNA, lipids and proteins all oxygen- consuming organisms are endowed with a well-integrated antioxidant system, including enzymatic and non-enzymatic components. The 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. Bananas have been proved to contain a potent antioxidant potential apart from other health benefits. Though their potency as antioxidants has been studied, comparative analysis is yet to be performed. This study aims to evaluate and compare the antioxidant and phytochemical activity of the pulp extracts of nine local varieties of banana, viz., Kadali, Karpooravalli, Monthan, Nendran, Pachainadan, Poovan, Rasthali, Red and Robusta .Ethanolic extraction was performed for the pulp of the banana varieties and the extracts were subjected to *in vitro* free radical scavenging assays like DPPH radical scavenging assay, ABTS cation radical scavenging assay, βcarotene bleaching assay in linoleic acid emulsion, Superoxide radical scavenging assay and lipid peroxidation inhibition assay. Total antioxidant assay to initially confirm the antioxidant potential was also carried out. 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 alkaloids, tannins and total sugars. In addition, antimicrobial potency of pulp extracts of banana was investigated with antibacterial activity test. The data obtained were subjected to statistical analysis and results were compared. All the nine banana varieties showed significant antioxidant and phytochemical content with Rasthali, Pachanadan, Poovan, Robusta topping the list. Appreciable antimicrobial activity in the Nendran, Kadali, Karpooravalli, Rasthali pulp extracts for the *Pseudomonas aeroginosa* , *Bacillus subtilis, Escherichia coli* strains. Thus, the pulp extracts obtained from these varieties may be useful to combat free radical- related diseases.

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LIST OF ABBREVIATIONS

µg	microgram
µl	microliter
µm	microgram
ABTS	2, 2,-azinobis (3-ethylbenzoline-6- sulfonic acid)
ANOVA	Analysis of Variance
ATP	Adenosine triphosphate
CAT	Catalase
CDNB	1-Chloro-2,4-dinitro phenyl hydrazine
DMRT	Duncun's Multiple Range Test
DNA	Deoxyribose Nucleic Acid
DPPH	1, 1-diphenyl -2-picryl hydrazyl
DNPH	2,4 dinitro phenyl hydrazine
EDTA	Ethylene Diamine Tetra Acetic acid
FDA	Food and Drug Administration
$FeCl_3$	Ferric Chloride
g	gram
H_2O_2	Hydrogen Peroxide
H_2SO_4	Sulphuric acid
HOCl	Hypochlorous acid
LDL	Low Density Lipoprotein
LPO	Lipid peroxidation

MDA	Malondialdehyde
mg	milligram
min.	Minute
ml	milliliter
NaOH	Sodium hydroxide
NBT	Nitro Blue Tetrazolium
NO	Nitric oxide
O_2	Oxygen
OFR	Oxygen-Free Radicals
$^{\cdot}OH$	Hydroxyl radical
PUFA	Polyunsaturated fatty acid
RBC	Red blood cells
ROM	Reactive Oxygen Metabolites
ROOH	Organic hydroperoxide
ROS	Reactive Oxygen Species
SOD	Superoxide dismutase
TBA	Thiobarbituric acid
TCA	Trichloro Acetic acid

Introduction

1. INTRODUCTION

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

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

There are numerous types of free radicals that can be formed within the body. The most common ROS include: the superoxide anion (O_2^-), the hydroxyl radical ($OH^·$), singlet oxygen ($^1O_2$), and hydrogen peroxide (H_2O_2). Superoxide anions are formed when oxygen (O_2) acquires an additional electron, leaving the molecule with only one unpaired electron. Within the mitochondria $O_2^{·-}$ is continuously being formed. Hydroxyl radicals are short-lived, but the most damaging radicals within the body. Hydrogen peroxide is produced *in vivo* by many reactions. (Acworth *et al.*, 1997).

When free radicals and other reactive oxygen species accumulate in the body, they cause damage on cells, DNA, lipid, sugar, and protein. The damage caused by free radicals and reactive oxygen species, in plants and animals, could lead to deterioration of

foods, cell membrane dysfunction, protein modification, enzyme inactivation, break of DNA strands, brain damage and dementia. Free-radical induced oxidative damages may be precursors to aging and diseases such as cancer, heart disease, diabetes mellitus, atherosclerosis, hypertension, sleep apnea, brain damage and dementia related diseases such as Alzheimer's disease and Parkinson's disease.

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

One important line of defense is a system of enzymes, including glutathione peroxidases, superoxide dismutases and catalase, which decrease concentrations of the most harmful oxidants in the tissues. Several essential minerals including selenium, copper, manganese and zinc are necessary for the formation or activity of these enzymes. Hence, if the nutritional supply of these minerals is inadequate, enzymatic defenses against free radicals may be impaired.

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

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

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

Among the fruits, banana has a lot of health benefits. It is a rich source of potassium, dietary fiber, manganese, Vitamin B_6 and C. A few suppliers claim that bananas are also a rich source of fructooligosaccharide.

Fructooligosaccharide is prebiotic because it nourishes prebiotic (beneficial) bacteria in the colon. These beneficial bacteria produce vitamins and digestive enzymes. These digestive enzymes improve our ability to absorb nutrients including calcium and the compounds that may protect us against pathogens. In addition, banana may reduce the gastrointestinal transit time, which may decrease the risk of colon cancer. On the other hand, a recent issue of FDA consumer magazine recommends bananas as a potassium-rich food because potassium counteracts some of sodium's effect on blood pressure.

Banana is also found to be a rich source of provitamin A carotenoids. Bananas are a great food with all round health benefits, for the average person, dieter or even athletes bananas may provide greater benefits than most fruits. The combination of carbohydrates and B vitamins present in a banana helps provide an energy boost which makes them great to eat 30 minutes before a workout, or even at breakfast to help boost energy at the start of every day.

Bananas are not only a good source of B Vitamins, they also contain Vitamin C, A and high levels of Potassium. Bananas provide antioxidants that can help keep your brain and heart healthy. Although the body produces antioxidants of its own, it seems to benefit from extra antioxidants provided in the diet, especially from whole grains, vegetables and fruits. This is still an emerging science, but the evidence suggests that the banana is a key player.

Banana, a tropical plant may protect itself from the oxidative stress caused by strong sunshine and high temperature by producing large amounts of antioxidants. Banana should be considered to be a good source of natural antioxidant for foods and functional food source against cancer and heart disease. Therefore, attention in recent times has been focused on the isolation characterization and utilization of the natural antioxidants especially growing interest in polyphenols as potential disease preventive agents. Bananas are one of the most popular foods on the world and it will be known that fruits contain various antioxidants compounds such as gallocatechin and dopamine. Since the bananas fruits are widely available, they have been used as food without apparent toxic effect.

Banana has been proved to contain antioxidant potential apart from health benefits. Though their potency as antioxidants and antimicrobial has been studied extensively, scientific literature pertaining to comparative analysis between their varieties is not yet documented. Hence, this project has been aimed to evaluate and compare the phytochemical, antioxidant and antimicrobial activities of pulp extract in nine local varieties of banana viz., Kadali, Karpooravalli, Nendran, Monthan, Pachinadan, Poovan, Rasthali, Red banana, Robusta.

Objective of the Study

2. OBJECTIVES

- To evaluate and compare *in vitro* antioxidant potential in pulp extracts of nine local varieties of Banana [*Musa sp.*]
- To determine and compare the endogenous antioxidants (enzymatic and non enzymatic) in pulp extracts of nine local varieties of Banana [*Musa sp.]*
- To evaluate and compare phytochemical composition in pulp extracts of nine local varieties banana [*Musa sp.*]
- To assess and compare antimicrobial potency in pulp extracts of nine local varieties of banana [*Musa sp.*]

Review of Literature

3. LITERATURE REVIEW

3.1. Free radicals

Free radicals can be defined as chemical species possessing an unpaired electron, which is formed by homolytic cleavage of a covalent bond of a molecule by the loss of a single electron from a normal molecule or by the addition of a single electron to a normal molecule. Free radicals are a group of active molecules with unpaired electrons that react with biological systems, resulting in cell damage. Antioxidants are capable of taking up these free radicals. In doing so, they protect healthy cells from damage and abnormal growth.

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

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

3.2 Reactive oxygen species

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

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

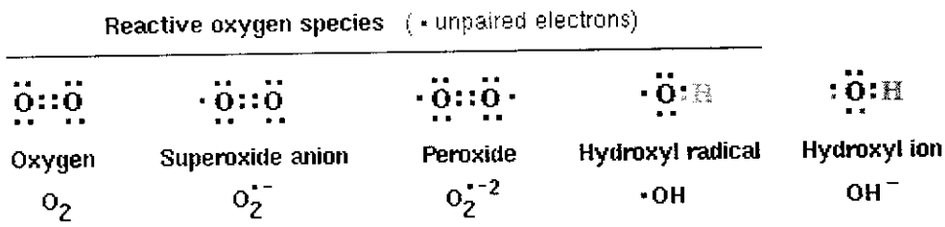


Figure 3.1: Reactive oxygen species

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

3.3 Reactive oxygen system

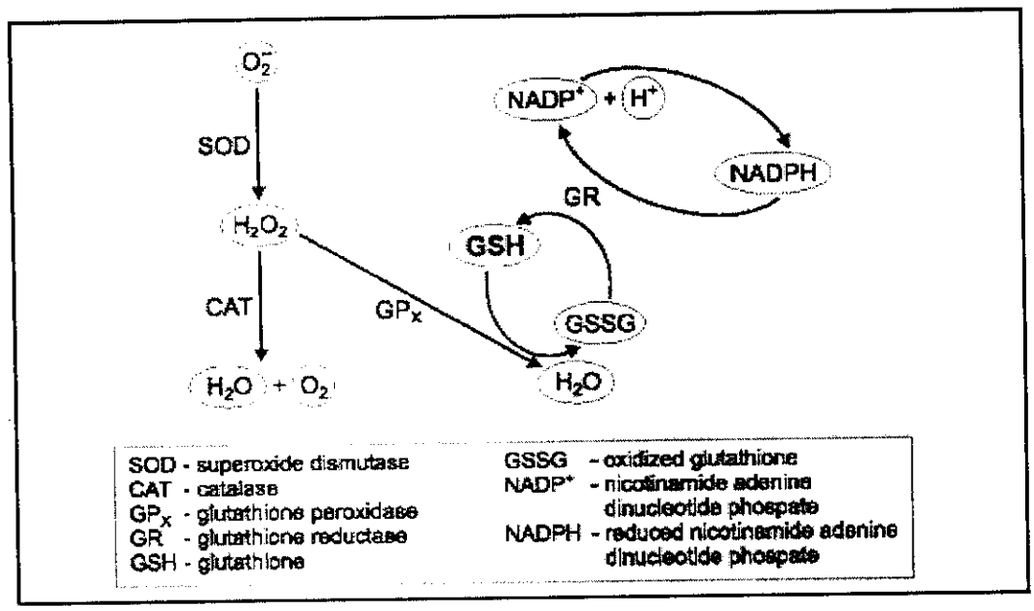


Figure 3.2: Reactive oxygen system

Three types of superoxide dismutase (SOD) can be distinguished: cytoplasmatic, mitochondrial and extracellular. SOD catalyzes the dismutation of superoxide radical anion ($O_2^{\bullet}$) into less noxious hydrogen peroxide (H_2O_2), that is further degraded by catalase or glutathione peroxidase. Catalase is an enzyme which accelerates degradation of H_2O_2 into water and oxygen .The second pathway of H_2O_2 metabolism depends on activity of glutathione peroxidase (GPx) and cooperating glutathione reductase. The reduction of H_2O_2 into water by GPx is accompanied by the conversion of glutathione from reduced form (GSH) into oxidized form (GSSG)

3.4 Formation of free radicals

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

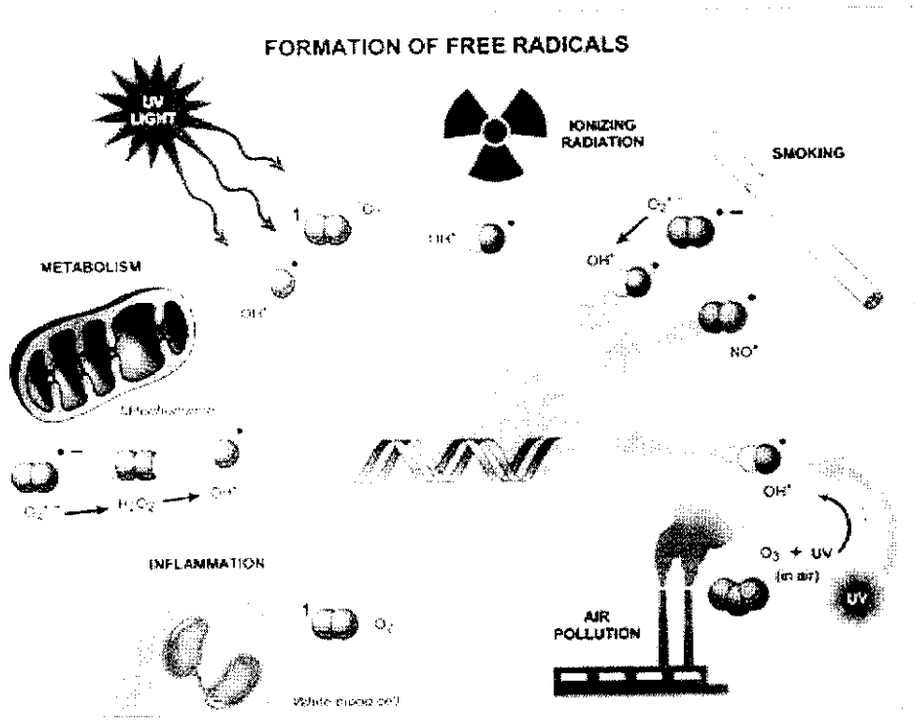


Figure 3.3: Formation of free radicals

3.5 Types of free radicals

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

- **Superoxide ion** (O_2^{-}.) (or reactive oxygen species) is an oxygen molecule with an extra electron. This free radical can cause damage to mitochondria, DNA and other molecules. Our body can neutralize superoxide ions by producing superoxide dismutase.

- **Hydroxyl radical (OH·)** is formed by the reduction of an oxygen molecule in the electron transport chain. Because of its high reactivity it will damage most organic molecules such as carbohydrates, DNA, lipids and proteins.

- **Singlet oxygen** is formed by our immune system. Singlet oxygen causes oxidation of LDL cholesterol.

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

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

3.6 Free radicals and human diseases

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

3.7 Antioxidant as Scavengers

To deal with the free radicals or so called ROS, the body is equipped with an effective defense system which includes various enzymes and high and low molecular weight antioxidants. Antioxidants neutralize free radicals by donating one of their own

electrons, ending the electron stealing reaction. The antioxidants do not themselves become free radical by donating electrons because they are stable in other form. These act as scavenger and play the housekeeper's role by mopping up free radicals before they get a chance to create havoc in a body. Thus, they may be well defined as the substances that are capable of quenching or stabilizing free radicals.

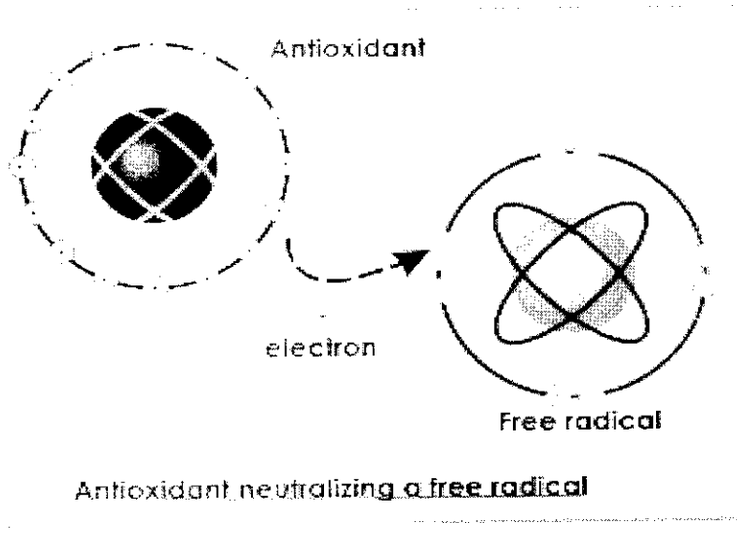


Figure 3.4: Antioxidant neutralizing free radical

Antioxidants therefore, according to their mode of action, have also been classified as the compounds that terminate the free radical chain in lipid peroxidation by donating electrons or hydrogen to fat containing a free radical and to the formation of a complex between the chain and a free radical. Antioxidants stop the reactions by contributing hydrogen from the phenolic hydroxyl hydrogen from the phenolic hydroxyl groups, themselves forming stable free radicals that do not initiate or propagate further oxidation of lipids (free radical terminators). Some of the important synthetic antioxidants of this class are butylated hydroxyanisole (BHA), butylated hydroxy toluene, terbutyl hydroquinone (TBHQ), propyl gallate (PG) and tocopherols

In recent years, the use of some synthetic antioxidants has been restricted because of their possible toxic and carcinogenic effects. This concern has resulted in an increased interest in the investigation of the effectiveness of naturally occurring compounds with antioxidant properties. Foods rich in antioxidants have been shown to play an essential

role in the prevention of cardiovascular diseases; cancers, neurodegenerative diseases, the most well known of which are Parkinson's and Alzheimer's diseases, inflammation and problem caused by cell and cutaneous aging (Shahidi and Wanasundara, 1992).

3.8 Types of antioxidants

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

3.8.1 Enzymatic antioxidants

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

3.8.1.1 Superoxide Dismutase

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

$$2O_2^- + 2H \xrightarrow{\text{SOD}} H_2O_2 + O_2$$

3.8.1.2 Catalase

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

dangerous superoxide radical to hydrogen peroxide, which catalase converts to harmless water and oxygen.

$$2H_2O_2 \xrightarrow{\text{Catalase}} H_2O + O_2$$

3.8.1.3 Glutathione peroxidase

Glutathione peroxidase also known as gamma - glutamylcysteinylglycine or GSH, the body's primary antioxidant, which is in virtually every cell, is one of the most powerful free radical fighters that the body has in its arsenal. Glutathione helps maintain the integrity of red blood cells, as well as protecting white blood cells, assists in carbohydrate metabolism and breaking down oxidized fats.

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

3.8.1.4 Glutathione-S-transferase

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

3.8.1.5 Glutathione reductase

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

3.8.1.6 Polyphenol oxidase

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

3.8.1.7 Glucose-6-phosphate dehydrogenase (G6PD)

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

3.8.2 Non enzymatic antioxidants

3.8.2.1 Vitamin A (β – Carotene)

Beta-carotene is a member of the carotenoid family, a group of powerful antioxidants that also includes alpha-carotene, lycopene, zeaxanthin, and lutein. However, of all the carotenoids, only alpha-carotene and beta-carotene are converted to significant amounts of vitamin A in the body, and beta-carotene is by far the most plentiful carotenoid found in fruits and vegetables. Beta-carotene acts as a precursor of vitamin A, and is therefore called a provitamin A compound. Foods or supplements containing beta-carotene are converted to vitamin A for the maintenance of healthy skin, good vision, and a robust immune system. Beta-carotene is also a powerful antioxidant, and has been shown to help guard against cancer and heart disease.

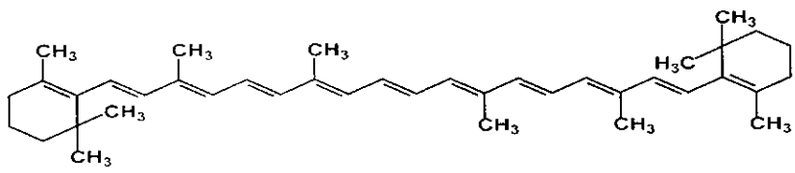


Figure 3.5: Vitamin A

3.8.2.2 Vitamin C (Ascorbic acid)

Vitamin C is an important dietary antioxidant, it significantly decreases the adverse effect of reactive species such as reactive oxygen and nitrogen species that can cause oxidative damage to macromolecules such as lipids, DNA and proteins which are

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

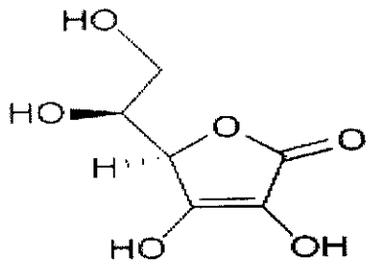


Figure 3.6: Vitamin C

3.8.2.3 Vitamin E (α-Tocopherol)

Vitamin E is a fat-soluble antioxidant that stops the production of reactive oxygen species formed when fat undergoes oxidation (Herrera *et al.*, 2001 and Packer *et al.*, 2001).Of these, α-tocopherol (also written as alpha-tocopherol) has been most studied as it has the highest bioavailability (Brigelius and Traber, 1999). It has been claimed that α-tocopherol is the most important lipid-soluble antioxidant, and that it protects cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction (Traber and Atkinson, 2007). This would remove the free radical intermediates and prevent the oxidation reaction from continuing. The oxidized α-tocopheroxyl radicals produced in this process may be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, retinol or ubiquinol.

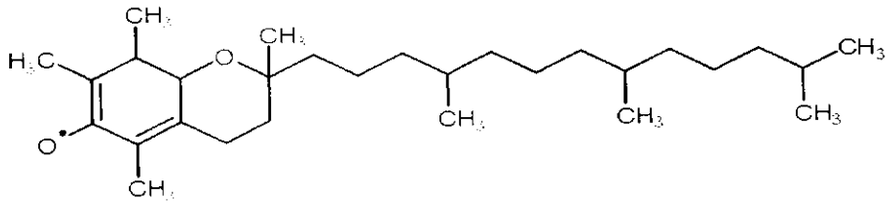


Figure 3.7: VitaminE

3.8.2.4 Glutathione

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

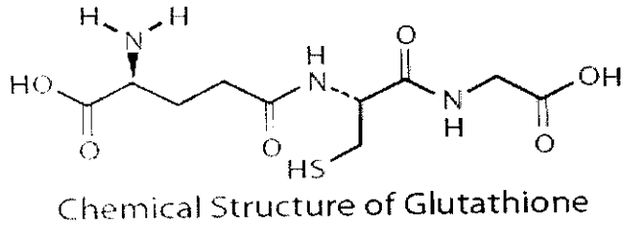


Figure 3.8: Glutathione

3.8.2.5 Melatonin

Melatonin is an antioxidant that can easily cross cell membranes and the blood-brain barrier (Hardeland, 2005). Melatonin is a direct scavenger of OH, O_2^-, and NO (Poeggeler *et al.*, 1994). Unlike other antioxidants, melatonin does not undergo redox cycling, the ability of a molecule to undergo reduction and oxidation repeatedly. Redox

cycling may allow other antioxidants (such as vitamin C) to regain their antioxidant properties. Melatonin, on the other hand, once oxidized, cannot be reduced to its former state because it forms several stable end-products upon reacting with free radicals. Therefore, it has been referred to as a terminal (or suicidal) antioxidant (Tan *et al.*, 2000)

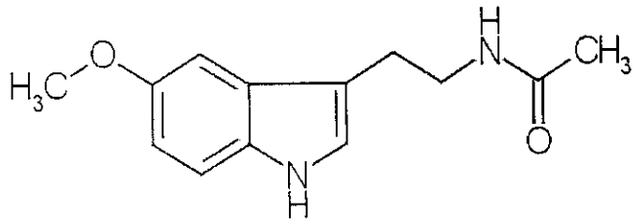


Figure 3.9: Melatonin

3.9 Phytochemicals

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. There are more than thousand known phytochemicals. It is well-known that plants produce these chemicals to protect itself, but recent research demonstrate that they can protect humans against diseases. Some of the well-known phytochemicals are lycopene in tomatoes, isoflavones in soy and flavonoids in fruits. They are not essential nutrients and are not required by the human body for sustaining life.

3.9.1 Flavonoids

Flavonoids are polyphenols abundantly found in fruits, vegetables, and herbs. Flavonoids are synthesized only in plants. They are a diverse group of phytochemicals, exceeding four thousand in number. From human nutrition perspective, flavonoids are important components of a healthy diet because of their antioxidant activity. Nevertheless, the antioxidant potency and specific effect of flavonoids in promoting human health varies depending on the flavonoid type (chemical, physical, and structural properties). Among the potent antioxidant flavonoid types are quercetin, catechins and xanthohumol. Because of the antioxidative property, it is suggested that flavonoids may delay or prevent the onset of diseases (such as cancer) induced by free radicals. They also

inhibit low density lipoprotein (LDL) oxidation by free radicals. Flavonoids have been reported to have negative correlation with incidence of coronary heart disease. Furthermore, flavonoids have anti-bacterial, anti-viral, anti-tumor, anti-inflammatory, antiallergenic and vasodilatory effect. They also inhibit platelet aggregation. (Verena *et al.*, 2006 and Subramani Sellappan *et al.*,2002)

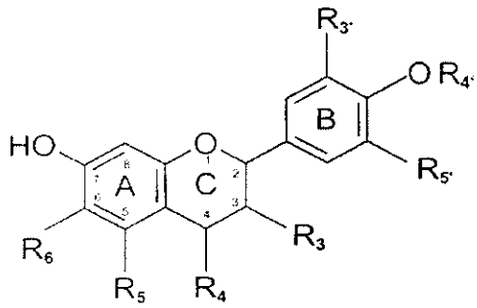


Figure 3.10: General structure of flavonoids

3.9.2 Alkaloids

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

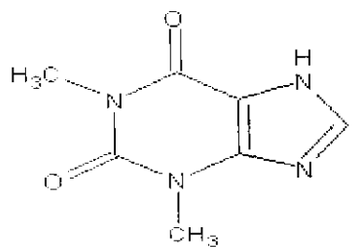


Figure 3.11: General structure of alkaloids

3.9.3 Tannins

Tannins are polyphenolics that make cranberries and pomegranates bitter. Tannins, along with Vitamin C, help build and strengthen collagen. Tannins prevent

urinary tract infection by preventing bacteria from adhering to the walls. Combination of tannin plus anthocyanins (as in pomegranate juice) can break-down oxidized cholesterol in the bloodstream and in atherosclerotic plaques. Most of the active compounds in black tea are tannins which are 90% catechins. Epicatechin is the major component of natural tannin in grapes.

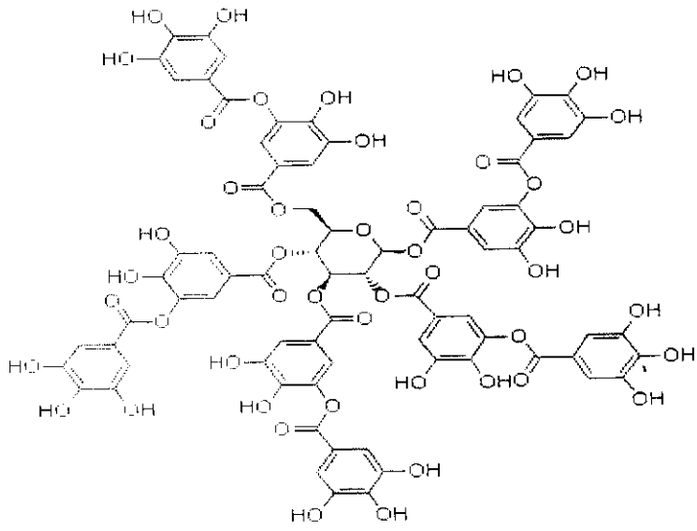


Figure 3.12: General structure of Tannin

3.9.4 Carotenoids

Carotenoids represent one of the most widespread groups of naturally occurring pigments. These compounds are largely responsible for the red, yellow, and orange color of fruits and vegetables, and are also found in many dark green vegetables. In recent years, carotenoids have received a tremendous amount of attention as potential anti-cancer and anti-aging compounds. Carotenoids are powerful antioxidants, protecting the cells of the body from damage caused by free radicals. Carotenoids, and specifically beta-carotene, are also believed to enhance the function of the immune system.

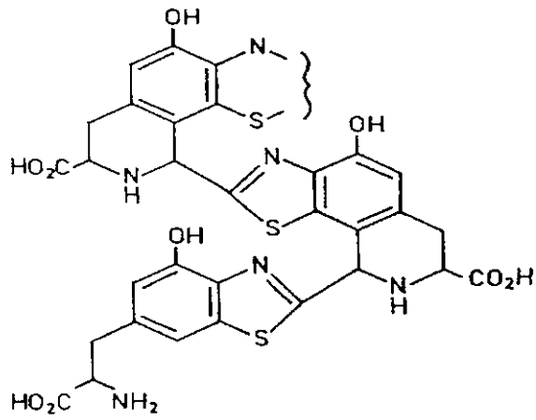


Figure 3.13: General structure of Carotenoids

3.9.5 Lycopene

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

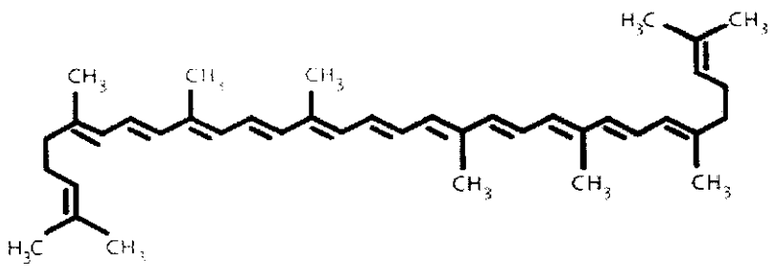


Figure 3.14: General structure of Lycopene

3.9.6 Total sugars

Mainly sugars and starches, together constituting one of the three principal types of nutrients used as energy sources (calories) by the body. Carbohydrates can also be defined chemically as neutral compounds of carbon, hydrogen and oxygen.

Carbohydrates come in simple forms such as sugars and in complex forms such as starches and fiber. The body breaks down most sugars and starches into glucose, a simple sugar that the body can use to feed its cells. Complex carbohydrates are derived from plants. Dietary intake of complex carbohydrates can lower blood cholesterol when they are substituted for saturated fat.

Carbohydrates are classified into mono, di, tri, poly and heterosaccharides. The smallest carbohydrates are monosaccharides such as glucose whereas polysaccharides such as starch, cellulose and glycogen can be large and even indeterminate in length. The energy produced by carbohydrates is 4 calories per gram.

3.10 Antioxidant capacity assays

3.10.1 DPPH radical scavenging activity:

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

3.10.2 Trolox Equivalent Antioxidant Capacity Assay

In this improved version, ABTS-, the oxidant is generated by persulfate oxidation of 2, 2-azinobis (3-ethylbenzoline-6-sulfonic acid)-(ABTS2-). Specifically, 7 mM of ABTS ammonium was dissolved in water and treated with 2.45 mM of ammonium persulphate and the mixture was then allowed to stand at room temperature for 12-16 h to give a dark blue solution. This solution was diluted with ethanol or buffer (pH 7.4) until the absorbance reached 0.7 at 745 nm. 3ml of the resulting solution was mixed with 1.0 ml of sample. The absorbance was read for every minute for 6 minutes. The difference of the absorbance reading is plotted versus the antioxidant concentrations to give a straight line.

3.10.3 Hydroxyl radical and Nitric oxide scavenging assay

The hydroxyl radical scavenging activity was measured as the percentage of inhibition of hydroxyl radicals generated in the Fenton's reaction mixture. Hydroxyl radical scavenging activity was measured by studying the competition between deoxy ribose and the extract for hydroxyl radicals generated from the Fe^{3+}/Ascorbate/EDTA/H_2O_2 system. The hydroxyl radicals attack deoxyribose, which eventually results in TBARS formation.

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

3.10.4 Lipid peroxidation inhibition assay

Initiation of lipid peroxidation by ferrous sulphate takes place either through hydroxy radical by Fenton's reaction. The inhibition could be caused by absence of ferryl-perferryl complex or by scavenging the hydroxy radical or the superoxide radicals or by changing the Fe^{3+}/Fe^{2+} or by reducing the rate of conversion of ferrous to ferric or by chelating the iron itself.

Iron catalyzes the generation of hydroxyl radicals from hydrogen peroxide and superoxide radicals. The hydroxyl radical is highly reactive and can damage biological molecules, when it reacts with polyunsaturated fatty acid moieties of cell membrane. Lipid hydroperoxides can be decomposed to produce apoxy and peroxy radical they eventually yield numerous carbonyl products such as malondialdehyde (MDA). The carbonyl products are responsible for DNA damages, generation of cancer and aging related disease (Ohkawa et al., 1979). Thus, decrease in MDA level with increase in the concentration of the extracts indicates the role of the extracts as an antioxidant.

3.10.5 Ferric Reducing Antioxidant Potential Assay

The reducing capacity was investigated by measuring Fe^{3+}- Fe^{2+} conversion. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The antioxidant activities of putative antioxidants have been attributed to various mechanisms such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued proton abstraction and radical scavenging.

3.10.6 β-carotene bleaching inhibition assay

Linoleic acid, an unsaturated fatty acid gets oxidized by "Reactive oxygen species" (ROS), which are produced in water. The products formed initiate the Carotene oxidation, which leads to discoloration of the emulsion. The extent of discoloration is measured at 470 nm. The antioxidant activity was expressed as percent inhibition relative to control i.e. linoleic acid emulsion.

3.11 Antioxidants and peroxidation

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

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

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

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

3.12 Antioxidants from natural dietary sources

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

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

important sources of antioxidants are green tea, black tea, herbal tea, spices, red wine, ginger, and garlic.

3.13 Importance of Antioxidant rich dietary sources

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

3.14 BANANA SPECIES

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

Kingdom: Plantae

Division: Magnoliophyta

Class: Liliopsida

Order: Zingiberales

Family: Musaceae

Genus: *Musa*

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

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

3.14.1 Calories in a Banana

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

3.14.2 Nutritional values of Banana

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

Table 3.1: Macronutrient Values of Banana

Micronutrients	Banana/ 1 fruit
Potassium (mg)	467
Sodium (mg)	1
Calcium (mg)	9.2
Magnesium (mg)	44.1
Phosphorus (mg)	25
Zinc (mg)	18
Iron (mg)	0.31
Vitamin C (mg)	10.3
Thiamin (mg)	0.037
Riboflavin (mg)	0.086
Niacin (mg)	0.785
Pantothenic acid (mg)	0.394
Vitamin B6 (mg)	0.433
Vitamin B12 (mcg)	0
Folate (mcg)	24
Vitamin A (IU)	76
Vitamin E (mg)	0.12
Vitamin K (mcg)	0.6
Beta carotene (mcg)	31

Table 3.2: Micronutrient Values of Banana

3.14.3 Nutritional benefits of Banana

3.14.3.1 Vitamins

The banana is the best fruit source of vitamin B_6 (one medium banana provides about 25% of daily needs; one big banana provides about a third of daily needs). Vitamin B_6 helps with the production of neurotransmitters including serotonin and GABA and the production of amino acids. Vitamin B_6 bioavailability from bananas is high i.e. the vitamin is easy to absorb from bananas (Roth-Maier, 2002).

Eating plenty of vitamin B_6 may have its benefits for expectant mums. According to a study (Ronnenberg, 2007) on 364 young Chinese women, those that had adequate vitamin B_6 had more chance of conceiving and less chance of losing their baby early in pregnancy. With sufficient levels of B_6 the odds of conception improved by 40% and lowered the odds of early pregnancy loss by 30%, compared to women with low B6.

Vitamin B_6 appears to have anti-cancer properties according to a Scottish study that looked at 2000 people with colon cancer and compared them to 2700 controls (Theodoratou, 2008). Vitamin B_6 is a co-enzyme involved in folate metabolism, which is reported to be associated with a lower risk of colon cancer. The results showed that there was an inverse association between B_6 intake and the risk of colon cancer. There was a mild inverse association with eating bananas themselves, but the strongest effect was through vitamin B_6, especially in younger people.

Although it appears that many US citizens may be low on Vitamin B_6 (Morris, 2008). Vitamin B_6 needs increase with age and during pregnancy and lactation, making the banana an ideal fruit for these people. Adults 31-50 years are recommended to have 1.3 mg B6 daily. This rises to 1.5 mg and 1.7 mg for women and men respectively aged 51 years and over, which is an increase of about 20%.

A medium banana will provide about a quarter of an adults vitamin C needs. Vitamin C is well known for it antioxidant and anti-scurvy effects. Less well known is

that Vitamin C is needed to make collagen, an important connective structure on which we make our bones and teeth.

Bananas are a modest source of folate (about 5% RDI in a small banana and 10% RDI in a large banana) making it ideal for young women and the health of the foetus, as well as its potential benefit in avoiding heart disease and dementia later in life. Bananas also provide some other essential B Vitamins, like Thiamine, Riboflavin, Niacin and Pantothenic acid.

3.14.3.2. Antioxidants in banana

Antioxidants are well known to be playing a role, possibly a significant role, in reducing the risk of succumbing to early disease. The antioxidant component of a banana is playing a small, but significant role in keeping our body healthy, in addition to its valuable nutrition contribution of B_6, fibre, folate and potassium.

One study suggested that twenty healthy volunteers underwent a series of blood measurements before and two hours after eating a banana. Eating the banana reduced the plasma oxidative stress and enhanced the resistance to oxidative changes in LDL cholesterol (Yin, 2008). Another study indicated that the phenolic compounds in bananas (and oranges and apples) prevented oxidative stress-induced neurotoxicity, suggesting that the protection of nerve cells by the phenolics could reduce the risk of Alzheimers Disease (Heo, 2008).

A further study gave rats banana flavonoid extracts. The rats received either a normal diet or a high fat diet, both with and without banana flavonoids. Those that received the flavonoids had higher levels of superoxide dismutase and catalase, natural antioxidant compounds produced by the body (Vijayakumar, 2008).

Although all three studies were small, each one was suggestive that bananas may be boosting the natural antioxidant processes within the body.

3.14.3.3 Fibre and resistant starch

It is no surprise that bananas, like all fruit, contain fibre. According to the National Nutrition Survey, only around 1 in 4 people eat the recommended Dietary Intake for fibre. A medium banana will provide 2.7g of fibre or around about 10% of an adult's fibre needs for a day.

What is unique about the banana is that it also provides resistant starch, which is starch that resists digestion and goes all the way through the small intestine and passes into the large intestine to act much like fibre (McCleary, 2003 and Cummings, 2007). In fact, resistant starch is now considered to be a type of fibre.

There are three types of resistant starch; raw bananas have type B (Mann & Truswell, 2007 and Cummings, 2007), or type 2 (Cummings, 1996 and Topping, 2003) based on the structure of the starch granules. Banana starch is high in a type of starch called amylose, with most of this starch not being digested in the small intestine and therefore passing into the large intestine unchanged (Faisant, 1995).

There is more resistant starch in "just ripe" bananas than found in "very ripe" bananas. As the banana ripens, the starch granules convert to sugars, about half of which is sucrose, a quarter fructose and a quarter glucose (Cordenunsi, 1995; Bowes & Church, 2005). The starch is converted to sugars and the amount of resistant starch declines by about half (Faisant, 1995).

Resistant starch offers many benefits as it is used as a food by the friendly bacteria in the bowel, helping to protect the bowel from becoming cancerous. It also increases stool weight with every 1g increase in banana RS causing a 1.68g increase in stool weight (Cummings, 1996). As the starch in unripe bananas does pass into the large bowel where it may increase bacterial gas production, it could explain why some people experience abdominal discomfort after eating barely ripe bananas.

3.14.4 Therapeutic value of banana

3.14.4.1 Cancer

Bananas could be helping to reduce the risk of kidney cancer. Research on 61 000 Swedish women aged 40-76 years found that, of all the fruits eaten, bananas gave the greatest protection against renal cancer. Women eating 5 bananas a week nearly halved their risk of renal cancer. Indeed, a high consumption of all fruits and vegetables was associated with less renal cancer(Rashidkhani, 2005)

Researchers from the University of California found that the regular consumption of bananas and oranges in the first two years of life consistently reduced a child's risk of childhood leukemia before the age of 15 years (Kwan, 2004). The authors speculate that the vitamin C and potassium may be playing a protective role.

Bananas contain lectins (bio-active proteins), which have been strongly linked to a reduction in the risk of cancer and potentially helpful in the treatment of cancer (De Mejía, 2005). Plant lectins are known to resist digestion and enter the blood stream intact. They can affect the growth and proliferation of cancer cells, including causing their ultimate death. Lectins appear to have anti-tumour and anti-carcinogenic effects that may benefit cancer treatment. It may be that the lectins in bananas also help reduce the risk of renal cancer and childhood leukemia.

3.14.4.2 Weight control

People will often claim that avoiding carbohydrate foods will help with weight control. Avoiding carbohydrate foods, such as grains and fruit, is not healthy in the short-term or long-term. As there is no fat in a banana and because bananas have a low energy density, it is almost impossible to get fat on a banana. Bananas are a high satiety food. For example, four bananas have the same number of kilojoules as a medium serve of fries. Not many people could eat four bananas at once, yet many people will eat a medium fries and follow up with a hamburger and a soft drink, giving you nearly three times the kilojoules as four bananas.

If you are trying to control your body weight, then enjoy fruits like the banana as a snack. They are filling and will help you to make it through to the next meal without overeating.

3.14.4.3 Fitness

Bananas are ideal for athletes, school children, truck drivers, office workers, gardeners etc as they are easy to transport, peel and eat. As bananas are so easy to eat, they are a favorite choice for cyclists, footballers and other athletes looking for a quick source of carbohydrate. The riper a banana, the quicker it is digested and converted to muscle fuel.

Athletes love to eat bananas before sport, at breaks in the tennis and cricket, and as a recovery food immediately after sport. Bananas are ideal as a snack, in a smoothie or on breakfast cereal.

3.14.4.4 Fatigue

Carbohydrate snacks are very useful for bringing low blood glucose levels back to normal and help avoid the mid-afternoon slump that many experience, especially during work or after school. Carbohydrate snacks are very useful for clear thinking, improved memory and normalisation of mood.

3.14.4.5 Diabetes and blood sugar

The Glycaemic Index of bananas is variable depending upon type and ripeness. The average GI of bananas is 52, less for under-ripe bananas as they have high starch content and low sugar content (Foster-Powell, 2002). A GI of 52 is classified as low, making them a good fruit choice for people with diabetes. As the banana ripens, the starch gets converted to sugars.

Very ripe bananas may raise blood glucose levels quicker than less ripe bananas, so we recommend that people with diabetes choose bananas that have just become ripe as they are digested slower with the least effect on blood glucose levels (Lintas, 1995).

3.14.4.6 Potassium and blood pressure

Bananas are often cited as being a good source of potassium. This is true, although many other fruits and vegetables also provide potassium too. A medium banana will provide around 320 mg of potassium, which is about 10% of daily requirements. That leaves a lot of room for high potassium foods like the banana. Eating more fruit and vegetables will bump up our potassium intake. In fact, adding an extra banana and a small side salad will get nearly half the population to a healthy potassium level of 4700mg.

Following 10000 people for 17 years showed a lower risk of stroke in hypertensive men who had the highest potassium intake (Fang, 2000). A Deakin University study showed a significant fall in blood pressure when subjects ate more fruit and vegetables and reduced their sodium intake (Nowson *et al.*, 2004). A review of potassium in blood pressure (Adrogue 2007) reiterated to role of the lower sodium, increased potassium diet in controlling blood pressure.

3.15 BANANA VARIETIES

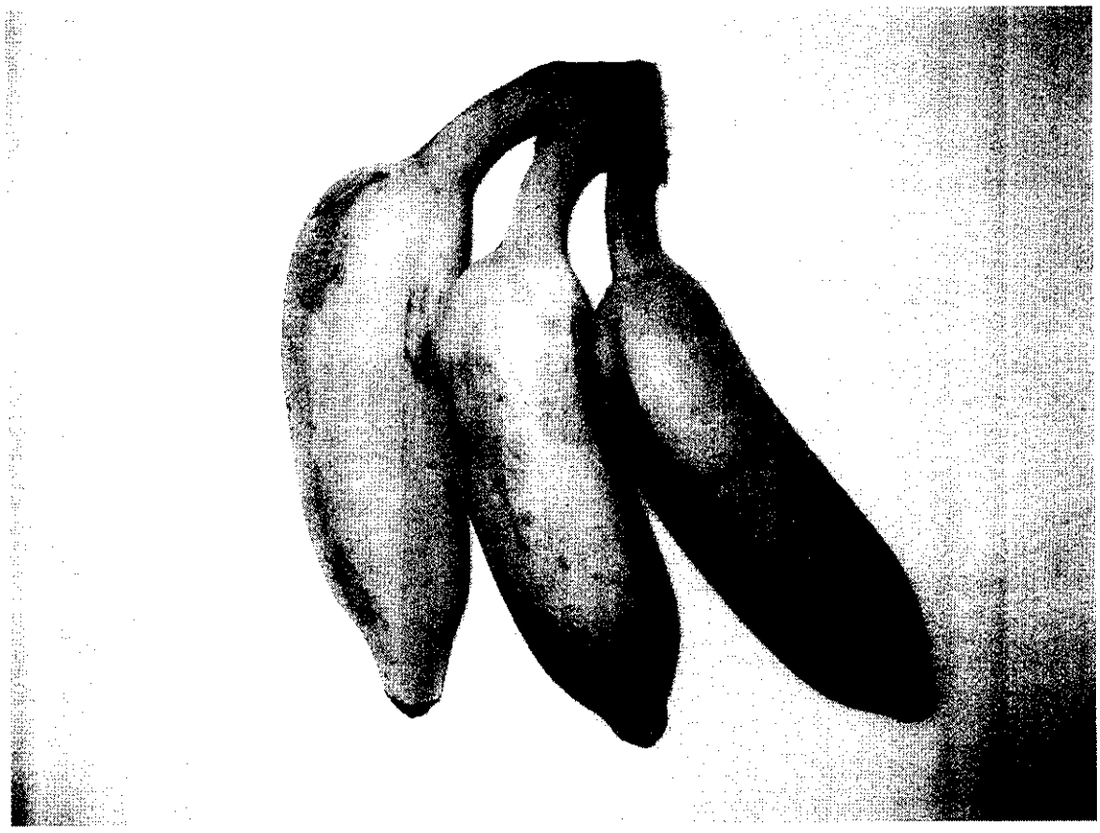


Figure 3.15: Banana cv. Kadali [*Musa spp* - Ney Poovan - AB].

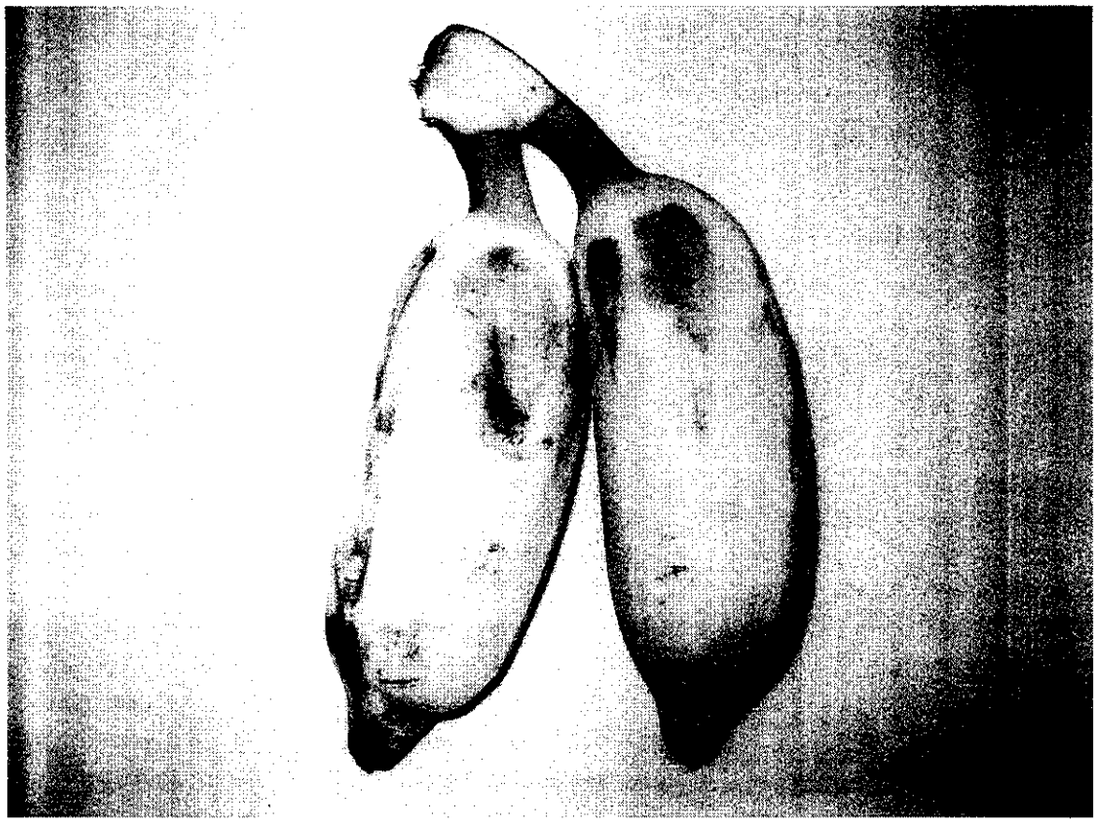


Figure 3.16: Banana cv. Karpooravalli [*Musa spp* –Karpooravalli - ABB].

Figure 3.17: Banana cv. Monthan [*Musa spp* - Bluggoe – ABB].

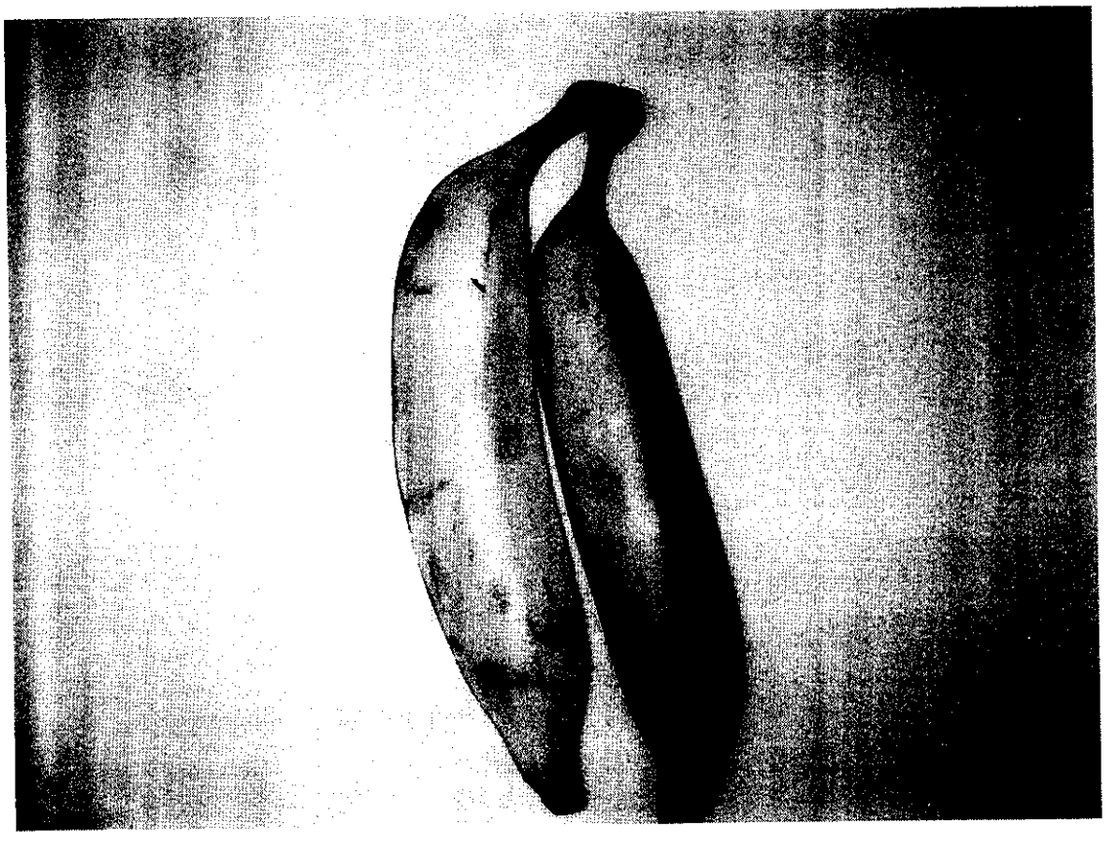


Figure 3.18: Banana cv. Nendran [*Musa spp* - French Plantaini - AAB]

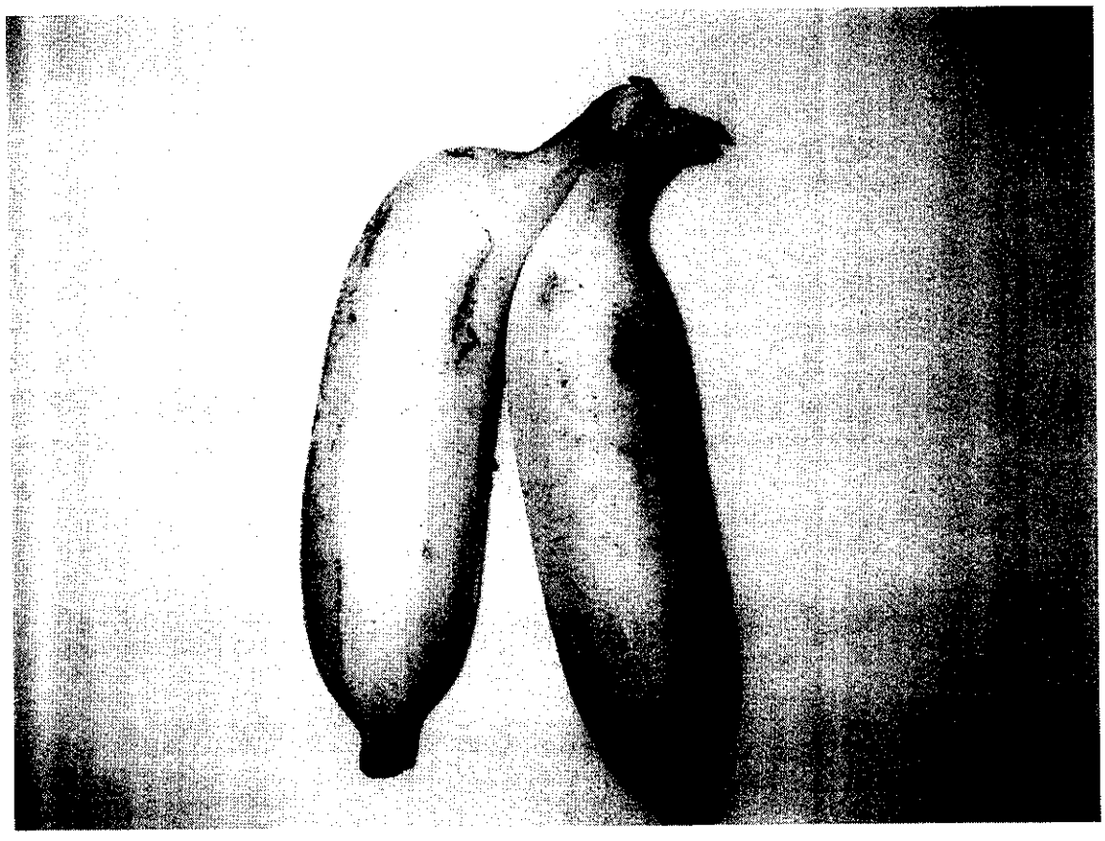


Figure 3.19: Banana cv. Poovan [*Musa spp* - Mysore - AAB].

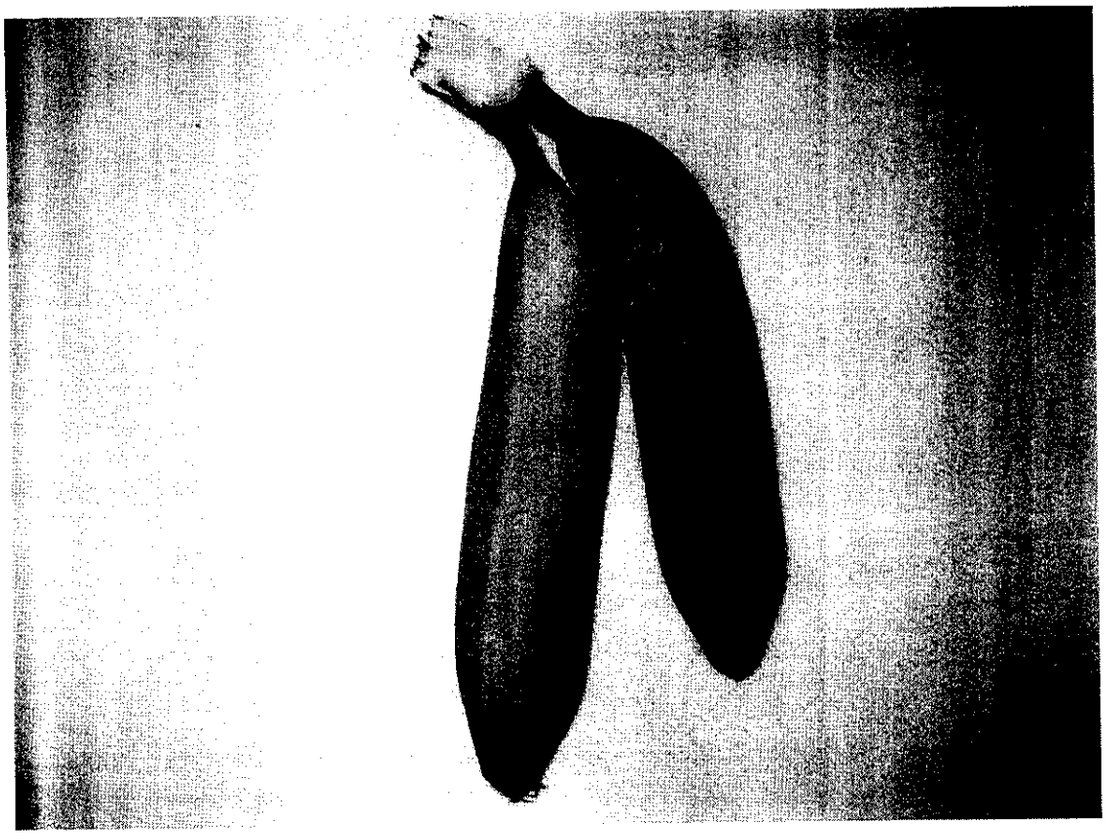


Figure 3.20: Banana cv. Pachanadan [*Musa spp* - Pachanadan - AAA].

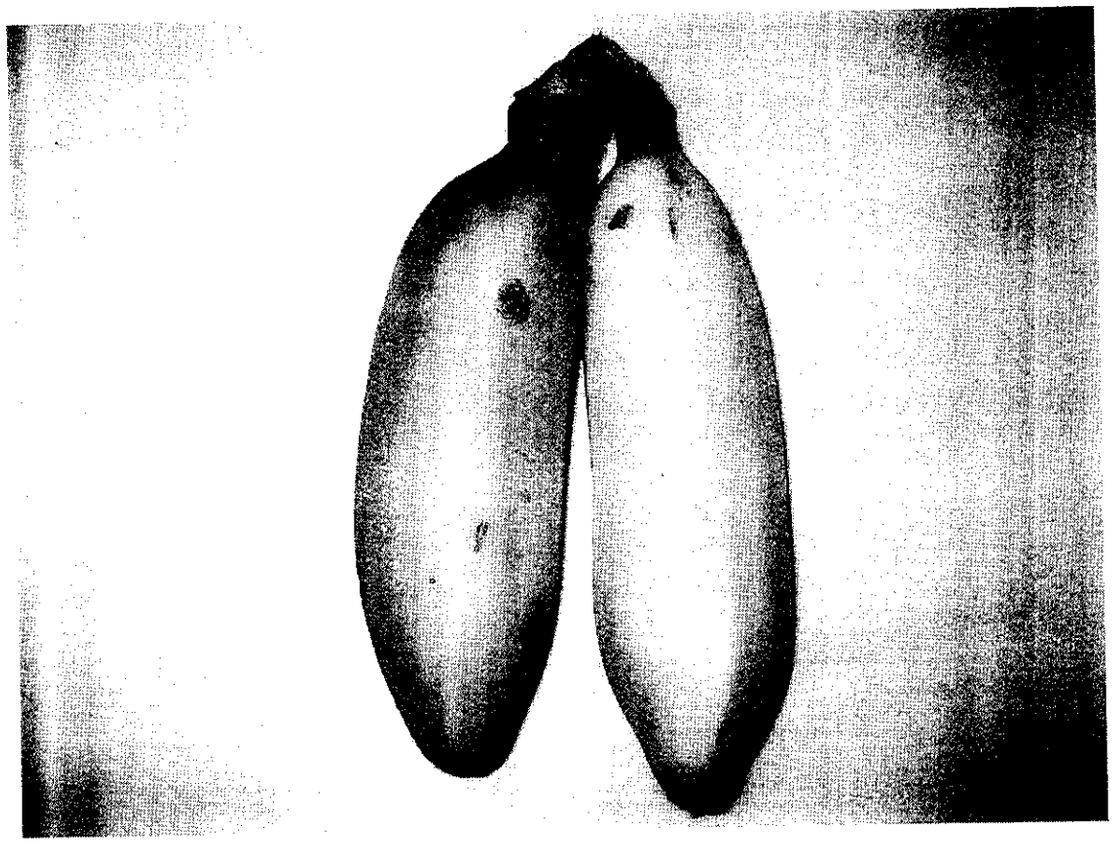


Figure 3.21: Banana cv. Rasthali [*Musa spp* - Rasthali - AAB].

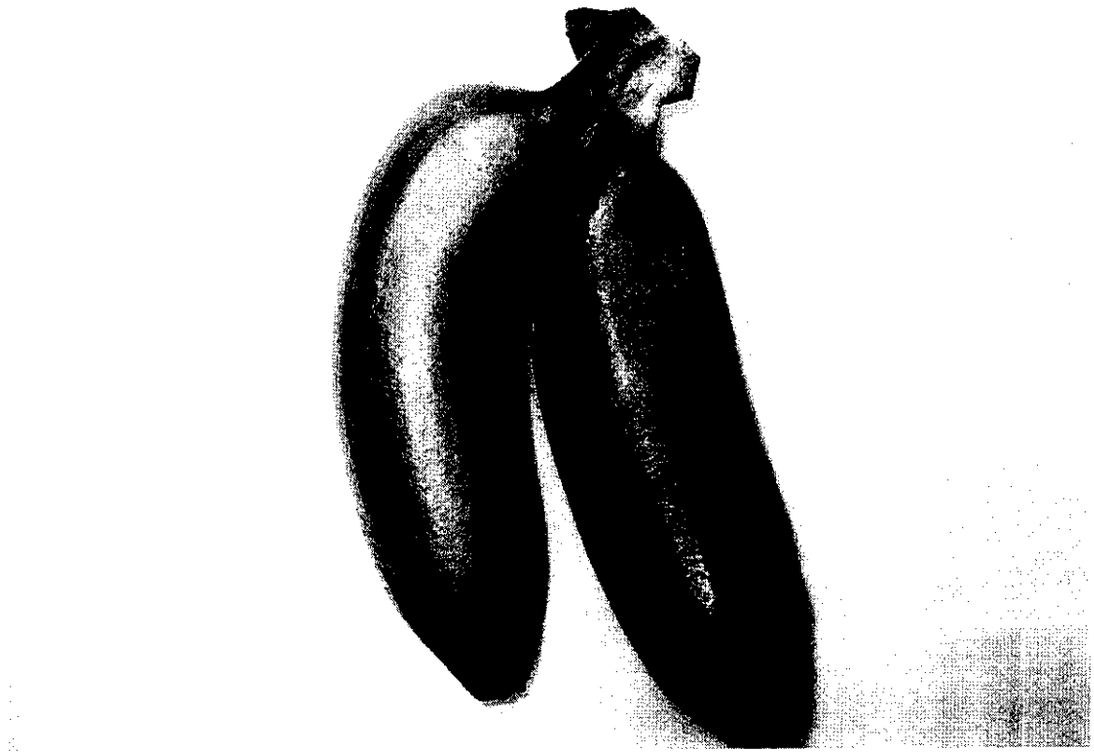


Figure 3.22: Banana cv. Red banana - [*Musa spp* – Red banana - AAA

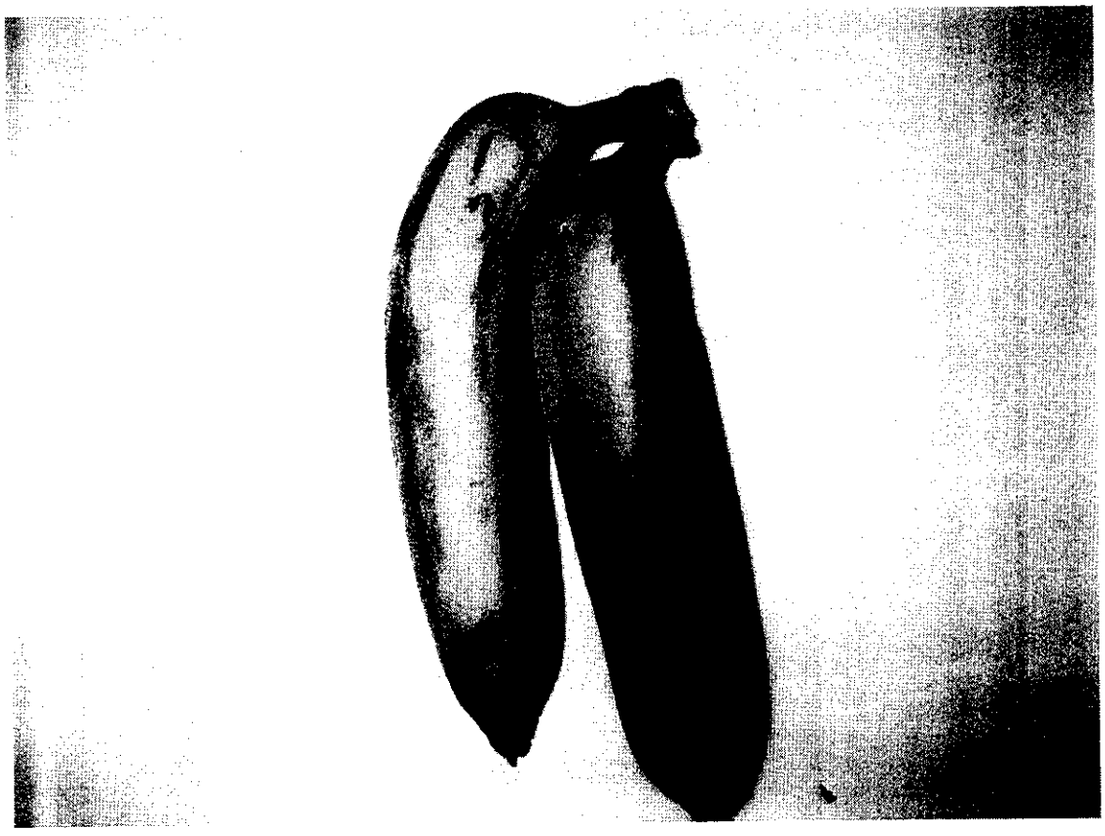


Figure 3.23: Banana cv. Robusta- Cavendish sub group [*Musa spp* - Robusta - AAA]

Materials and Methods

4. MATERIALS AND METHODS

4.1 Chemicals used

1,1-Diphenyl-2-Picryl hydrazyl (DPPH), methanol, Ammonium persulfate, 2,2-azobis-3-ethylbenzthiazoline-6-sulfonicacid(ABTS),sodiumnitroprusside,sulphanilamide, napthyl ethylene diamine dihydrochloride, o-phosphoric acid, deoxyribose, Ethylene diamine tetraacteic acid (EDTA), ferric chloride, trichloroaceticacid(TCA), thiobarbituric acid (TBA), ferrous sulphate, acetic acid, sulfuric acid, ammonium molybdate, disodium hydrogen phosphate, potassium ferricyanide, hydrogen peroxide, sodium nitrate, aluminium chloride, sodium hydroxide, potassium hydroxide, ascorbic acid, calcium carbonate, triton x100,hydroxylamine hydrochloride, riboflavin, potassium dichromate ,sodiumazide, reduced glutathione, ethanol, anthrone, CDNB, catechol, MBTH, theophylline, sodiumperiodate, trifluroaceticacid, xylene, NADP,NADPH, glucose 6 phosphate, magnesium chloride, DTNB, thiourea, oxalic acid, 2,2'dipyridyl,petroleum ether, chloroform. All reagents were used for the analytical grade.

4.2 Banana varieties

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

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

4.2.1 Preparation of the banana pulp extracts

The banana powder was prepared from dried banana pulp. Ethanol volume equal to 10% of the weight of each banana powder was added. Then it was kept in an orbital shaker overnight at 30°C, after which the solution was filtered and the filtrate was dried completely. The dried extract was then scraped off and weighed. This was used for further *in vitro* assays.

4.3 METHODS

4.3.1 PHASE I

4.3.1.1 *IN VITRO* ANTIOXIDANT CAPACITY ASSAYS

4.3.1.1.1 Total Antioxidant Capacity Assay (Prieto, 1999)

Principle

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

Reagents

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

Procedure

The working solutions (1-10 mg/ml) of the samples were prepared by dissolving the extracts in water. 0.2 ml of the extracts were mixed with 2ml of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate).The tubes were capped with silver foil and incubated at 95°C for 90 minutes. The tubes were then cooled to room temperature and the absorbance was measured at 695nm against a blank. Ascorbic acid was used as the standard. The total antioxidant capacity was expressed as ascorbic acid equivalent (Raghavan Govindarajan *et al.*, 2003 and Umamaheshwari *et al.*, 2008)

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

Principle

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

Reagents

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

Procedure

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

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

4.3.1.1.3 Determination of ABTS Cation Radical Scavenging Activity (Re *et al*., 1999)

Principle

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

Reagents

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

Procedure

The assay was performed by a slightly modified protocol. ABTS radical was produced by reacting ABTS solution (7mM) with ammonium persulfate (2.45mM) and the mixture was allowed to stand in the dark at room temperature for 12-16 hours to give a dark coloured solution. The absorbance was measured at 745nm. The initial absorbance was found to be around 2.99. This stock solution was diluted with methanol to give a final absorbance value of around 0.7(±0.02) and equilibrated at 30°C. Different concentrations of the sample (1-10 mg/ml) were prepared by dissolving the extracts in water. About 0.3ml of the sample was mixed with 3ml of ABTS working standard in a microcuvette. The decrease in the absorbance was measured after mixing the solution in one minute intervals up to 6min. The final absorbance was noted. A solution of ABTS working standard and 0.3ml of methanol was used as the control. About 3ml of methanol was used as blank. The percentage inhibition was calculated according to the formula:

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

4.3.1.1.4 Determination of Nitric Oxide Radical Scavenging Activity

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

Principle

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

Reagents

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

Procedure

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

4.3.1.1.5 Determination of Hydroxyl Radical Scavenging activity

(Umamaheshwari and Chatterjee, 2008)

Principle

Hydroxyl radical scavenging activity was measured by the ability of the different fractions of banana samples to scavenge the hydroxyl radicals generated by the Fe^{3+}-ascorbate–EDTA–H_2O_2 system (Fenton reaction). These damage the substrate deoxyribose, which undergoes degradation to form malondialdehyde. This produces a

pink chromogen with TBA. The antioxidants inhibit this reaction and hence there is a decrease in the colour intensity of the chromogen as the antioxidant potential increases (Umamaheshwari *et al.*, 2008).

Reagents

1. 2-deoxyribose (28mM in 20mM KH2PO4 buffer, pH 7.4)
2. 1.04 mM EDTA
3. $FeCl_3$
4. 1.0mM hydrogen peroxide
5. 1.0mM ascorbic acid
6. 1.0% TBA
7. 2.8% TCA

Procedure

The reaction mixture with a final volume of 1.0 ml contained 100 µl of 2-deoxy-2-ribose (28mM in 20mM KH_2PO_4 buffer, pH 7.4), 500 µl of the fractions at various concentrations (1 -10 mg/ml) in water,200 µl of 1.04 mM EDTA and 200 µM $FeCl_3$, 100 µl of 1.0mM hydrogen peroxide (H_2O_2) and 100 µl of 1.0mM ascorbic acid. Test samples were kept at 37°C for 1 hr. The free radical damage imposed on the substrate, deoxyribose was measured using the thiobarbituric acid test.1 ml of 1% thiobarbituric acid (TBA) and 1 ml of 2.8% trichloro acetic acid (TCA) were added to the test tubes and incubated at 100°C for 20 min. After cooling, the absorbance was measured at 532 nm (Umamaheshwari et al., 2008) against a blank containing deoxyribose and buffer. Deoxyribose degradation was measured as TBARS against a control with buffer instead of the sample. The percentage inhibition was calculated.

4.3.1.1.6 Determination of Superoxide Radical Scavenging Activity

Reagents

1. Phosphate buffer

2. Riboflavin

3. EDTA

4. NBT

5. Sodium cyanide

Procedure

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

4.3.1.1.7 Determination of Lipid Peroxidation Inhibition Activity

(Ohkawa *et al.*, 1979)

Principle

Initiation of lipid peroxidation by ferrous sulphate takes place through the hydroxyl radical formation by Fenton's reaction. These produce malondialdehyde (MDA), which reacts with TBA to form a pink chromogen. The inhibition of lipid peroxidation could take place due to the scavenging of the hydroxyl radicals/ superoxide radicals or by reducing the rate of conversion of ferrous to ferric or by chelating the iron itself.

Reagents

1) Phosphate buffer saline (pH 7.4)
2) 0.07M Ferrous sulphate
3) 20% acetic acid (pH 3.5)
4) 0.8%TBA in 1.1% SDS
5) 20% TCA

Procedure

Goat liver was washed thoroughly in cold phosphate buffered saline (pH 7.4). This was then minced in a mortar and pestle with a measured volume of cold buffer in ice. The minced liver was then homogenized in a homogenizer to give a 10% homogenate. The homogenate was filtered using cheese cloth to remove unwanted residue. The filtrate was then centrifuged at 10,000rpm for 10 min. under refrigerated conditions. The supernatant was used for the assay. 0.5ml of this 10%homogenate was taken. To this, 0.5ml of the extracts (1-10 mg/ml) in water was added. The volume of the reaction mixture was made upto 1ml with distilled water. To this, 0.05ml of 0.07M ferrous sulphate was added. The solution was incubated at room temperature for 30 min. To the incubated solution, 1.5ml of 20% acetic acid (pH 3.5), 1.5ml of 0.8% TCA (in 1.1% SDS) and 0.05ml of 20% TCA were added. The tubes were vortexed to ensure appropriate mixing. Then the tubes were incubated at 100°C for 1 hour. The tubes were then cooled to room temperature. The absorbance was read at 532nm.The control contained PBS instead of the sample. The percentage inhibition was calculated.

4.3.1.1.8 Determination of Ferric Reducing Antioxidant Potential Activity

(Oyaizu, 1986)

Reagents

1. Phosphate buffer (0.2M, pH 6.6)
2. 1% Potassium ferricyanide
3. 10% TCA
4. 0.1% Ferric chloride

Procedure

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

4.3.1.1.9 Determination of β-Carotene Bleaching Inhibition Activity

(Mi-Yae *et al.*, 2003)

Principle

Linoleic acid, an unsaturated fatty acid gets oxidized by "reactive oxygen species" (ROS), which are produced in water. The products formed initiate the carotene oxidation, which leads to discoloration of the emulsion. The extent of discoloration is measured at 470 nm. The antioxidant activity was expressed as percent inhibition relative to control i.e. linoleic acid emulsion.

Reagents

1. β-Carotene
2. Chloroform
3. Linoleic acid
4. Tween 80

Procedure

A solution of β-carotene was prepared by dissolving 2mg of β-carotene in 10ml of chloroform.2ml of this solution is pipetted into a 100ml round bottomed flask. After removal of chloroform at 40°C under vacuum, 40mg of linoleic acid, 400mg of tween 80 emulsifier and 100ml of distilled water are added to the flask with vigorous shaking. Aliquots (2.4ml) of this emulsion are transferred into different test-tubes containing 0.1ml of different concentrations of the sample (1-5 mg/ml).The tubes are shaken and incubated at 50°C in a water bath. As the emulsion is added to each tube, the zero time soon as absorbance is measured at 470nm using a spectrophotometer. Absorbance readings are then recorded at 20min interval until the control sample had changed colour. A blank, devoid of β-carotene, is prepared for background subtraction. Antioxidant activity is calculated using the following equation:

Antioxidant activity = (β-carotene content after 2 hrs of assay/initial β-carotene content) x 100

4.3.2 PHASE II

4.3.2.1 ENZYMATIC ANTIOXIDANTS

The enzymatic antioxidants, catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase, glucose 6 phosphate dehydrogenase, polyphenol oxidase, were assessed in banana pulp extracts.

4.3.2.1a Extraction of the Sample

1g of banana pulp was weighed and homogenized with 10ml of 0.2 M phosphate buffer pH 7.4. The sample was centrifuged at 4°C and the supernatant was used for estimation of enzymatic antioxidants.

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

Principle

This 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 diazonium compound which subsequently reacts with naphthylamine to produce a red azo compound whose absorbance is measured at 543nm.

Reagents

1. 50m M phosphate buffer. pH 7.4
2. 20 mM L-Methionine
3. 1%(v/v) Triton X-100
4. 10m M Hydroxylamine hydrochloride
5. 50µM EDTA
6. 50µM Riboflavin
7. Griess reagent: 1%sulphanilamide, 2%phosphoricacid, and 0.1% naphthylethylene diamine dihydrochloride.

Procedure

Pipetted 1.4ml of reaction mixture containing 1.1ml phosphate buffer, 75 µl methionine, 40 µl triton X-100, 75 µl hydroxylamine hydrochloride, 100µl EDTA and 100µl of sample into a test tubes. The tubes were pre incubated at 37° C for 5 minutes. After incubation 80µl of riboflavin was added. The control tube contain buffer instead of sample. The test and control tubes were exposed to UV light for 10 minutes. After the exposure 1ml of Griess reagent was added to all the tubes and absorbance of pink colour developed was measured at 543nm.

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

4.3.2.1.2 Estimation of Catalase (Sinha, 1972)

Principle

Catalase causes rapid decomposition of hydrogen peroxide to water.

$$H_2O_2 \rightarrow H_2O + O_2$$

The method is based on the fact that dichromate in acetic acid reduces to chromic acetate when heated in the presence of H_2O_2 with the formation of perchloric acid as an unstable intermediate. The chromic acetate thus produced is measured colorimetrically at 610nm. Since 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 H_2O_2 for different periods of time. The reaction is stopped at specific time intervals by addition of dichromate/acetic acid mixture and the remaining H_2O_2 is determined by measuring chromic acetate colorimetrically after heating the reaction.

Reagents

1. 0.01M Phosphate buffer, pH 7.0
2. 0.2M Hydrogen peroxide
3. Stock dichromate/acetic acid solution: Mixed a 5% potassium dichromate with glacial acetic acid (1:3 by volume)
4. Working dichromate/acetic acid solution: The stock was diluted to 1:5 with distilled water to make the working dichromate/acetic acid solution.

Procedure

The assay mixture contained 0.5ml of H_2O_2, 1.0ml of buffer and 0.4ml water, 0.2ml of the enzyme was added to initiate the reaction. 2.0ml of the dichromate/acetic acid reagent was added after 0, 30, 60, 90 seconds of incubation. To the control tube the enzyme was added after the addition of the acid reagent. The tubes were then heated for 10 min and the colour developed was read at 530nm.

The activity of catalase was expressed as µmole of H_2O_2 decomposed/min/mg protein.

4.3.2.1.3 Estimation of Glutathione Peroxidase (Ellman, 1959)

Principle

Glutathione peroxidase catalyses the following reaction

$$2GSH + H_2O_2 \xrightarrow{\text{Se- GPx}} GSSH + 2H_2$$

Glutathione was measured by its reaction with DTNB to give a compound that absorbance at 412nm.

Reagents

1. 0.4M sodium phosphate buffer, pH 7.0
2. 10mM sodium azide
3. 2.5m M hydrogen peroxide
4. 4m M reduced glutathione
5. 10% TCA
6. 0.3 M phosphate solution
7. 0.04% DTNB in 1% sodium citrate
8. Reduced glutathione standard: 20 mg reduced glutathione was dissolved in 100ml of water.

Procedure

0.4ml of buffer, 0.1ml of sodium azide, 0.2ml of reduced glutathione, 0.1ml of H2O2, 0.2ml of enzyme and 1.0ml of water were added to a final incubation volume of 2.0ml. 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 supernatant was removed by centrifugation and added to 3.0ml disodium hydrogen phosphate solution and 1.0 ml of DTNB reagent. The colour developed was read at 412nm. Standards in the range of 200-1000 µg were taken and treated in the similar manner.

The activity was expressed in terms of µg of glutathione utilized/min/mg protein.

4.3.2.1.4. Estimation of Glutathione - S - Transferase (Habig, 1973)

Principle

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

$$CDNB + GSH \xrightarrow{GST} CDNB\text{-}S\text{-}glutathione$$

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. 0.5M phosphate buffer, p H 6.5
2. 30 mM CDNB in 95% ethanol
3. 30 mM reduced glutathione

Procedure

To 1.0 ml of buffer, 0.1 ml of sample, 1.7ml of water and 0.1 ml of CDNB were added and incubated at 37°C for 5 min. After incubation, 0.1 ml of reduced glutathione was added. The increase in the optical density of the enzyme for 1, 2 and 3 minutes were measured against blank at 340nm.

The enzyme activity is calculated in terms of µmoles of CDNB conjugate formed/min/mg protein.

4.3.2.1.5 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 340nm.

$$NADPH\ (NADH) + H^{+} + GSSG \longrightarrow NADP^{+} + 2GSH$$

Reagents

1. 0.3M phosphate buffer, pH 6.8
2. 25mM EDTA
3. 12.5mM oxidized glutathione
4. 3mM NADPH

Procedure

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

The enzyme activity is calculated in terms of µmoles of NADPH oxidized/min/mg protein.

4.3.2.1.6 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 340nm when NADP reduces to NADPH. This reaction takes place when electrons are transferred from glucose 6 phosphate to NADP in the reaction catalyzed by glucose 6 phosphate dehydrogenase.

Reagents

1. 0.1 M Tris Hcl buffer, pH 8.2
2. 0.2 mM NADP
3. 0.1M Magnesium chloride
4. 6 mM glucose 6 phosphate

Procedure

0.4ml of Tris Hcl buffer, 0.2ml of NADP, 0.2ml of magnesium chloride, 1.0 ml of water and 0.2ml of sample were taken in a test tube. The reaction was started by the addition of 0.2 ml of glucose 6 phosphate and the increase in OD was measured at 340nm.

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

4.3.2.1.7 Estimation of Polyphenol Oxidase

(Rocha and Morais, 2001; Yemenicioglu, 2002)

Principle

Polyphenol activity was determined by measuring the increase in absorbance 495nm.

Reagents

1. 0.1M Phosphate buffer, pH 6.0
2. 0.01M Catechol

Procedure

The reaction mixture contained 2.0ml of sample, 3.0ml of buffer and 1.0ml of catechol. The contents were mixed well and increased absorbance was measured at every 30sec up to 3 min.

The enzyme activity was calculated in terms of 0.001 OD change / min / mg protein.

4.3.3 PHASE III

4.3.3.1 NON ENZYMATIC ANTIOXIDANTS

The non enzymic antioxidants reduced Glutathione, Vitamin A, Vitamin C, Vitamin E were assessed in banana pulp.

4.3.3.1.1 Estimation of Total Reduced Glutathione (Moran *et al.*, 1979)

Principle

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

Reagents

1. 0.1M Phosphate buffer p H 7.0
2. 0.4M Na_2HPO_4
3. DTNB reagent : 40 mg DTNB in 100 ml of 1% tri sodium citrate
4. Standard glutathione: 20 mg reduced glutathione was dissolved in 100 ml water.

Procedure

0.5 ml of 20% tissue homogenate was mixed with 0.5 ml of 5% TCA. The precipitate was removed by centrifugation.0.1 ml of the supernatant was made up to 1.0ml with 0.4M Phosphate solution and 2.0 ml of the freshly prepared DTNB reagent was added. The absorbance was read after 10 minutes at 412nm against a reagent blank. A set of standards were also treated in the above manner. The amount of glutathione was expressed as µg/mg protein.

4.3.3.1.2 Estimation of Vitamin A

Reagents

1. 2N KOH
2. 90% alcohol
3. Petroleum ether(40 – 60°C)
4. Chloroform
5. Trifluroacetic acid (TFA)

Procedure

1ml of 10% homogenate was mixed with 1ml of 2N KOH in 90% alcohol. The mixture was refluxed for 20min at 60°C. Then safonified mixture was cooled to room temperature 25ml of water was added and mixed well and transferred to separating funnel. Extracted thrice with 25,15,10ml petroleum ether. Ether extract was pooled and washed with 50-100ml of distilled water repeatedly. Petroleum ether extract was dried by adding anhydrous sodium sulphate. The aliquots were evaporated to dryness at 60°C in a

water bath. Residue taken immediately and 2.0ml of TFA reagent was added. The blue colour developed was read at 620nm.

4.3.3.1.3 Estimation of Vitamin C (Sadasivam and Manickam, 2008)

Principle

Bromine water oxidizes ascorbic acid into dehydroascorbic in the presence of oxalic acid. After coupling with 2,4-dinitrophenyl hydrazine at 37°C for three hours, the solution is treated with 80% H_2SO_4 to produce a red colour complex and the absorbance is measured spectrometrically at 540nm.

Reagents

1. Oxalic acid
2. Activated charcoal solution
3. DNPH
4. 80% H_2SO_4

Procedure

5g of sample was ground in 25-50ml of 4% oxalic acid. The sample was centrifuged at 10000 rpm for 20 min.10ml aliquots were transferred to conical flask. A few drops of activated charcoal solution were added. The solution was made up to 25 ml with 4% oxalic acid.1 ml aliquots of the above solution were taken and 3ml of the distilled water was added. To the above solution, 1 ml of DNPH was added. This was incubated at 37°C for 3 hours. 7ml of 80% H_2SO_4 (in ice) was added to the test tubes and they were incubated at room temperature for 30 min. The absorbance was read at 540nm. (Poteggente *et al*., 2002).

4.3.3.1.4 Estimation of Vitamin E

Principle

Tocopherol 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 and

carotenes are firs extracted with xylene and the extinction read at 460nm to measure carotenes. A correlation is made for this after adding ferric chloride and read at 520nm.

Reagents

1. Absolute ethanol
2. Xylene
3. 2, 2` dipyridyl
4. Ferric chloride
5. Standard D, L-α - tocopherol
6. **Sample extraction** : 1g of banana pulp was homogenized in a mortar and pestle and transferred to a conical flask. 50ml of 0.1N sulphuric acid was added slowly without shaking. Stoppered and allowed to stand overnight. The next day contents of the 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

1.5ml of pulp extract was pipetted into a Stoppered centrifuge tube, 1.5ml of the standard and 1.5ml of water respectively. To the test and blank added 1.5ml of ethanol and to the standard added 1.5ml of water. 1.5ml xylene was to all the tubes, stoppered, mixed well and centrifuged. Transferred 1.0ml of xylene layer into another stoppered tube, take care not to include any ethanol or protein. Added 1.0ml 2,2` dipyridyl reagent to each tube, Stoppard and mixed. Pipetted out 1.5ml of the mixtures into spectrophotometer cuvettes and read absorbance of test and standard against the blank at 460nm. Then in turn beginning with the blank, 0.33ml of ferric chloride solution was added. Mixed well and after exactly 1.5minutes read test and standard against the blank at 520nm. The amount of vitamin E can be calculated using the formula

$$\text{Vitamin E } (\mu g/g) = \frac{\Delta A520nm - \Delta A450nm \times \text{conc[S]} \times \text{OD of std} \times \text{Total volume}}{\Delta A520nm \times \text{Vol for experiment} \times \text{wt of sample}}$$

4.3.4 PHASE IV

4.3.4.1 PHYTOCHEMICAL ASSAYS

4.3.4.1.1 Estimation of Total Carotenoids and Lycopene

(Sadasivam and Manickam, 2008)

Principle

The total carotenoids in the sample are extracted with petroleum ether. The total carotenoids are estimated spectrometrically at 450nm. Lycopene has absorption at 473 nm 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 is safonified for 30min in a shaking water bath at 37°C in 12% KOH in ethanol. Transferred the sample to a separating funnel containing 10-15ml of Petroleum ether. Mixed gently in the separating funnel. Allowed it to stand till the layers separate completely. Collected the pigments in the petroleum layer. Transferred this to separating funnel and extract using petroleum ether-homogenous phase. Repeated the extraction of the aqueous phase with petroleum ether until it was colorless. Discarded the aqueous layer. To the extract, added a small quantity of sodium sulfide to remove turbidity. Final volume of extract noted to known volume. Read absorbance at 450nm and 503nm.

4.3.4.1.2. Estimation of Total Phenols (Singleton and Rossi, 1965)

Principle

Phenols react with phosphomolybdic acid in Folin- Ciocalteau reagent in alkaline medium and produce a blue colored complex (molybdenum blue) that can be estimated at 650nm.

Reagents

1. Ethanol
2. Folin- Ciocalteau reagent
3. Sodium carbonate

Procedure

1 g of the banana pulp was weighed and ground with 10ml of 80% aqueous ethanol in a mortar and pestle. The ground sample was then centrifuged at 10000 rpm for 20minutes. The supernatant obtained was poured into separate tubes. Reextract the pellets in 50ml ethanol. This sample was then again centrifuged at 5000nm for 20minutes. The pooled 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 phenol 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 3minutes. 2.0ml of 20% sodium carbonate was added. Then the sample was boiled in water bath for a minute and the blue colour developed was read at 650nm.

4.3.4.1.3. Estimation of Flavonoids

Reagents

1. Methanol
2. Sodium nitrite
3. Aluminium chloride
4. 1M NaOH

Procedure

1g of the banana sample was weighed and ground with 10ml of 80% aqueous methanol using mortar and pestle. The grinded sample was filtered with the help of a Whatmann filter paper no.42 and clear sample solution was obtained. 0.5ml 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 allowed the mixture to stand for 5 min. Then 0.15ml of 10% aluminium chloride was added. After 6 min, 0.5ml of 1M NaOH was added into test tube. The solution in the test tube was then diluted with 0.275ml of distilled water. The absorbance was read at 510nm.

4.3.4.1.4 Estimation of Alkaloids (Singh and Archana Sahu, 2006)

Principle

Alkloids were oxidized by sodium metaperiodate under mild acidic conditions to from an intermediate, N, N- dimethyl alloxan which then reacted with MBTH (3-methyl-2-benzo thiazolinone hydrazone hydrochloride) to yield a blue coloured product. This complex was spectrophtometrically measured at 630nm.

Reagents

1. 0.1M Acetic acid
2. 0.01M Sodium meta periodate
3. 0.01M 3-methyl 2-benzo thiazolinone hydrazone hydrochloride (MBTH)

Procedure

In a clean dry conical flask, weighed 0.5g of dried banana powder and added 50ml of 10% acetic acid in ethanol. Kept this in an orbital shaker for 4hours and filtered. 1.5ml of this sample was pipetted out into the boiling tube. To all the tubes 1ml of 0.01M sodium meta periodate and 0.5ml of 0.1M acetic acid were added. Then10ml of distilled water was added and kept in boiling water bath for 10min. Then 2ml of 0.01M MBTH added to the tubes and kept in the boiling water bath for 2 min. The tubes were cooled and made up to 25ml with distilled water. The blue colour formed measured at 630nm.

4.3.4.1.5 Estimation of Tannins (Kirk and Sawyer, 1998)

Reagents

1. Sodium carbonate

Procedure

1g of sample was dispersed in 10ml of distilled water and shaken. The mixture was allowed to stand for 30minat 28°C and was filtered through whatmann filter paper. 2ml of the extract was dispersed in to a 50ml standard flask and 2.5ml of saturated sodium carbonate was added. The content was made up to 50ml with distilled water and incubated at 28°Cfor 90min. The absorbance was measured at 260nm.

4.3.4.1.6 Determination of Total Sugars by Anthrone Method

Principle

Carbohydrates are first hydrolyzed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxy methyl furfural. This compound forms with anthrone a green coloured product with an absorption maximum at630nm.

Reagents

1. 2.5N Hydrochloric acid.
2. Anthrone reagent: Dissolve 200mg anthrone in 100ml of ice cold 95% sulphuric acid (freshly prepared).

Procedure

100mg of sample was hydrolyzed with 5ml of 2.5N Hydrochloric acid in boiling water bath for 3 hours. The sample was neutralized with solid sodium carbonate until the effervescence ceases and made up to 10ml with distilled water. The sample was centrifuged and supernatant was collected. 25µl of the sample aliquot was made up to 1ml with distilled water. Standards were prepared by taking 0.2- 1.0ml of working standard and volume was made up to ml with distilled water. 4ml of anthrone reagent was

added to all the tubes and heated in boiling water bath for 8 minutes. The tubes were cooled and green developed was read at 630nm.

4.3.5 PHASE V

4.3.5.1 Antibacterial Activity

4.3.5.1.1 Bacterial Strains

1. E.coli
2. Pseudomonas aeroginosa
3. Staphylococcus aureus
4. Bacillus subtilis

4.3.5.1.2 Preparation of bacterial inoculum

Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to the conical flask containing nutrient broth and autoclaved at 121°C.Then incubated with agitation for 24 hrs at 37°C.

4.3.5.1.3 Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) is defined as the lowest concentration (ppm) of the extract in agar plates showing no visible bacterial growth. 3g of nutrient agar was dissolved in 100ml of distilled water and autoclaved.20ml of nutrient agar was transferred into autoclaved petri plates and allowed to solidify. 100mg of extract was dissolved in 1ml of 10% DMSO. 150µl of bacterial culture was spread on the plate. The well was made on agar plate; the extract was transferred into wells. The plate was incubated at 37°C.

4.4 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 and Discussion

5. RESULTS AND DISCUSSION

A free radical is nothing more than a molecular structure which contains an unpaired electron. Electrons tend to stay in pairs. Electron pairs make up the chemical bond which keeps molecules from flying apart. An unpaired electron is driven by a potent chemical force which compels it to find a mate. This molecular instinct to merge with another electron is so powerful that the searching molecule behaves erratically, moving about much like a weapon within cellular structures. Its random and wild molecular movements within cellular material can create cellular damage, which can eventually result in degeneration or mutation.

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

Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Although there are several enzyme systems within the body that scavenge free radicals, the principle micronutrient (Vitamin) antioxidants are Vitamin E, Beta-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.

Bananas are one of the most popular fruits on the world and it well be known that fruits contain various antioxidants compounds such as gallocatechin and dopamine. Since the banana fruits are widely available, they been used as food without apparent toxic effect. Bananas are a great food for all round health benefits, for the average person, dieter or even athletes bananas may provide greater benefits than most fruits. The combination of carbohydrates and B Vitamins present in a banana helps provide an energy boost which makes them great to eat 30 minutes before a workout, or even at

breakfast to help boost energy at the start of every day. Bananas are not only a good source of B vitamins they also contain Vitamin C, A and high potassium.

5.1 PHASE I

5.1.1 *IN VITRO* ANTIOXIDANT CAPACITY ASSAYS

5.1.1.1 Total antioxidant capacity assay

The total antioxidant assay gives an estimate of the overall antioxidant potential of the plant. There is a formation of phosphomolybdenum complex, the intensity of which indicates the potential of the banana as a scavenger of free radicals.

Extracts	Ascorbic acid equivalent (AAE) µM/g
Kadali	$6.00^{f} \pm 0.759$
Karpooravalli	$6.00^{f} \pm 0.597$
Nendran	$5.60^{e} \pm 0.514$
Monthan	$6.20^{g} \pm 0.323$
Pachanadan	$5.20^{d} \pm 0.792$
Poovan	$3.80^{a} \pm 0.611$
Rasthali	$\mathbf{6.60}^{h} \pm 0.660$
Red	$4.00^{b} \pm 0.892$
Robusta	$4.41^{c} \pm 0.601$

Table 5.1 Total antioxidant activity in Ascorbic acid equivalents of ethanolic extracts of pulp extracts of banana varieties.

Values represent mean ± SD of 3 replicates.

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

Different pulp extracts of banana showed various degrees of antioxidant capacity. The ethanolic extracts of Rasthali banana showed highest activity in the range of 6.60 μmol g^{-1} compared to other varieties of banana pulp, whereas the ethanolic extract of Poovan banana showed least activity in the range of 3.80 μmol g^{-1}.

Studies by Sun *et al.*, (2002) depicted vitamin C contribution to total antioxidant activity to be 1.58% compared to other fruits. According to the Wang *et al.*, 1996 suggestion the estimated contribution of Vitamin C to the total antioxidant activity is relatively low. Therefore, the major contribution to the total antioxidant activity in fruits was from the combination of phytochemicals, not from the vitamin C.

5.1.1.2 DPPH radical scavenging activity

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

Extent of DPPH radical scavenged was determined by the decrease in intensity of violet colour in the form of EC_{50} values. Lower EC_{50} value represents higher antioxidant activity. The antioxidant activity was compared with ascorbic acid as standard Yamaguchi *et al.*, (1998).

Banana extract	Sample concentration (mg/ml)									
	1	2	3	4	5	6	7	8	9	10
Kadali	50.54^{a}±1.56	53.13^{a}±0.80	55.05^{a}±0.73	56.76^{a}±0.76	58.63^{a}±0.99	61.63^{a}±0.95	64.78^{a}±1.01	67.54^{b}±0.94	70.14^{a}±0.91	73.72^{a}±1.17
Karpooravalli	31.37^{a}±1.45	34.26^{a} ±1.29	36.83^{a}±1.46	40.24^{a}±0.95	42.83^{a}±1.09	44.94^{a}±0.91	48.55^{a}±1.14	53.89^{b}±1.1	57.35^{a}±1.30	62.10^{a}±1.67
Nendran	3.84^{a}±0.2	27.21^{a}±2.33	37.23^{a}±2.84	41.36^{a}±1.45	46.00^{a} ± 2.0	52.28^{a}±2.14	57.86^{a}±1.88	62.20^{b}±2.32	68.54^{a}±1.34	72.94^{a}±2.53
Monthan	49.97^{a}±2.47	57.29^{a}±1.96	62.57^{a}±1.63	68.54^{a}±1.87	74.14^{a}±2.42	81.14^{a}±2.89	85.61^{a}±2.06	90.70^{b}±1.21	93.42^{a}±1.96	**97.87^{a}±1.10**
Pachanadan	40.50^{a}±1.97	48.30^{a}±1.48	52.13^{a}±1.45	55.51^{a}±1.48	60.04^{a}±2.22	64.36^{a}±1.28	69.84^{a}±2.87	74.56^{b}±0.88	77.05^{a}±0.96	81.15^{a}±2.32
Poovan	33.82^{a}±0.91	38.49^{a}±1.36	45.55^{a}±2.70	53.26^{a}±2.80	60.06^{a}±2.23	64.53^{a}±2.20	71.63^{a}±1.75	77.31^{b}±2.31	81.21^{a}±1.07	87.63^{a}±2.25
Rasthali	59.92^{a}±2.29	63.17^{a}±1.31	67.44^{a}±1.60	71.18^{a}±0.96	73.26^{a}±1.1	76.50^{a}±1.11	79.52^{a}±1.35	82.12^{b}±1.15	85.74^{a}±1.10	88.39^{a} ±1.44
Red	70.48^{a}±1.65	75.30^{a}±0.88	78.18^{a}±1.10	81.80^{a}±1.03	84.23^{a}±1.22	87.06^{a}±1.1	89.76^{a}±0.92	91.87^{b}±0.93	94.27^{a}±1.0	96.62^{a} ±1.1
Robusta	17.98^{a}±1.68	25.65^{a}±2.36	33.12^{a}±1.91	40.53^{a}±2.40	49.01^{a}±1.73	55.92^{a}±1.68	60.50^{a}±2.21	66.54^{b}±1.49	73.03^{a}±2.20	79.13^{a}±2.22
Standard (µg/ml)	10	20	30	40	50	60	70	80	90	100
Ascorbic acid	49.66±2.08	55.59±2.43	65.41±1.71	73.98±2.59	79.30±1.80	84.38±2.06	87.99±0.99	90.33±0.92	93.33±0.88	96.37±1.31

Table 5.2a Scavenging activity (%) on DPPH radicals by ethanolic extracts of nine banana pulp varieties.

Values represent mean ± SD of 3 replicates.

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

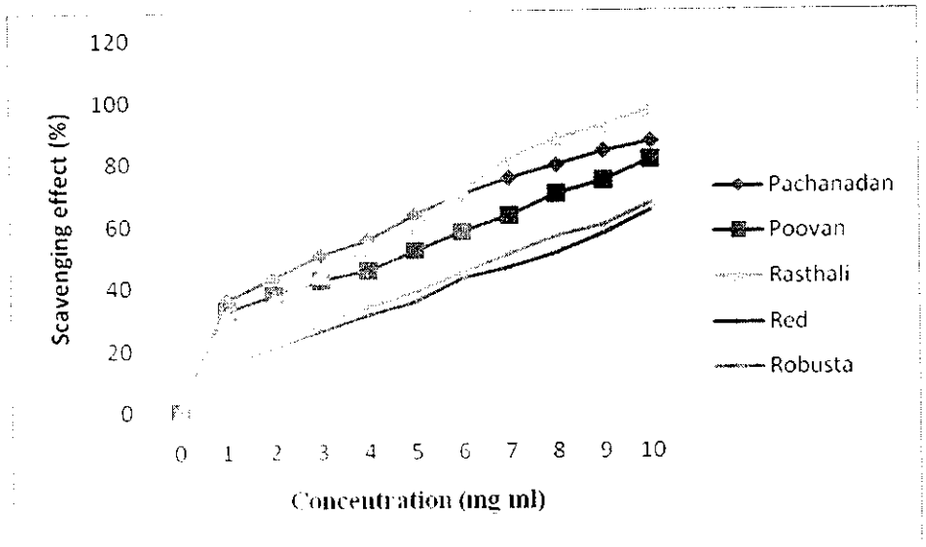


Figure 5.1a Scavenging activity (%) on DPPH radical by ethanolic extracts of *acuminate* type banana varieties.

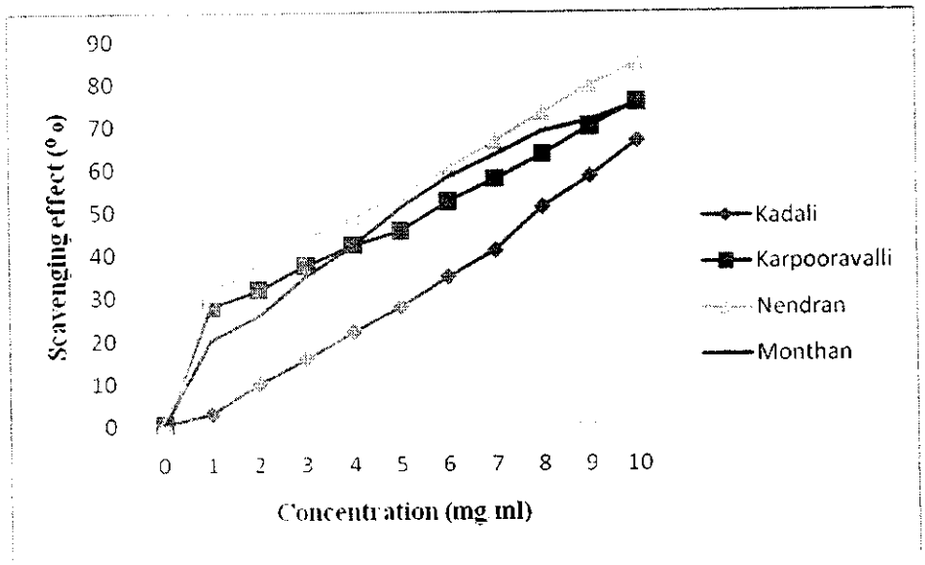


Figure 5.1b Scavenging activity (%) on DPPH radical by ethanolic extracts of *balbisiana* type banana varieties.

Free radical scavenging potential of the ethanolic extracts of the nine varieties banana pulp shown in Table 5.2a which increases with the increase in concentration.

At 1-10mg ml^{-1}, scavenging abilities of ethanolic extracts of Kadali, Karpooravalli, Nendran, Monthan, Pachanadan, Poovan, Rasthali, Red, and Robusta bananas on DPPH radicals exhibited 50.54-73.72%, 31.37-62.10%, 3.84-72.94%, 49.97-97.87%, 40.5-81.15%, 33.82-87.63%, 59.92-88.39%, 70.48-96.62%, 17.98-79.13% respectively. However, at 10mg ml^{-1}, Monthan banana pulp extract exhibited highest DPPH radical scavenging activity and Karpooravalli banana showed least DPPH radical scavenging activity with respect to EC_{50} values.

Statistically, the scavenging effect of banana pulp extracts was effective in the order of Monthan >Red> Rasthali>Poovan>Pachanadan>Robusta> Kadali> Nendran > Karpooravalli at 10 mg ml^{-1}.

Banana varieties	Kadali	Karpooravalli	Nendran	Monthan	Pachanadan	Poovan	Rasthali	Red	Robusta	Ascorbic acid
EC_{50} (mg/ml)	1.0	7.3	5.8	**0.9**	2.5	3.6	1.0	1.0	5.3	1.2

Table 5.2b Comparison of EC_{50} values of DPPH inhibition activity.

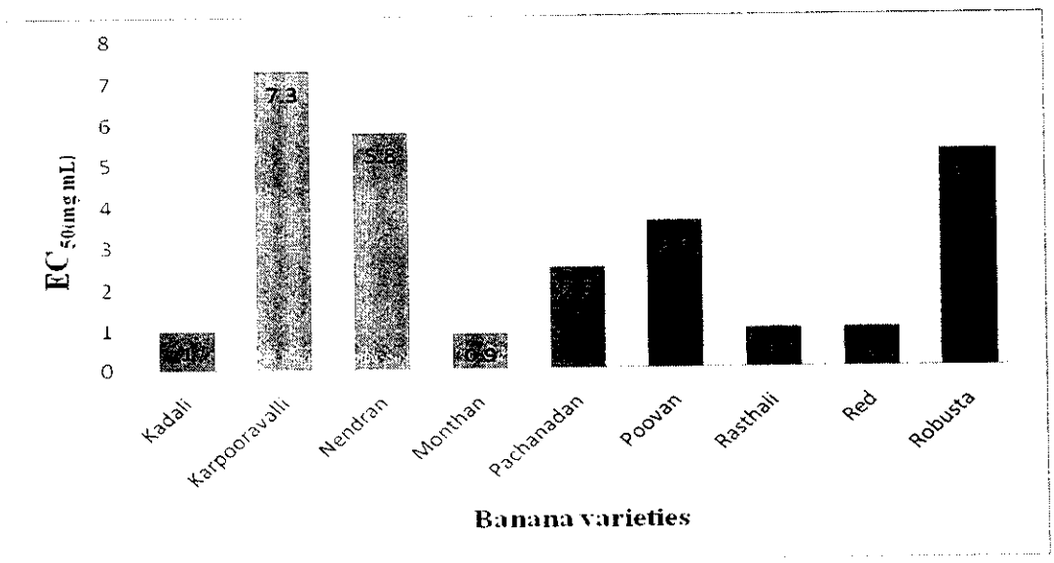


Figure 5.1c Comparison of EC_{50} values of DPPH inhibition activity.

An EC_{50} value is the concentration of sample required to scavenge 50% free radical or to prevent lipid peroxidation by 50%. These values were calculated for the *in vitro* assays and the antioxidant activity is observed for each of the banana pulp extracts and the standard. The extracts were found to have different levels of antioxidant activity in different systems tested.

Among the ethanolic extracts of the banana varieties, Monthan extract exhibits the highest potential for DPPH radical scavenging as it has the least EC_{50} value 0.9mg/ml as shown in Figure 5.1c.

Similar studies by Rungnapa Meechaona *et al.*, (2007) showed the scavenging activity of DPPH radicals by Thai bananas.

5.1.1.3. ABTS cation radical scavenging activity

ABTS assay measures the relative antioxidant ability to scavenge the radical ABTS and is an excellent tool for determining the antioxidant activity of hydrogen donating antioxidants and chain breaking antioxidants. The ABTS may be generated through an enzymatic or a chemical oxidation reaction (Miller and Rice Evens, 1997).

This method measures the relative antioxidant ability to scavenge the radical ABTS as compared with a standard ascorbic acid, and is an excellent tool for determining the antioxidant activity of hydrogen donating antioxidants and chain breaking antioxidants. This method involves the scavenging effect of secondary radicals.

Banana extract	Sample concentration (mg/ml)									
	1	2	3	4	5	6	7	8	9	10
Kadali	35.80^{C}±1.58	45.67^{C}±1.30	54.79^{C}±1.64	65.91^{C}±1.43	73.03^{C}±1.36	77.38^{b}±1.38	81.86^{b}±1.48	86.18^{bc}±1.54	89.33^{c}±1.13	93.83^{c}±1.44
Karpooravalli	28.33^{d}±1.41	36.53^{d}±1.75	41.31^{f}±1.23	50.26^{f}±1.69	56.60^{f}±1.18	61.11^{e}±1.35	67.37^{d}±0.75	72.71^{e}±2.25	80.52^{e}±1.64	87.10^{e}±1.39
Nendran	45.40^{b}±1.20	51.19^{b}±0.80	61.57^{b}±2.21	69.06^{b}±1.12	75.19^{b} ±0.99	78.33^{h}±1.02	82.55^{b}±1.1	85.27^{c}±.93	91.99^{b}±1.68	96.27^{ab}±0.79
Monthan	3.67^{f}±0.641	7.73^{f}±1.043	15.04^{h}±1.58	23.26^{h}±1.57	32.24^{h}±2.01	40.77^{g}±1.63	50.65^{f}±1.18	65.00^{f}±1.18	85.03^{d}±1.52	94.89^{bc}±1.83
Pachanadan	20.29^{e}±1.32	28.33^{e}±1.25	33.51^{g}±0.86	37.71^{g}±1.1	44.64^{g}±1.42	49.71^{f}±1.11	54.86^{e}±1.48	63.61^{f}±0.92	68.92^{f}±1.57	78.21^{f}±1.47
Poovan	28.09^{d}±1.1	35.58^{d}±1.11	44.27^{e}±1.10	57.18^{e}±0.86	63.41^{e}±1.32	68.69^{d}±0.93	84.16^{b}±0.91	87.66^{b}±1.31	90.47^{bc}±1.24	94.29^{bc}±1.53
Rasthali	36.60^{c}±1.81	45.02^{c}±1.03	52.86^{c}±0.99	61.27^{d}±1.66	68.39^{d}±1.22	77.78^{b}±1.62	83.07^{b}±1.1	87.40^{bc}1.51	92.51^{b}±0.60	96.54^{ab}±1.53
Red	50.48^{a}±1.65	60.73^{a}±0.88	73.92^{a}±1.10	78.56^{a}±1.03	82.41^{a}±1.22	86.56^{a}±1.1	90.71^{a}±0.92	94.44^{a}±0.93	96.87^{a}±0.85	**98.12^{a} ±0.21**
Robusta	30.02^{d}±1.73	44.57^{c}±0.61	47.33^{d}±1.10	55.33^{e}±1.11	64.38^{e}±1.60	71.80^{c}±1.41	78.88^{c}±2.21	81.71^{d}±4.1	85.54^{d}±1.11	90.13^{d}±0.66
Standard (µg/ml)	10	20	30	40	50	60	70	80	90	100
Ascorbic acid	17.60±1.59	24.61±1.93	34.69±2.04	42.23±1.41	46.43±0.68	51.84±1.70	57.27±1.91	67.54±2.23	77.81±1.82	88.65±2.64

Table 5.3a Scavenging effect (%) on ABTS radical by ethanolic extract of nine banana varieties

Values represent mean ± SD of 3 replicates.

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

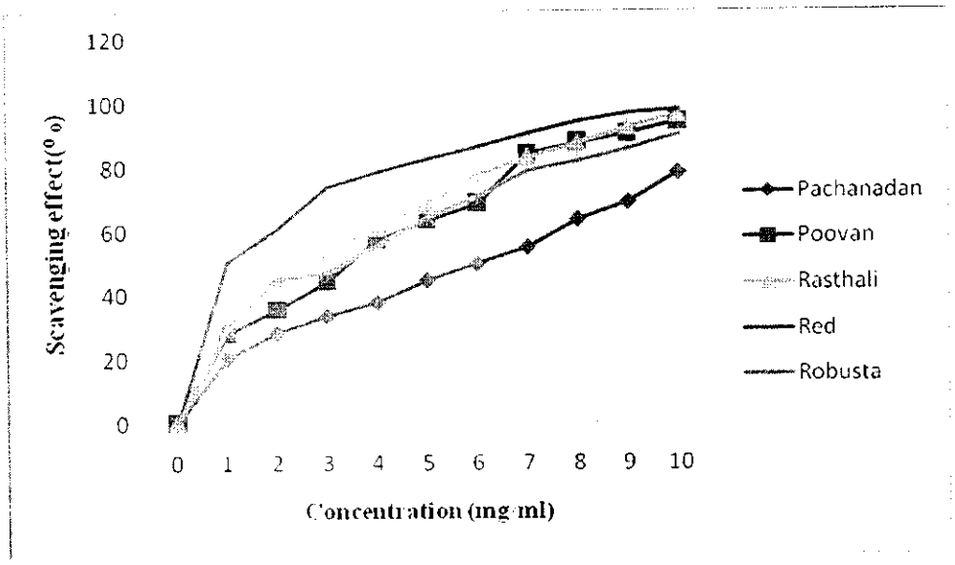


Figure 5.2a Scavenging activity (%) on ABTS radical by ethanolic extracts of *acuminate* type banana varieties.

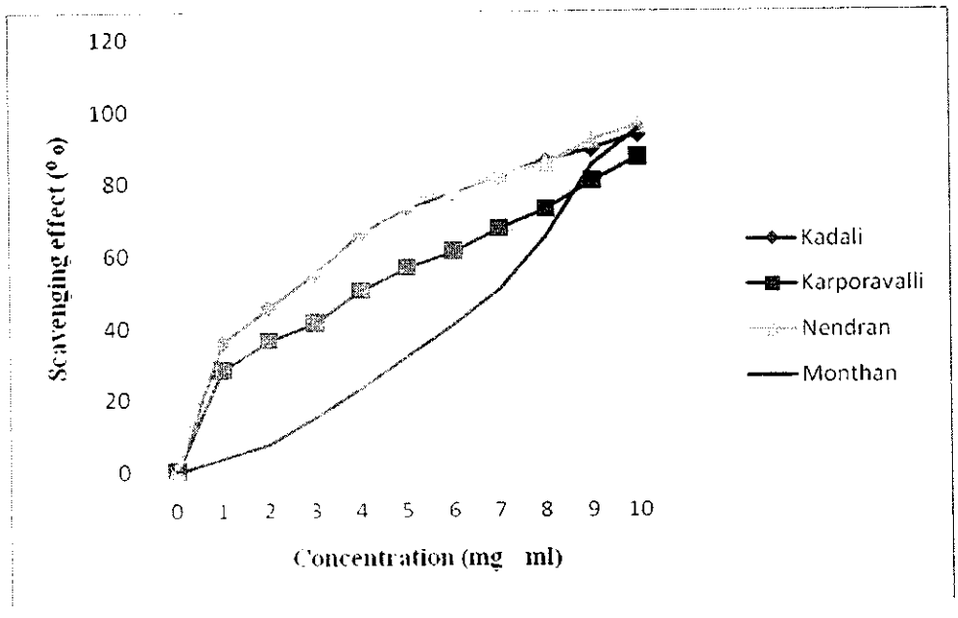


Figure 5.2b Scavenging activity (%) on ABTS radical by ethanolic extracts of *balbisiana* type banana varieties.

The percentage inhibition of the ABTS radical by the ethanolic extracts of banana varieties increases with the increase in concentration as depicted in the Table 5.3a

At 1-10mg ml^{-1}, scavenging abilities of ethanol extracts of Kadali, Karpooravalli, Nendran, Monthan, Pachanadan, Poovan, Rasthali, Red, and Robusta bananas on ABTS radicals showed inhibition of 35.80-93.83%, 28.33-87.10%, 45.40-96.27%, 3.67-94.89%, 20.29-78.21%, 28.09-94.29%, 36-60-96.64%, 50.48-98.21%, 30.02-90.13%, respectively. However, at 10mg ml^{-1}, Red banana extract exhibited highest ABTS scavenging activity and Pachanadan banana extract showed least ABTS scavenging activity. Statistically, the scavenging effect of banana pulp extracts was effective in the order: Red>Rasthali>Nendran>Monthan>Poovan>Kadali>Robusta>Karpooravalli>Pachanadan at10mg ml^{-1}.

Banana varieties	Kadali	Karpooravalli	Nendran	Monthan	Pachanadan	Poovan	Rasthali	Red	Robusta	Ascorb c acid
EC_{50} (mg/ml)	2.5	4.0	1.8	7.0	6.0	3.5	2.7	**1.0**	3.3	5.7

Table 5.3b Comparison of EC_{50} values of ABTS radical scavenging activity.

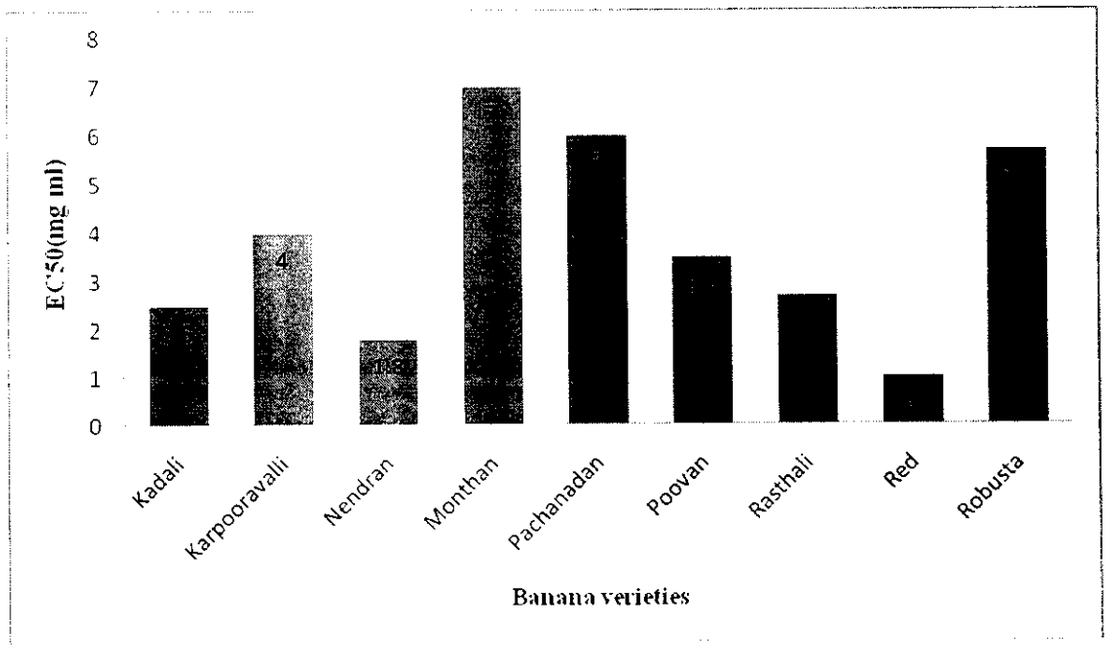


Figure 5.2c Comparison of EC_{50} values of ABTS radical scavenging activity

Among the ethanolic extracts of the nine banana varieties, Red banana extract exhibits the highest potential for ABTS radical scavenging as it has the least EC_{50} value 1.0mg/ml as shown in Figure 5.2c.

Studies by Ingrid *et al.* (2006) showed scavenging activity on ABTS radicals by different kinds of selected fruits.

5.1.1.4. Nitric oxide radical scavenging activity

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

Extent of nitric oxide radical scavenged was determined by the decrease in intensity of pink coloured chromophore in the form of EC_{50} values at 546nm. Lower EC_{50} value represents higher antioxidant activity.

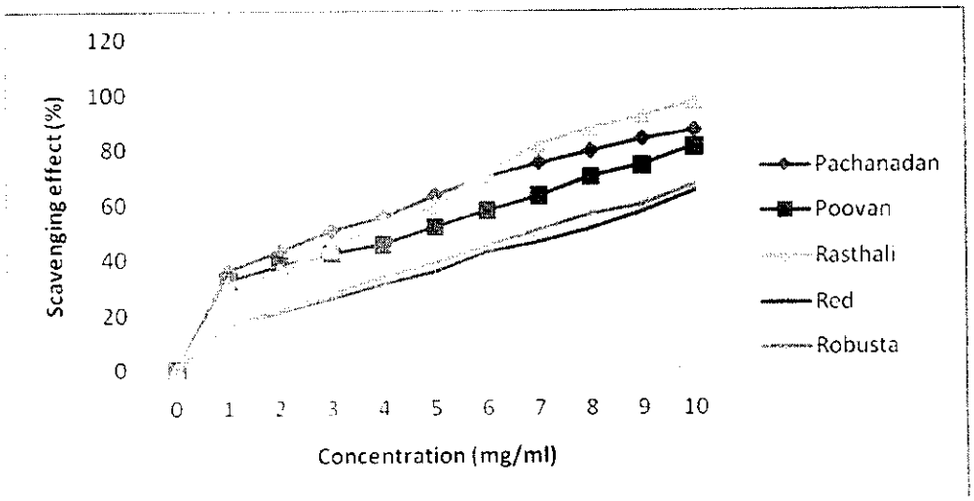


Figure 5.3a Scavenging effect (%) on nitric oxide radical by ethanolic extract of *acuminate* type banana varieties.

Banana extract	Sample concentration (mg/ml)									
	1	2	3	4	5	6	7	8	9	10
Kadali	2.55^{f}±0.25	9.68^{f}±1.79	15.57^{e}±2.51	21.79^{e}±1.92	27.66^{f}±1.74	34.68^{e}±1.53	40.8h^{h}±2.07	50.98^{g}±2.45	58.19^{f}±2.89	66.42^{e}±2.55
Karpooravalli	27.77^{c}±1.97	31.91 ^{c}±1.95	37.44^{c}±1.64	42.20^{c}±1.61	45.57^{d}±1.54	52.42^{c}±2.38	57.70^{d}±1.93	63.39^{e}±2.17	69.80^{c}±3.79	75.61^{d}±1.82
Nendran	31.36^{b}±2.83	37.55^{b}±2.46	43.71^{b}±1.96	48.63^{b}±1.67	54.00^{c} ±1.36	59.95^{b}±2.43	66.41^{c}±2.14	73.05^{c}±2.43	79.42^{c}±2.25	84.66^{b}±1.71
Monthan	20.01^{d}±2.35	25.83^{d}±2.35	34.87^{c}±1.76	41.94^{c}±1.84	50.86^{c}±4.50	58.00^{b}±2.14	63.22^{cd}±2.01	68.67^{d}±0.94	71.14 ^{de}±0.99	75.24^{d}±1.82
Pachanadan	36.00^{a}±2.60	43.04^{a}±2.05	50.46^{a}±2.03	55.53^{a}±1.68	63.33^{a}±2.53	69.98^{a}±2.58	74.95^{b}±1.12	79.16^{b}±1.57	83.60^{b}±1.44	86.63^{b}±1.84
Poovan	32.61^{b}±1.84	37.83^{b}±2.04	42.28^{b}±1.33	45.46^{b}±1.11	51.82^{c}±2.08	57.69^{b}±2.17	62.96^{d}±1.51	69.92^{cd}±1.81	74.02^{d}±1.37	80.75^{c}±1.84
Rasthali	30.65^{bc}±1.9	35.82^{b}±2.71	44.35^{b}±2.67	52.97^{a}±2.04	59.29^{b}±1.93	71.29^{a}±2.77	81.64^{a}±2.85	87.33^{a} ±1.99	91.98^{a}±1.74	**97.22^{a}±1.74**
Red	16.14^{e}±1.72	21.15^{e}±2.08	25.92^{d}±1.92	31.25^{d}±2.12	35.60^{e}±1.84	42.74^{d}±1.35	46.36^{g}±1.18	50.87^{g}±1.81	57.30^{f}±1.83	64.64^{ae}±2.8
Robusta	16.35^{e}±1.36	21.89^{e}±1.69	28.08^{d}±2.35	33.87^{d}±1.91	38.62^{e}±1.85	44.83^{d}±2.34	50.75^{f}±1.88	56.62^{f}±2.39	59.83^{f}±2.70	67.03^{e}±2.31
Standard (µg/ml)	10	20	30	40	50	60	70	80	90	100
Ascorbic acid	43.81±2.42	44.64±1.45	55.35±1.33	58.28±1.87	61±1.12	66.29±1.57	71.0±1.14	76.39±2.60	80.54±1.54	85.65±1.97

Table 5.4a Scavenging effect(%) on nitric oxide radical by ethanolic extract of nine banana varieties

Values represent mean ± SD of 3 replicates.

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

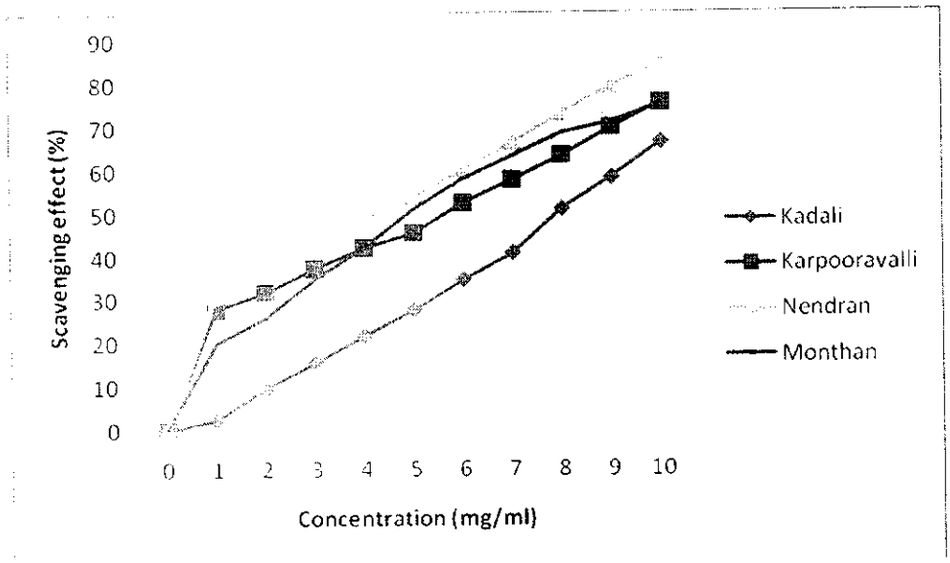


Figure 5.3b Scavenging effect (%) on nitric oxide radical by ethanolic extract of *balbisiana* type banana varieties.

Free radical potential of the ethanolic extracts of nine varieties of banana pulp is furnished in Table 5.4a which increases with the increase in concentration.

At 1-10mg ml^{-1}, scavenging abilities of ethanol extracts of Kadali, Karpooravalli, Nendran, Monthan, Pachanadan, Poovan, Rasthali, Red, and Robusta bananas on nitric oxide radicals are 2.55-66.42%, 27.77-75.61%, 31.65-85.36%, 20.01-75.24%, 36.00-86.63%, 32.61-80.75%, 30.65-97.22%, 16.22-64.64%, 16.35-67.03%, respectively. However, at 10mg ml^{-1}, Rasthali banana exhibited highest nitric oxide radical scavenging activity and Red banana exhibited the lowest scavenging activity.

Statistically, the scavenging effect of banana pulp extract antioxidants was effective in the order of Rasthali>Pachanadan>Nendran > Poovan > Karpooravalli > Monthan > Robusta> Kadali> Red at 10 mg ml^{-1}.

Banana varieties	Kadali	Karpooravalli	Nendran	Monthan	Pachanadan	Poovan	Rasthali	Red	Robusta	Ascorbic acid
EC_{50} (mg/ml)	8.0	6.0	4.2	5.0	3.0	4.5	**2.6**	7.8	6.8	2

Table 5.4b Comparison of EC_{50} values of nitric oxide radical scavenging activity.

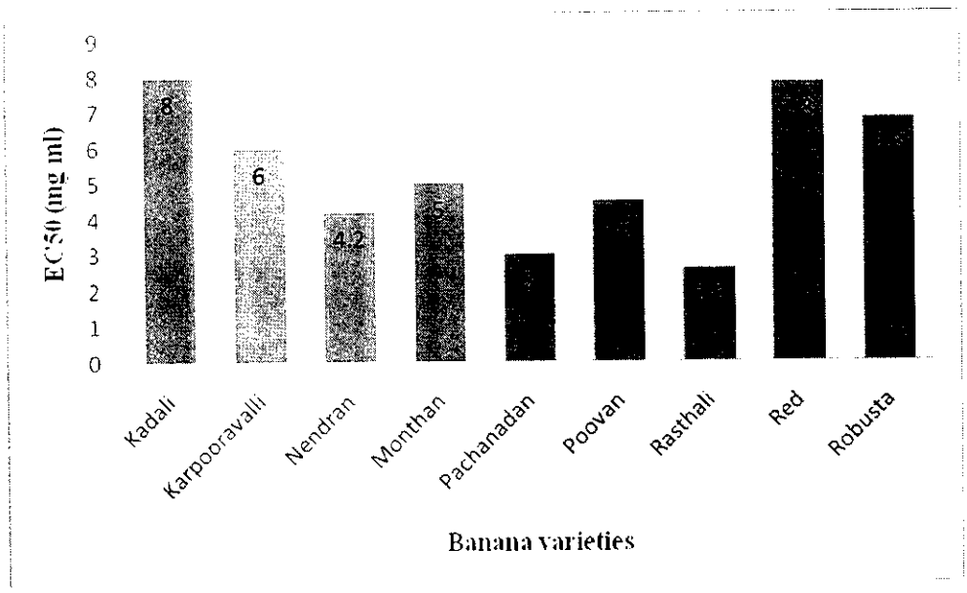


Figure 5.3c Comparison of EC_{50} values of nitric oxide radical scavenging activity.

Among the ethanolic extract of nine banana varieties, Rasthali banana exhibited a highest potential for nitric oxide scavenging activity as it has a least EC_{50} value 2.6 mg ml^{-1} as represented in Figure 5.3c.

5.1.1.5. Hydroxyl radical scavenging activity

An antioxidants ability to scavenge hydroxyl radical is an important activity. The highly reactive OH' can cause oxidative damage to DNA lipids and proteins. As is the case for many other free radicals, OH' can be neutralized if it is provided with the hydrogen atom.

The effect of pulp extract on scavenging hydroxyl radicals was measured by studying the competition between pulp extract and deoxyribose for the hydroxyl radical generated from the ferric-ascorbate-EDTA-H_2O_2 system and can inhibit deoxyribose degradation depending on its concentration and rate constant for reaction with OH '.The hydroxyl radical attacks deoxyribose and sets off series of reactions that eventually result in TBARS formation. When a molecule scavenges a hydroxyl, it decreases TBARS formation.

Banana extract	Sample concentration (mg/ml)									
	1	2	3	4	5	6	7	8	9	10
Kadali	$40.29^{d}\pm2.64$	$47.54^{d}\pm2.84$	$54.46^{d}\pm3.12$	$62.58^{d}\pm3.66$	$69.27^{de}\pm1.75$	$73.48^{d}\pm1.78$	$78.04^{d}\pm1.64$	$81.72^{c}\pm1.57$	$86.55^{c}\pm1.57$	$90.48^{b}\pm1.21$
Karpooravalli	$64.04^{a}\pm3.84$	$72.52^{a}\pm2.06$	$79.88^{a}\pm2.72$	$85.70^{a}\pm2.06$	$89.09^{a}\pm0.98$	$91.43^{a}\pm0.98$	$93.22^{a}\pm0.55$	$94.26^{a}\pm0.46$	$95.95^{a}\pm0.85$	**$98.39^{a}\pm1.31$**
Nendran	$44.19^{c}\pm1.88$	$51.37^{c}\pm2.39$	$58.71^{c}\pm2.09$	$65.41^{d}\pm1.73$	$70.74^{d}\pm1.80$	$78.41^{c}\pm2.41$	$82.87^{c}\pm1.41$	$87.13^{b}\pm1.28$	$91.55^{b}\pm1.73$	$96.55^{a}\pm1.47$
Monthan	$3.83^{f}\pm0.25$	$9.38^{f}\pm1.01$	$14.26^{f}\pm1.80$	$20.17^{f}\pm2.43$	$24.11^{h}\pm1.08$	$29.73^{g}\pm2.22$	$34.72^{g}\pm1.47$	$39.87^{e}\pm1.73$	$46.77^{e}\pm2.64$	$54.33^{d}\pm3.30$
Pachanadan	$35.51^{e}\pm2.32$	$43.52^{e}\pm2.95$	$49.43^{e}\pm1.64$	$52.91^{e}\pm1.88$	$56.91^{g}\pm1.37$	$60.84^{f}\pm1.74$	$66.61^{f}\pm2.95$	$74.09^{d}\pm1.49$	$79.71^{d}\pm2.08$	$85.89^{c}\pm2.73$
Poovan	$39.48^{d}\pm2.53$	$53.15^{c}\pm2.95$	$59.01^{c}\pm1.87$	$71.95^{c}\pm1.70$	$75.74^{c}\pm2.08$	$79.58^{c}\pm1.45$	$83.38^{c}\pm1.68$	$87.67^{b}\pm1.65$	$91.94^{b}\pm1.15$	$96.58^{a}\pm1.72$
Rasthali	$35.24^{e}\pm3.05$	$42.55^{e}\pm2.42$	$50.56^{e}\pm2.29$	$55.76^{e}\pm1.69$	$62.09^{f}\pm2.21$	$66.70^{e}\pm1.07$	$71.14^{e}\pm1.32$	$74.36^{d}\pm1.38$	$79.10^{d}\pm2.13$	$85.95^{c}\pm1.60$
Red	$50.27^{b}\pm1.90$	$61.55^{b}\pm2.45$	$70.41^{b}\pm2.11$	$76.44^{b}\pm1.43$	$81.47^{b}\pm2.30$	$86.04^{b}\pm1.29$	$89.82^{b}\pm0.97$	$92.74^{a}\pm1.42$	$96.26^{a}\pm0.61$	$97.98^{a}\pm0.97$
Robusta	$45.48^{c}\pm2.11$	$52.54^{c}\pm1.90$	$61.72^{c}\pm1.43$	$62.24^{d}\pm1.25$	$66.83^{e}\pm1.19$	$70.03^{e}\pm1.04$	$73.61^{e}\pm1.46$	$78.98^{c}\pm1.72$	$83.43^{c}\pm1.64$	$87.74^{bc}\pm1.38$
Standard (µg/ml)	10	20	30	40	50	60	70	80	90	100
Ascorbic acid	7.97±1.84	14.61±1.75	21.85±1.36	28.30±1.86	33.93±1.82	37.78±1.64	44.2±1.72	50.94±2.78	58.86±1.43	65.43±1.56

Table 5.6a Scavenging effect(%) on hydroxyl radical by ethanolic extract of nine banana varieties

Values represent mean ± SD of 3 replicates.

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

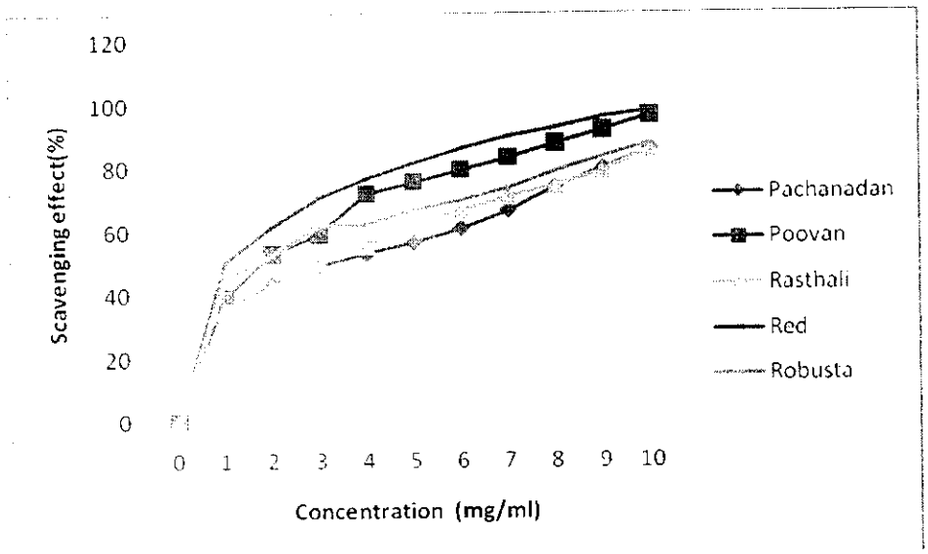


Figure 5.4a Scavenging effect (%) on hydroxyl radical by ethanolic extract of *acuminate* type banana varieties.

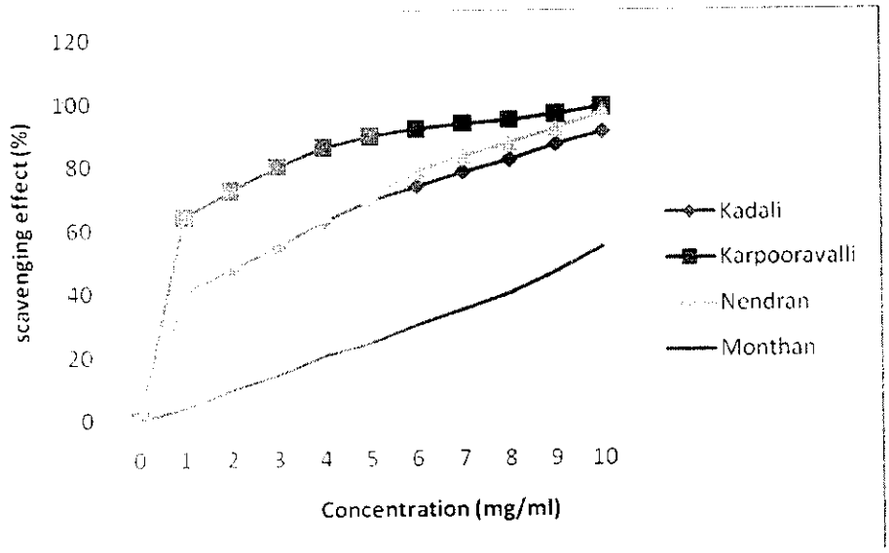


Figure 5.4b Scavenging effect (%) on hydroxyl radical by ethanolic extract of *balbisiana* type banana varieties.

At 1-10mg ml^{-1}, scavenging abilities of ethanol extracts of Kadali, Karpooravalli, Nendran, Monthan, Pachanadan, Poovan, Rasthali, Red, and Robusta bananas on hydroxyl radical 40.29 -90.48% ,64.04- 98.39%, 44.19-96.55%, 3.83-54.33%, 35.51-85.89%, 39.48 – 96.58 %, 35.24-85.95%, 50.27- 97.9%, 45.48-87.74%, respectively.

However, at 10mg ml^{-1}, Karpooravalli banana exhibited highest hydroxyl radical scavenging activity and Monthan banana exhibited least activity which have been shown in Table 5.5a

Statistically, the scavenging effect of banana pulp extract antioxidants was effective in the order Karpooravalli> Red> Poovan> Nendran> Kadali> Robusta> Rasthali> Pachanadan> Monthan at 10 mg ml^{-1}. A significant difference in hydroxy radical scavenging activity was observed with different sample concentration.

Banana varieties	Kadali	Karpooravalli	Nendran	Monthan	Pachanadan	Poovan	Rasthali	Red	Robusta	Ascorbic acid
EC_{50} (mg/ml)	2.4	**0.8**	1.8	9.5	3.0	1.9	3.0	1.0	1.7	8.0

Table 5.5b Comparison of EC_{50} values of hydroxyl radical scavenging activity.

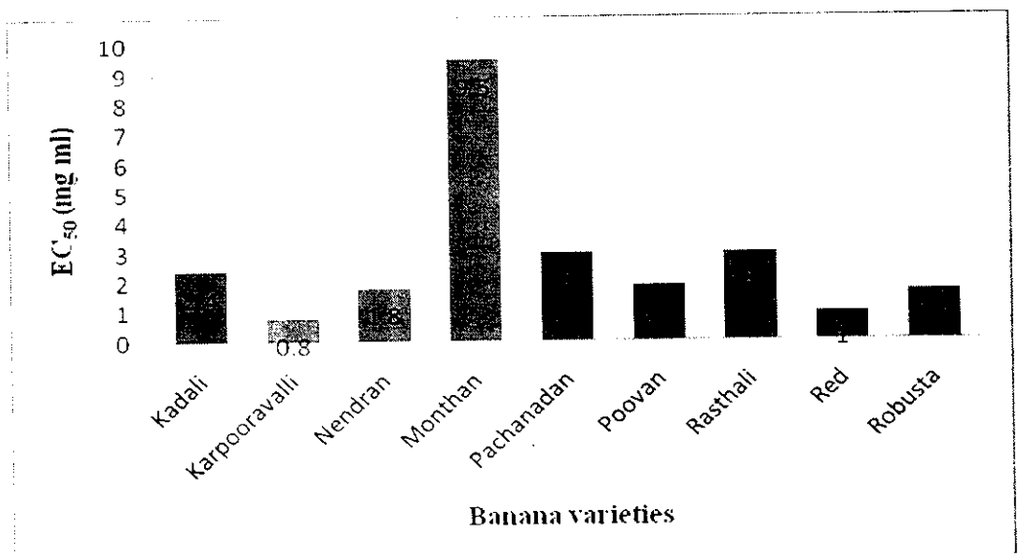


Figure 5.4c Comparison of EC_{50} values of hydroxyl radical scavenging activity.

Among the ethanolic extract of nine banana varieties Karpooravalli banana exhibit a highest potential for hydroxyl radical scavenging activity as it has a least EC_{50} value 0.8 mg ml^{-1} as depicted in Figure 5.4c

Similar studies by Perez Captole *et al.*, (2007) determined the scavenging effect on hydroxyl radicals by *Musa paradisiacal* L. extract.

5.1.6 Superoxide radical scavenging activity

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

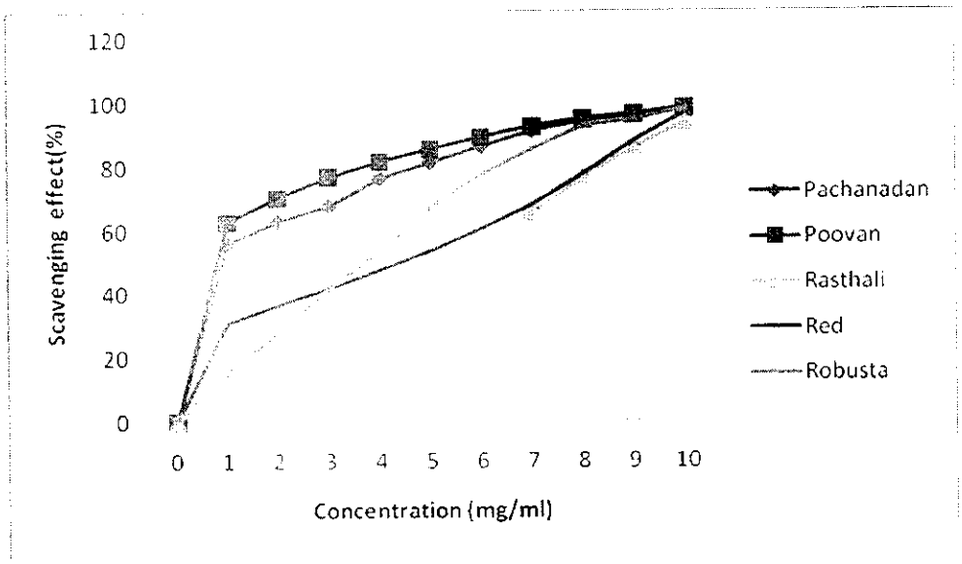


Figure 5.5a Scavenging effect (%) on super oxide radical by ethanolic extract of *acuminate* type banana varieties.

Banana extract	Sample concentration (mg/ml)									
	1	2	3	4	5	6	7	8	9	10
Kadali	33.01^{cd}±2.0	42.29^{d}±2.6	48.53^{d}±3.54	57.84^{d}±2.26	68.62^{c}±2.69	75.17^{b}±1.64	79.76^{c}±1.96	85.02^{b}±1.86	90.68^{b}±1.70	96.80^{ab}±1.96
Karpooravalli	35.02^{c}±1.66	47.84^{c}±1.68	56.93^{c}±1.24	67.94^{c}±0.90	73.93^{b}±0.99	77.81^{b}±1.18	82.48^{bc}±0.88	87.40^{b}±1.43	91.45^{b}±0.92	94.82^{ab}±1.55
Nendran	23.34^{h}±1.30	34.56^{e}±1.02	44.52^{e}±1.31	54.46^{e}±0.85	63.61^{d} ±0.76	75.22^{b}±1.21	81.86^{c}±1.01	86.39^{c}±0.99	89.43^{bc}±0.91	94.93^{ab}±1.67
Monthan	10.19^{g}±1.0	22.89^{g}±2.85	30.78^{f}±2.28	36.48^{g}±1.47	44.14^{f}±2.58	52.27^{e}±2.90	64.77^{d}±3.72	72.15^{d}±1.39	83.74^{d}±2.30	90.59^{c}±1.69
Pachanadan	55.90^{b}±1.27	62.74^{b}±0.93	67.72^{b}±1.18	76.49^{b}±0.91	81.45^{g}±1.22	86.47^{a}±0.87	91.38^{a}±0.63	94.20^{a}±0.77	96.08^{a}±0.68	**98.48^{a}±0.82**
Poovan	62.79^{a}±1.36	70.31^{a}±1.06	76.89^{a}±1.31	81.71^{a}±1.08	85.45^{c}±1.03	89.27^{a}±0.88	92.55^{a}±1.23	94.81^{a}±0.50	96.31^{a}±0.59	98.26^{a}±0.90
Rasthali	15.28^{f}±1.47	22.25^{g}±1.61	30.92^{f}±1.55	36.66^{g}±3.15	45.23^{f}±2.30	55.84^{d}±2.84	65.47^{d}±2.74	77.03^{c}±2.57	86.28^{cd}±3.22	94.06^{b}±1.88
Red	31.16^{d}±2.17	36.87^{e}±1.23	41.99^{e}±1.32	47.84^{f}±2.10	53.62^{e}±2.93	60.63^{c}±1.58	68.31^{d}±2.05	78.18^{c}±2.78	88.29^{bc}±2.90	96.98^{ab}±1.7
Robusta	15.78^{f}±1.07	28.17^{f}±1.56	42.24^{e}±1.75	53.70^{e}±0.95	67.66^{c}±1.10	77.64^{b}±1.49	85.60^{b}±1.39	92.76^{a}±1.25	94.98^{a}±0.945	98.24^{a}±0.61
Standard (µg/ml)	10	20	30	40	50	60	70	80	90	100
Ascorbic acid	35.25±1.58	42.63±2.06	51.42±1.36	55.75±1.65	62.66±2.14	69.18±1.39	75.32±1.81	82.73±1.96	86.52±1.42	95.95±2.16

Table 5.5a Scavenging effect(%) on super oxide radical by ethanolic extract of nine banana varieties

Values represent mean ± SD of 3 replicates

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

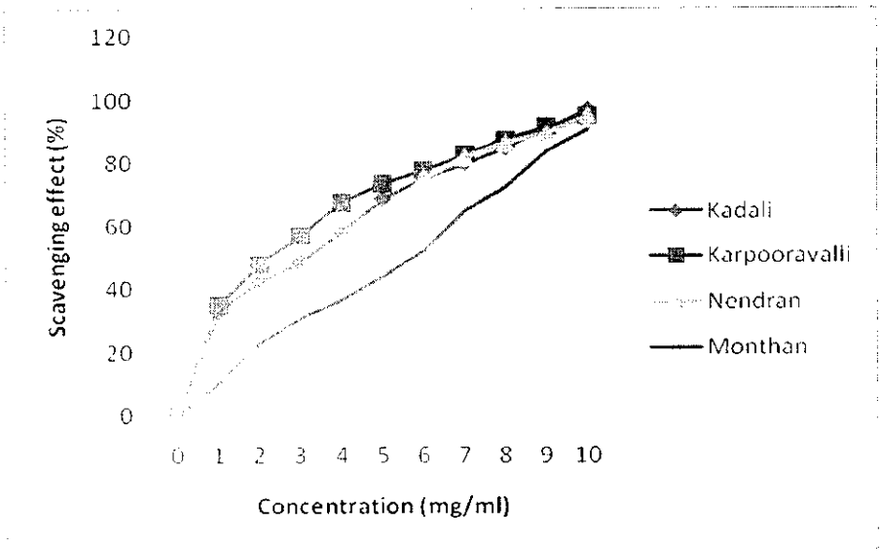


Figure 5.5b Scavenging effect (%) on super oxide radical by ethanolic extract of *balbisiana* type banana varieties.

At 1-10mg ml^{-1}, scavenging abilities of ethanol extracts of Kadali, Karpooravalli, Nendran, Monthan, Pachanadan, Poovan, Rasthali, Red, and Robusta bananas on super oxide radicals 33.01-96.80%, 36.80-94.82%, 23.34 - 94.93%, 10.19- 90.59% ,55.90-98.48%, 62.79- 98.26%, 15.28- 94.06%, 31.16- 96.98%, 15.78-98.24%, respectively.

However at 10mg ml^{-1} Pachanadan banana showed highest scavenging activity on super oxide radicals and Monthan banana showed least scavenging activity on super oxide radicals.

Statistically, the scavenging effect of antioxidants from banana pulp was effective in the order Pachanadan > Poovan > Robusta > Red > Kadali > Nendran > Karpooravalli > Rasthali > Monthan 10 mg ml^{-1}.

Banana varieties	Kadali	Karpooravalli	Nendran	Monthan	Pachanadan	Poovan	Rasthali	Red	Robusta	Ascorbic acid
EC_{50} (mg/ml)	3.2	2.3	3.6	5.9	**0.7**	0.9	5.4	4.3	3.6	3.0

Table 5.6b Comparison of EC_{50} values of super oxide radical scavenging activity.

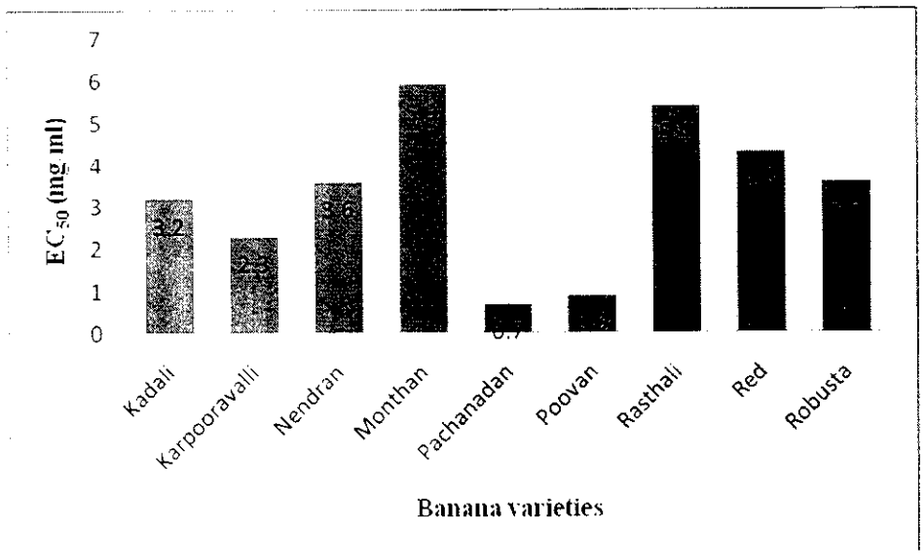


Figure 5.5c Comparison of EC_{50} values of super oxide radical scavenging activity.

Among the ethanolic extract of nine banana varieties Pachanadan banana exhibit a highest potential for superoxide radical scavenging activity as it has a least EC_{50} value 0.7 mg ml^{-1} have shown in Figure 5.5c.

Similar studies by Perez Captole *et al.*, (2007) determined the scavenging effect on super oxide radicals by *Musa paradisiacal* L. extract.

5.1.1.7 Inhibition of lipid peroxidation activity

Lipid peroxidation is the oxidative degradation of poly unsaturated fatty acids [PUFA] and involves free radicals. This is a basic membrane damage process and results in deleterious effects. Initiation of lipid peroxidation was carried out by the addition of ferrous sulphate. This occurs by the formation of hydroxy radicals by Fenton's reaction (Braughler *et al.*, 1986).These produce malondialdehyde (MDA), which reacts with thiobarbituric acid (TBA) to form a pink chromogen. This inhibition could be caused by the absorbance of ferryl-per ferryl complex or by scavenging the hydroxyl radical or the superoxide radicals or by changing the Fe^{3+} / Fe^{2+} ratio or by reducing the rate of conversion of ferrous to ferric or by chelating the iron itself.(Baskar, Rajeswari and Sathish kumar, 2007)

Banana extract	Sample concentration (mg/ml)									
	1	2	3	4	5	6	7	8	9	10
Kadali	33.01^{cd}±2.0	42.29^{d}±2.6	48.53^{d}±3.54	57.84^{d}±2.26	68.62^{c}±2.69	75.17^{b}±1.64	79.76^{c}±1.96	85.02^{b}±1.86	90.68^{b}±1.70	96.80^{ab}±1.96
Karpooravalli	35.02^{c}±1.66	47.84^{c}±1.68	56.93^{c}±1.24	67.94^{c}±0.90	73.93^{b}±0.99	77.81^{b}±1.18	82.48^{bc}±0.88	87.40^{b}±1.43	91.45^{b}±0.92	94.82^{ab}±1.55
Nendran	23.34^{b}±1.30	34.56^{e}±1.02	44.52^{e}±1.31	54.46^{e}±0.85	63.61^{d} ±0.76	75.22^{b}±1.21	81.86^{c}±1.01	86.39^{c}±0.99	89.43^{bc}±0.91	94.93^{ab}±1.67
Monthan	10.19^{g}±1.0	22.89^{g}±2.85	30.78^{f}±2.28	36.48^{g}±1.47	44.14^{f}±2.58	52.27^{e}±2.90	64.77^{d}±3.72	72.15^{d}±1.39	83.74^{d}±2.30	90.59^{c}±1.69
Pachanadan	55.90^{b}±1.27	62.74^{b}±0.93	67.72^{b}±1.18	76.49^{b}±0.91	81.45^{g}±1.22	86.47^{a}±0.87	91.38^{a}±0.63	94.20^{a}±0.77	96.08^{a}±0.68	98.48^{a}±0.82
Poovan	62.79^{a}±1.36	70.31^{a}±1.06	76.89^{a}±1.31	81.71^{a}±1.08	85.45^{c}±1.03	89.27^{a}±0.88	92.55^{a}±1.23	94.81^{a}±0.50	96.31^{a}±0.59	98.26^{a}±0.90
Rasthali	15.28^{f}±1.47	22.25^{g}±1.61	30.92^{f}±1.55	36.66^{g}±3.15	45.23^{f}±2.30	55.84^{d}±2.84	65.47^{d}±2.74	77.03^{c}±2.57	86.28^{cd}±3.22	94.06^{b}±1.88
Red	31.16^{d}±2.17	36.87^{e}±1.23	41.99^{e}±1.32	47.84^{f}±2.10	53.62^{e}±2.93	60.63^{c}±1.58	68.31^{d}±2.05	78.18^{c}±2.78	88.29^{bc}±2.90	96.98^{ab}±1.7
Robusta	15.78^{f}±1.07	28.17^{f}±1.56	42.24^{e}±1.75	53.70^{e}±0.95	67.66^{c}±1.10	77.64^{b}±1.49	85.60^{b}±1.39	92.76^{a}±1.25	94.98^{a}±0.945	98.24^{a}±0.61
Standard (µg/ml)	10	20	30	40	50	60	70	80	90	100
Ascorbic acid	35.25±1.58	42.63±2.06	51.42±1.36	55.75±1.65	62.66±2.14	69.18±1.39	75.32±1.81	82.73±1.96	86.52±1.42	95.95±2.16

Table 5.7a Lipid peroxidation inhibition (%) in goat liver homogenate by ethanolic extracts of nine varieties of banana.

Values represent mean ± SD of 3 replicates

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

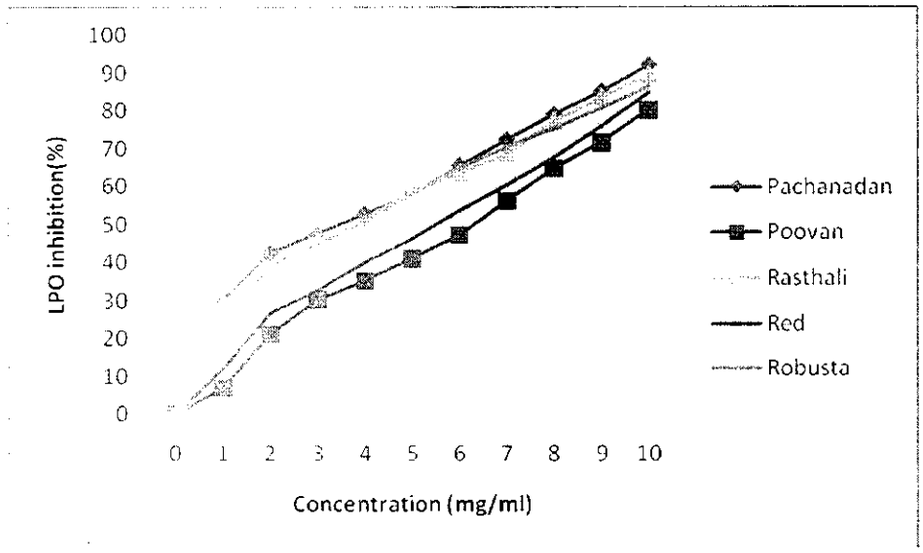


Figure 5.6a Lipid peroxidation inhibition (%) in goat liver homogenate by ethanolic extracts of *acuminate* type banana varieties.

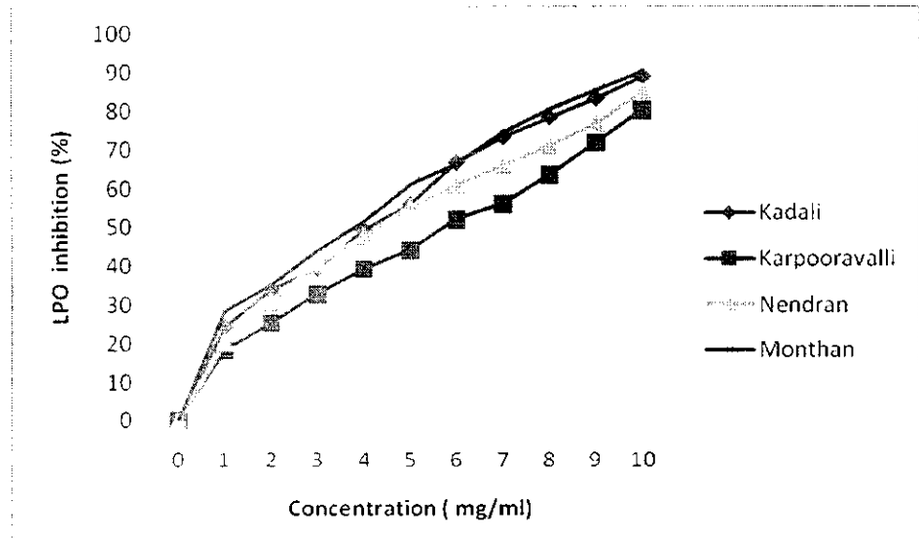


Figure 5.6b Lipid peroxidation inhibition (%) in goat liver homogenate by ethanolic extracts of *balbisiana* type banana varieties.

Free radical scavenging potential of the ethanolic extracts of local varieties of banana pulp is displayed in Table 5.7a which increases with the increase in concentration.

At 1-10mg ml^{-1}, lipid peroxidation inhibition of ethanol extracts of Kadali, Karpooravalli, Nendran, Monthan, Pachanadan, Poovan, Rasthali, Red, and Robusta bananas were 24.24 -89.03%,18.13 - 80.43%, 20.59- 85.20%, 28.24 - 90.14%,30.09 - 92.26%,6.90 - 80.23%, 29.07 -89.52%, 12.20 - 84.90%, 22.50 - 86.53%, respectively. However, at 10mg ml^{-1} Pachanadan banana exhibited highest inhibition on lipid peroxidation and Poovan banana exhibited the least inhibition activity.

Statiscally, the Lipid peroxidation inhibition of banana was effective in the order Pachanadan > monthan > Rasthali > Kadali > Robusta > Nendran > Red > Karpooravalli > Poovan at 10 mg ml^{-1}.

Banana varieties	Kadali	Karpooravalli	Nendran	Monthan	Pachanadan	Poovan	Rasthali	Red	Robusta	Ascorbic acid
EC_{50} (mg/ml)	4.0	5.9	4.3	3.8	**3.4**	6.3	4.0	5.6	3.9	7.8

Table 5.7b Comparison of EC_{50} values of lipid peroxidation inhibition activity.

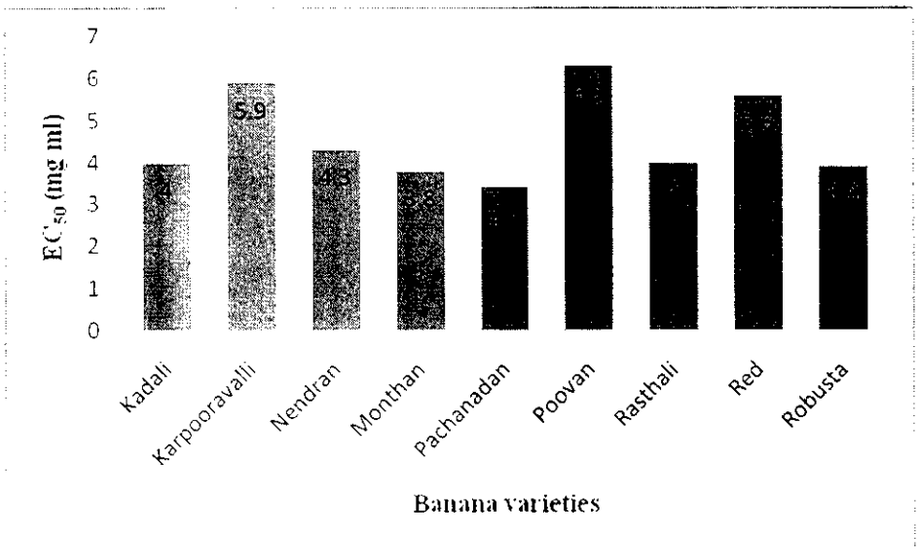


Figure 5.6c Comparison of EC_{50} values of lipid peroxidation inhibition activity.

Among the ethanolic extract of nine banana varieties Pachanadan banana exhibited a highest potential for lipid peroxidation inhibition activity as it has a least EC_{50} value 3.4 mg ml^{-1} have represented in Figure 5.7b.

5.1.1.8 Ferric Reducing Antioxidant Potential activity

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

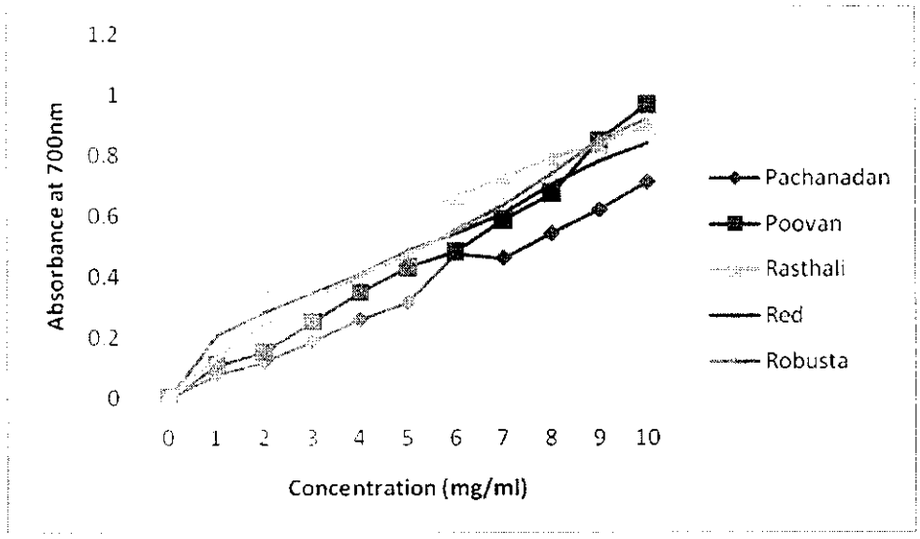


Figure 5.7a Reducing power (A_{700nm}) of ethanolic extract of *acuminate* type banana varieties.

Banana extract	Sample concentration (mg/ml)									
	1	2	3	4	5	6	7	8	9	10
Kadali	0.25^{ah}±0.01	0.437^{a}±0.01	0.531^{a}±0.02	0.658^{a}±0.03	0.756^{a}±0.02	0.929^{a}±0.03	1.05^{a}±0.04	1.13^{a}±0.01	1.18^{a}±0.01	**1.23^{a}**±0.02
Karpooravalli	0.040^{a}±0.01	0.115^{a}±0.01 5	0.186^{a}±0.01	0.303^{a}±0.03	0.449^{cd}±0.03	0.560^{c}±0.04	0.663^{c}±0.03	0.752^{e}±0.03	0.769^{e}±0.02	0.958^{d}±0.03
Nendran	0.196^{c}±0.01	0.25^{c}±0.034	0.34^{c}±0.039	0.454^{c}±0.04	0.549^{b} ±0.03	0.634^{b}±0.03	0.740^{b}±0.04	0.841^{h}±0.03	0.950^{h}±0.03	1.05^{b}±0.03
Monthan	0.17^{cd} ±0.01	0.239^{c}±0.03	0.321^{c}±0.02	0.386^{de}±0.02	0.458^{c}±0.01	0.526^{cd}±0.02	0.611^{cd}±0.01	0.694^{de}±0.01	0.759^{d}±0.01	0.916^{cd}±0.03
Pachanadan	0.07^{fg}±0.0	0.119^{d}±0.02	0.187^{c}±0.02	0.259^{g}±0.01	0.315^{d}±0.02	0.378^{d}±0.01	0.461^{e}±0.01	0.542^{f}±0.02	0.620^{e}±0.02	0.712^{f}±0.01
Poovan	0.110^{ef}±0.01	0.151^{d}±0.01	0.252^{d}±0.03	0.348^{ef}±0.02	0.431^{c}±0.01	0.483^{d}±0.02	0.588^{d}±0.02	0.675^{e}±0.01	0.848^{c}±0.01	0.970^{c}±1.83
Rasthali	0.306^{a}±0.01	0.374^{b}±0.01	0.444^{b}±0.01	0.536^{b}±0.03	0.595^{b}±0.02	0.664^{b}±0.01	0.728^{b}±0.02	0.791^{bc}±0.02	0.834^{c}±0.02	0.895^{d}±0.03
Red	0.21^{bc}±0.02	0.28^{c}±0.021	0.348^{c}±0.02	0.410^{cd}±0.01	0.489^{c}±0.01	0.546^{c}±0.01	0.607^{cd}±0.01	0.704^{de}±0.02	0.779^{d}±0.02	0.840^{e}±0.01
Robusta	0.14^{de}±0.01	0.249^{c}±0.03	0.329^{c}±0.03	0.40^{cde}±0.01	0.470^{c}±0.02	0.554^{c}±0.03	0.633^{cd}±0.03	0.737^{cd}±0.04	0.845^{cd}±0.02	0.918^{cd}±0.02
Standard (µg/ml)	10	20	30	40	50	60	70	80	90	100
Ascorbic acid	0.151±0.01	0.241±0.016	0.305±0.026	0.384±0.022	0.48±0.028	0.593±0.015	0.685±0.034	0.75 ±0.028	0.845±0.024	0.954±0.032

Table 5.8a Reducing power (A_{700nm}) 0f ethanolic extracts of nine banana varieties.

Values represent mean ± SD of 3 replicates

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

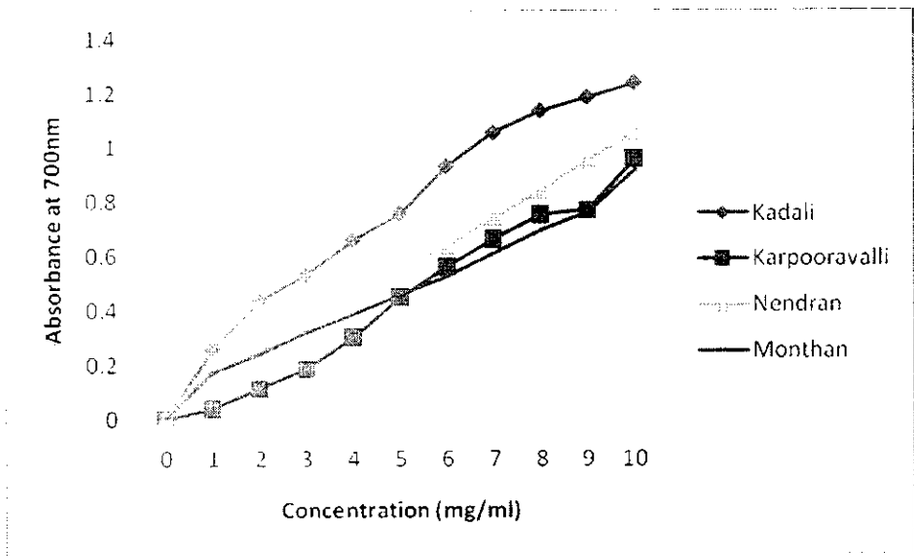


Figure 5.7b Reducing power (A_{700nm}) of ethanolic extract of *balbisiana* type banana varieties.

The reducing power of ethanolic extracts of banana varieties increases with the increase in concentration as depicted in Table 5.8. At 1-10mg ml^{-1}, reducing power of ethanol extracts of Kadali, Karpooravalli, Nendran, Monthan, Pachanadan, Poovan, Rasthali, Red, and Robusta bananas showed mean absorbance of 0.258 - 1.258, 0.040 - 0.958 , 0.196 -1.054 , 0.175 - 0.916 , 0.078- 0.712 , 0.110 - 0.970 , 0.306 - 0.895 , 0.207 - 0.840 , 0.137-0.918 respectively. At 10mg ml^{-1} Kadali banana showed highest reducing power and Pachanadan banana showed least reducing power.

5.1.1.9 β-carotene bleaching (BCB) activity

The free radical linoleic acid attacks the highly unsaturated β-carotene and the presence of different antioxidants can hinder the extent of β-carotene bleaching by neutralizing the linoleate free radical and other free radicals formed in the system.

In the β-carotene bleaching assay, linoleic acid produces hydroperoxides as free radicals during incubation at 50°C. Linoleic acid hydro peroxides attack the β-carotene molecule and as a result, it undergoes rapid decolorization. The corresponding decrease in absorbance can monitored spectrophotometrically. The presence of antioxidant extracts

can hinder the extent of β-carotene bleaching by acting on the free radicals formed in the system. (Jayaprakasha *et al.*, 2001 and Suja *et al.*, 2005).

Banana extract	**Sample concentration (mg/ml)**				
	1	2	3	4	5
Kadali	15.13^{cd}±2.6	19.72^{c}±2.02	25.44^{g}±1.65	30.71^{g}±1.94	37.16^{f}±2.70
Karpooravalli	27.94^{a}±3.07	35.33^{ab}±2.03	40.58^{ab}±2.85	48.30^{ab}±2.60	58.26^{ab}±2.93
Nendran	28.12^{a}±2.82	34.64^{abc}±3.2	37.88^{bc}±2.60	45.47^{bc}±3.28	54.53^{bc}±2.67
Monthan	14.31^{h}±2.18	20.49^{e}±2.29	28.24^{fg}±2.44	34.30^{fg}±2.58	43.71^{e}±3.57
Pachanadan	21.07^{b}±1.17	30.46^{c}±2.46	35.52^{cd}±2.37	42.78^{cd}±2.82	50.82^{cd}±2.78
Poovan	30.09^{a}±2.64	37.83^{a}±2.68	44.37^{a}±2.12	51.29^{a}±2.25	**60.25^{a}±2.59**
Rasthali	13.27^{d}±1.00	23.32^{de}±2.33	30.91^{ef}±2.36	37.72^{ef}±2.86	54.13^{bcd}±3.6
Red	19.22^{bc}±1.8	26.01^{d}±2.4	32.95^{de}±2.54	40.86^{de}±2.73	50.06^{d}±2.19
Robusta	23.11^{b}±2.86	32.56^{bc}±2.81	42.65^{a}±4.11	49.94^{a}±1.16	55.28^{bc}±1.41
Standard (µg/ml)	10	20	30	40	50
Ascorbic acid	14.65±2.22	17.63±2.11	28.03±2.59	36.06±3.16	59.40±3.79

Table 5.9a β-carotene bleaching inhibition activity (%) of ethanolic extracts of nine banana varieties.

Values represent mean ± SD of 3 replicates.

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

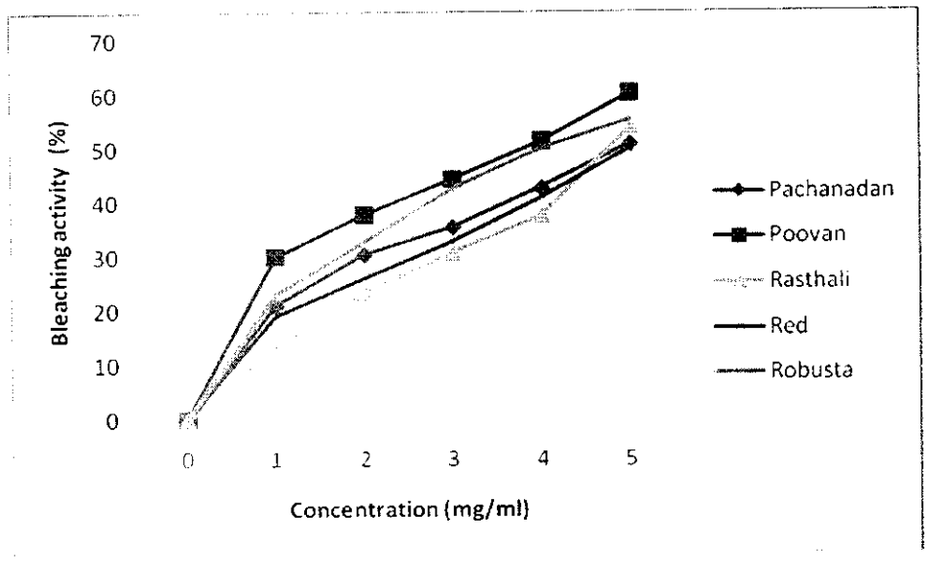


Figure 5.8a β-carotene bleaching (%) of ethanolic extracts of *acuminate* type banana varieties.

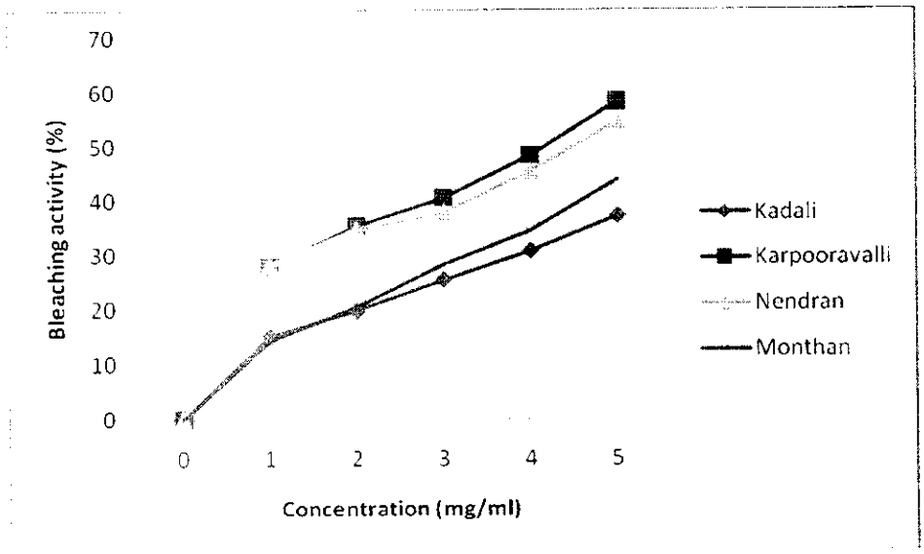


Figure 5.8b β - carotene bleaching (%) of ethanolic extracts of *balbisiana* type banana varieties.

The percentage bleaching β-carotene by the ethanolic extracts of egg yolk varieties increases with the increase in concentration as depicted in the Table 5.9a. At 1-5

mg ml^{-1}, β-carotene bleaching effect of ethanol extracts of Kadali, Karpooravalli, Nendran, Monthan, Pachanadan, Poovan, Rasthali, Red, and Robusta bananas were 15.13 – 37.16 %, 27.94 – 58.26% , 28.12 – 54.53% , 14.31 – 43.71%, 21.07 – 50.82%, 30.09 – 60.25%, 13.27 - 54.13%, 19.22 – 50.06%, 23.11 – 55.28%, respectively. However, at 5 mg ml^{-1} Poovan banana showed highest bleaching activity and Kadali banana showed least bleaching activity. Statistically, the bleaching activity of banana varieties were effective in the order of Poovan > Karpooravalli > Robusta > Nendran > Rasthali > Pachanadan > Red > Monthan > Kadali at 5 mg ml^{-1}.

Banana varieties	Kadali	Karpooravalli	Nendran	Monthan	Pachanadan	Poovan	Rasthali	Red	Robusta	Ascorbic acid
EC_{50} (mg/ml)	3.0	0.7	0.7	2.5	1.3	**0.6**	2.0	1.8	1.0	2.5

Table 5.9b Comparison of EC_{50} values of β- carotene bleaching activity.

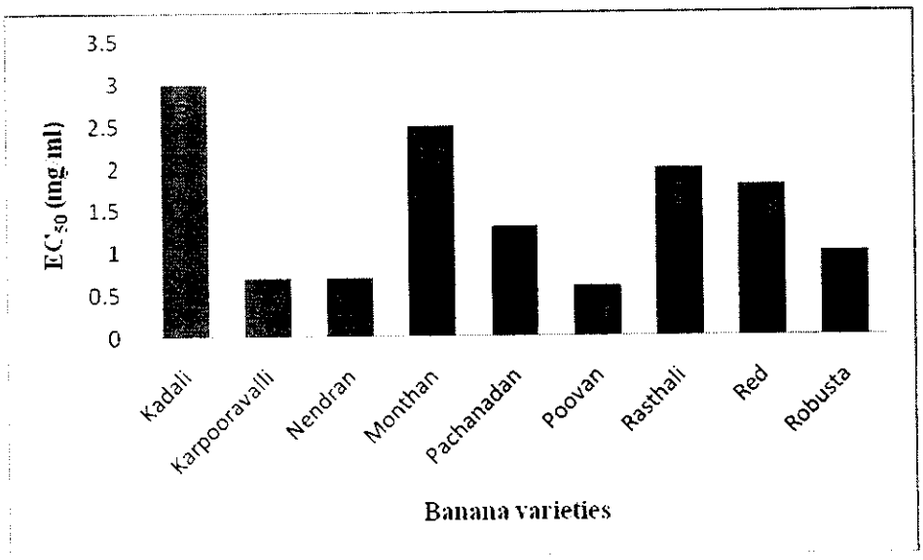


Figure 5.8c Comparison of EC_{50} values of β-carotene bleaching activity.

Among the ethanolic extract of nine banana varieties Poovan banana exhibited a highest potential for β-carotene bleaching activity as it has a least EC_{50} value 0.6 mg ml^{-1} have displayed in Figure 5.8c

5.2 PHASE II

5.2.1 Enzymatic antioxidants

5.2.1.1 Hydrogen peroxide metabolizing enzymes

Super oxide dismutase (known as SOD) is an enzyme which acts as a catalyst in the process of dismutation of superoxide into oxygen and hydrogen peroxide. It is therefore a critical antioxidant defense which is present in nearly all cells which are exposed to oxygen. SOD is a powerful enzyme and acts as a cellular antioxidant. In simple terms, SOD acts as a super-scavenger of potentially damaging free-radicals.

Catalase works closely with superoxide dismutase to prevent free radical damage to the body. SOD converts the dangerous superoxide radical to hydrogen peroxide, which catalase converts to harmless water and oxygen. Catalyses are some of the most efficient enzymes found in cells; each catalase molecule can convert millions of hydrogen peroxide molecules every second thus, preventing the formation of carbon dioxide bubbles in the blood. Catalase also uses hydrogen peroxide to break down potentially harmful toxins in the body, including alcohol, phenol, and formaldehyde.

Banana varieties	Superoxide dismutase	Catalase
Kadali	1.73 ^{c} ±0.0748	8.11 ^{b} ±0.105
Karpooravalli	0.86 ^{a} ±0.0117	12.67 ^{c} ±0.065
Nendran	1.57 ^{c} ±0.264076	13.69 ^{c} ±0.070
Monthan	3.83 ^{c} ±0.116762	6.19 ^{a} ±0.085
Pachanadan	2.05 ^{d} ±0.132035	16.59 ^{g} ±0.080
Poovan	1.38 ^{b} ±0.10535	14.59 ^{f} ±0.055
Rasthali	**4.80** ^{f} ±0.115902	12.80 ^{d} ±0.095
Red	1.61 ^{c} ±0.0680	19.74 ^{h} ±0.194
Robusta	1.94 ^{d} ±0.0240	**26.17** ^{i} ±2.217

Table 5.10 Activities of Hydrogen peroxide metabolizing enzymes in pulp extracts of local varieties of Banana

Values represent mean ± SD of 3 replicates.

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

Units: SOD - 50% inhibition of nitrate min^{-1} mg $protein^{-1}$.

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

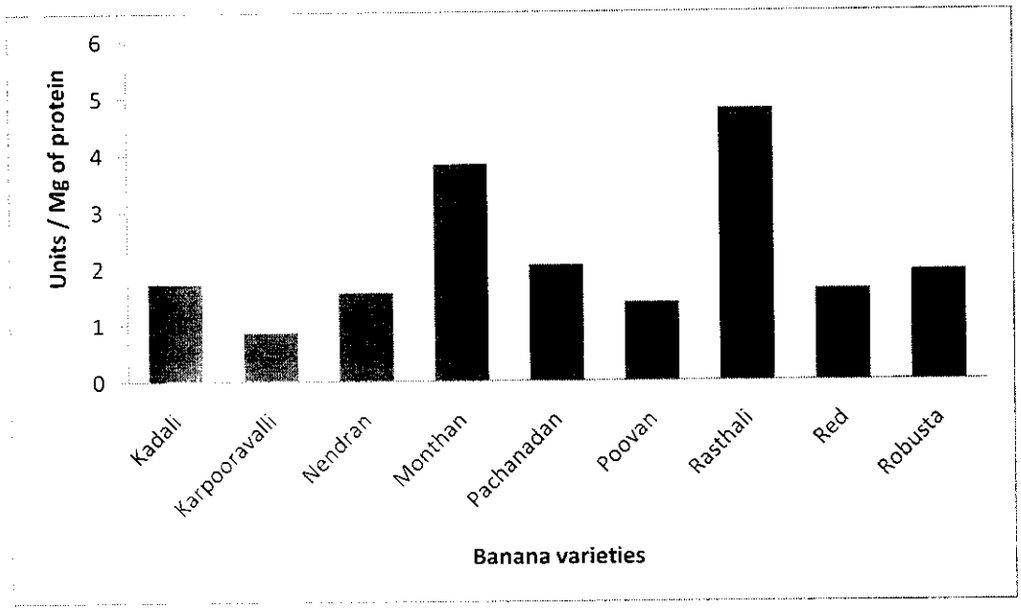


Figure 5.9 Activities of Superoxide Dismutase in pulp extracts of nine varieties of banana.

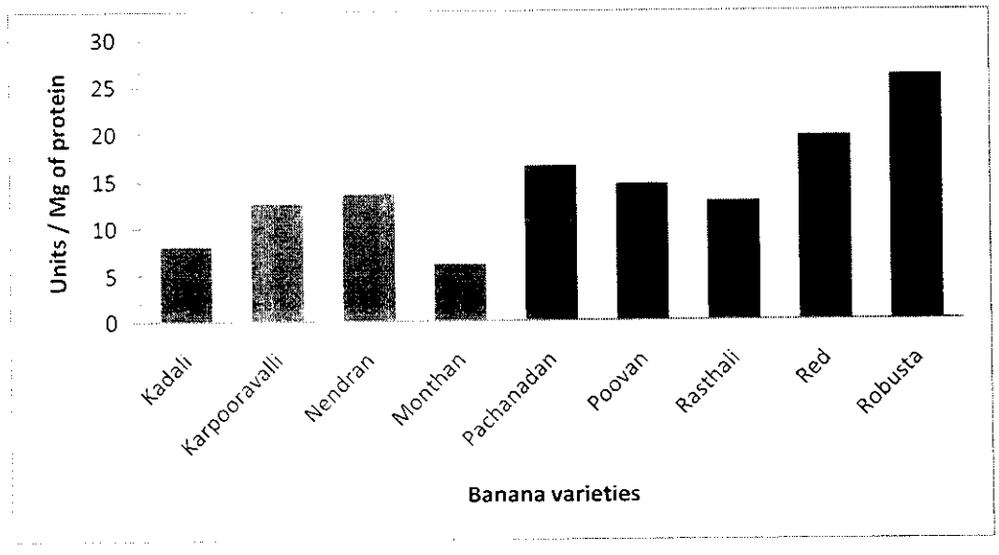


Figure 5.10 Activities of catalase in pulp extracts of nine varieties of banana.

The super oxide dismutase activity in pulp extracts of nine banana varieties varied from 0.86 - 4.80 units mg^{-1} protein as depicted in Table 5.10. The highest activity was found in Rasthali and lowest activity was found Karpooravalli banana. Therefore, Rasthali banana has the highest potential to scavenge superoxide radicals.

The activity of catalase enzyme in pulp extracts of nine varieties banana varied from 6.19- 26.17 units mg^{-1} protein as represented in Table 5.10. The highest hydrogen peroxide decomposing activity was recorded in Robusta banana and least activity was found in Kadali banana.

5.2.1.2. Activities of glutathione utilizing enzymes

Glutathione peroxidase is the general name of an enzyme family with peroxidase activity whose main biological role is to protect the organism from oxidative damage. The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water.

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

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

Banana varieties	Glutathione peroxidase	Glutathione reductase	Glutathione- S - transferase
Kadali	0.012 [a] ±0.002	**9.47** [f] ±0.113	22.16 [e] ±0.543
Karpooravalli	0.029 [b] ±0.002	5.60 [d] ±0.193	6.32 [c] ±0.190
Nendran	0.043 [c] ±0.004	2.27 [c] ±0.009	35.43 [f] ±0.292
Monthan	0.032 [b] ±0.002	2.17 [c] ±0.129	2.48 [a] ±0.405
Pachanadan	0.046 [c] ±0.003	1.74 [b] ±0.009	**50.61** [g] ±0.207
Poovan	**0.083** [e] ±0.004	1.29 [a] ±0.175	8.90 [d] ±0.166
Rasthali	0.057 [d] ±0.004	8.10 [e] ±0.159	2.58 [a] ±0.246
Red	0.044 [c] ±0.008	5.34 [d] ±0.345	8.54 [d] ±0.156
Robusta	0.055 [d] ±0.005	**9.47** [f] ±0.374	3.23 [b] ±0.156

Table 5.11 Activities of Glutathione utilizing enzymes in pulp extract of local varieties of Banana

Values represent mean ± SD of 3 replicates.

Means followed 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

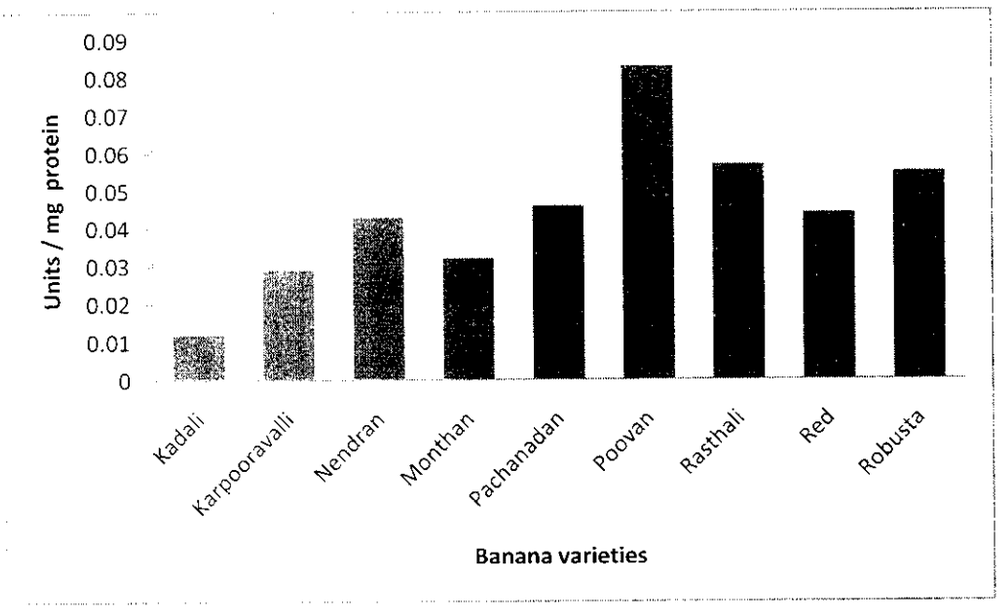


Figure 5.11 Activities of glutathione peroxidase enzyme in pulp extracts of nine varieties of banana.

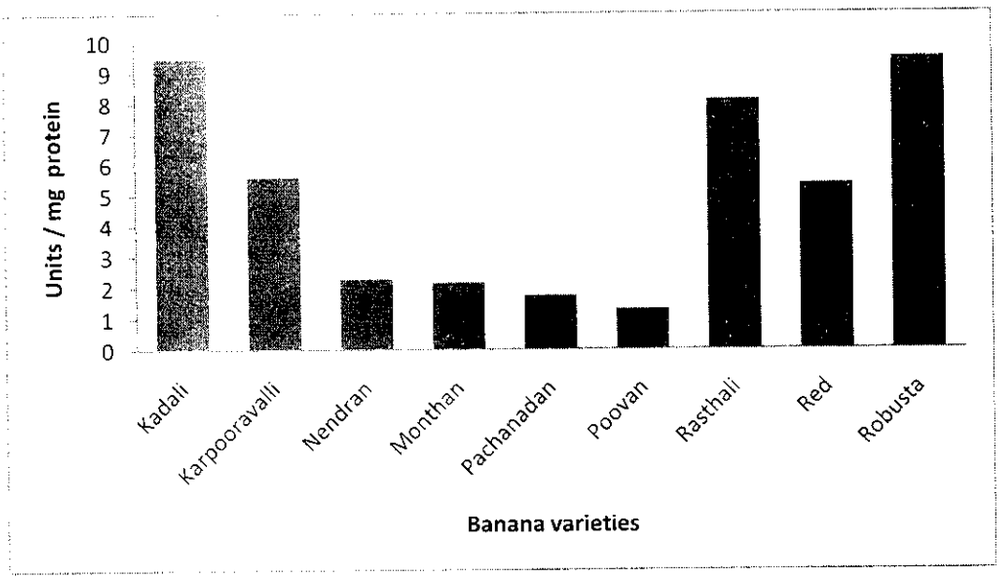


Figure 5.12 Activities of glutathione reductase enzyme in pulp extract of nine varieties of banana

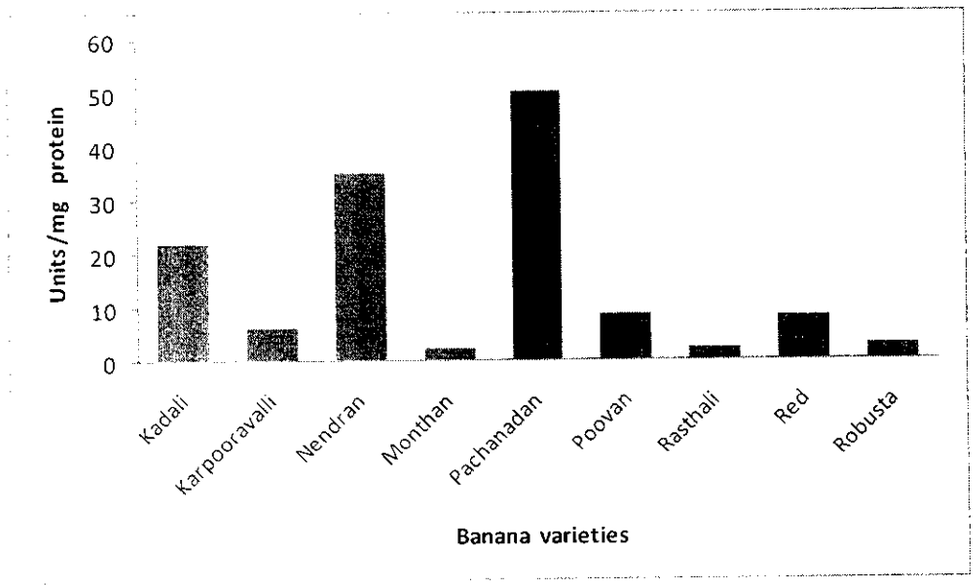


Figure 5.13 Activities of Glutathione - S - transferase enzyme in pulp extracts of nine varieties of banana.

Table 5.11 furnishes with activities of glutathione utilizing enzymes in pulp extracts of nine varieties of banana. The activity of glutathione peroxidase enzyme in pulp extracts of nine varieties of banana ranged from 0.012-0.083 units mg^{-1} protein. The highest activity was found in Poovan banana and least activity was found in Kadali banana.

The activities of glutathione reductase enzyme in pulp extracts of nine varieties of banana ranges from 1.29- 9.4 units mg^{-1} protein. The Robusta and Kadali banana varieties exhibit highest activity and lowest activity was recorded in Poovan banana.

The glutathione-S- transferase activity in pulp extracts of nine varieties of banana ranges from 2.58-50.61 units mg^{-1} protein. The highest activity was shown in Pachanadan banana and least activity was found in Rasthali banana.

5.2.1.3 Activities of glucose 6 phosphate dehydrogenase and polyphenol oxidase

G6PDH is a cytosolic enzyme in the pentose phosphate pathway, a metabolic pathway that supplies reducing energy to cells (such as erythrocytes) by maintaining the level of the co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH). The NADPH in turn maintains the level of glutathione in these cells that helps protect the red blood cells against oxidative damage.

Poly phenol oxidase is a copper- containing enzyme which oxidizes phenolic compounds to produce undesirable browning during storage and processing of fresh fruits and vegetables. 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.

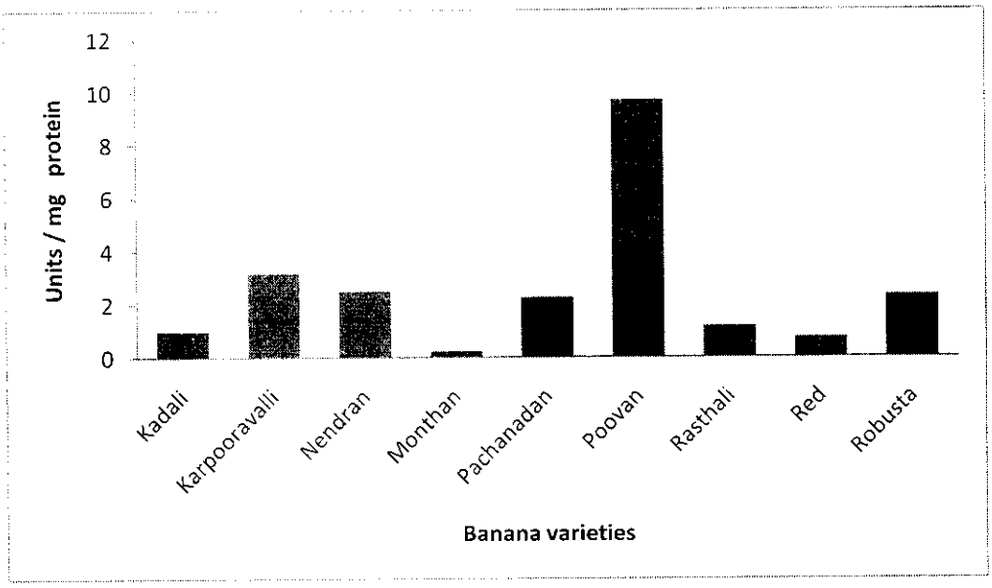


Figure 5.14 Activities of glucose 6 phosphate dehydrogenase in pulp extracts of nine varieties of banana.

Banana varieties	Glucose 6 phosphate dehydrogenase	Polyphenol oxidase
Kadali	0.99^{bc} ±0.083	0.08^{a} ±0.007
Karpooravalli	3.19^{c} ±0.180	0.09^{a} ±0.007
Nendran	2.49^{d} ±0.176	1.29^{c} ±0.1
Monthan	0.24^{a} ±0.017	**16.53^{h}** ±0.075
Pachanadan	2.26^{d} ±0.325	0.18^{b}±0.009
Poovan	**9.70^{f}** ±0.275	13.31^{f}±0.11
Rasthali	1.16^{c} ±0.529	13.93^{g} ±0.097
Red	0.75^{b} ±0.040	9.14^{d} ±0.097
Robusta	2.35^{d} ±0.105	12.84^{e} ±0.120

Table 5.12 Activities of Glucose-6-phosphate dehydrogenase and Polyphenol Oxidase in pulp extracts of nine varieties of Banana.

Values represent mean ± SD of 3 replicates.

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

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

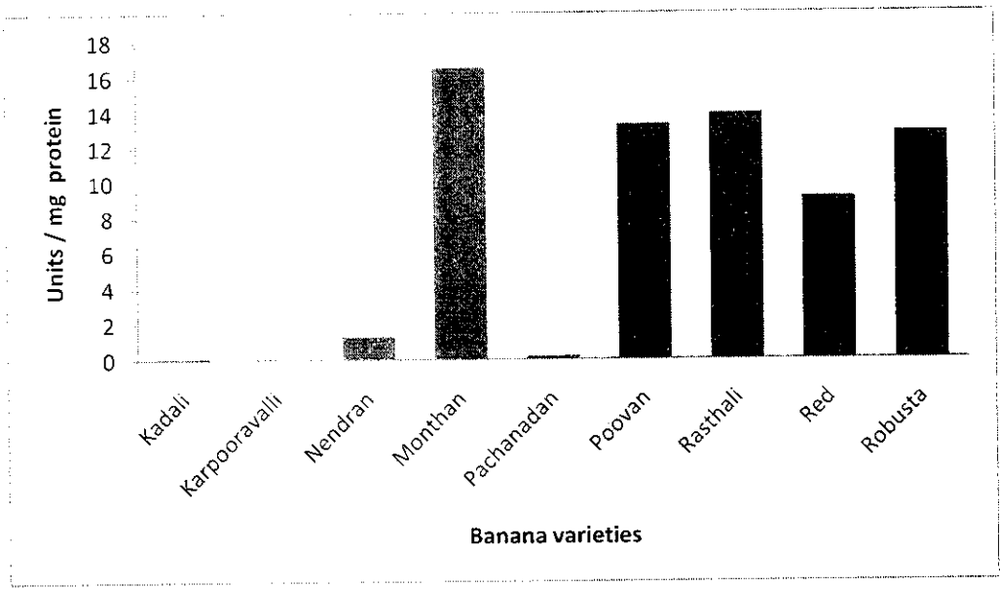


Figure 5.15 Activities of polyphenol oxidase in pulp extracts of nine varieties of banana.

The activities of glucose 6 phosphate dehydrogenase and polyphenol oxidase activities in pulp extracts of nine varieties of banana is represented in Table 5.12. The glucose 6 phosphate dehydrogenase activities in pulp extracts of nine banana varieties ranged from 0.75 – 9.70 units mg^{-1} protein. The highest activity was recorded in Poovan banana. Hence, it helps in efficient regeneration of reduced glutathione by maintaining NADPH levels. Reduced glutathione in turn forms conjugate with free radicals thereby combating several diseases and least activity was seen in Red banana.

The polyphenol oxidase activity in pulp extracts of nine banana varieties varied from 0.08–16.53 units mg^{-1} protein. Monthan banana topped among other varieties of banana and least activity recorded in Kadali banana.

5.3 PHASE III

5.3.1 Non enzymatic antioxidants

5.3.1.1 Total reduced Glutathione and Vitamin A

Glutathione (GSH) is a tripeptide. It contains an unusual peptide linkage between the amine group of cysteine and the carboxyl group of the glutamate side chain.

Glutathione, an antioxidant, helps protect cells from reactive oxygen species such as free radicals and peroxides. High levels of glutathione appear to protect against the dangers of cancer, heart disease, premature aging, autoimmune diseases, damage from many pharmaceutical drugs, and chronic illnesses.

Vitamin A or retinol is a polyisoprenoid compound containing a cyclohexanyl ring. It is stored mainly as retinol esters in liver. It plays a role in trapping peroxy free radicals in tissues at low partial pressure of oxygen. It also involved in the regulation of lipid peroxidation in plasma.

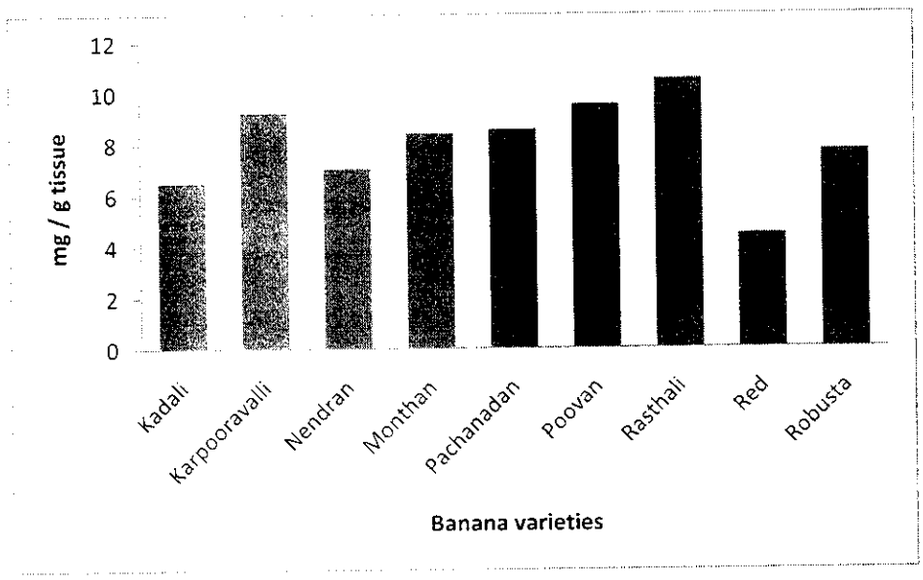


Figure 5.16 Total reduced glutathione content in pulp extracts of nine varieties of banana.

Banana varieties	Total reduced glutathione	Vitamin A
Kadali	6.53^{b} ±0.345	0.705^{f} ±0.006
Karpooravalli	9.26^{f} ±0.172	0.135 ^{c} ±0.005
Nendran	7.06 ^{c} ±0.165	0.069 ^{b}±0.006
Monthan	8.42 ^{e} ±0.225	0.050 ^{a} ±0.006
Pachanadan	8.57 ^{e} ±0.116	0.075 ^{b} ±0.008
Poovan	9.56^{f} ±0.23	0.448^{e} ±0.007
Rasthali	**10.55**^{g} ±0.346	**0.835** ^{h} ±0.005
Red	4.48^{a} ±0.401	0.348 ^{d} ±0.009
Robusta	7.74^{d} ±0.23	0.782 ^{g} ±0.003

Table 5.13 Total reduced glutathione and vitamin A content in pulp extracts of nine varieties of banana.

Values represent mean ± SD of 3 replicates.

Means followed by a common letter are not significantly different at the 5% Level by DMRT.

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

Vitamin A - mg Vitamin A acetate equivalent g^{-1} fresh tissue.

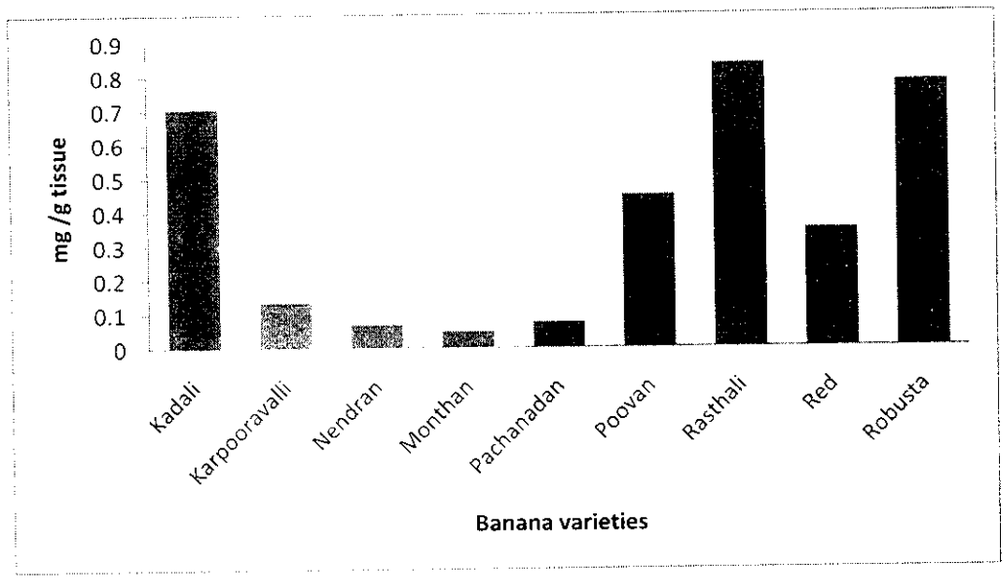


Figure 5.17 Vitamin A content in pulp extracts of nine varieties of banana.

Total reduced glutathione and vitamin A content in pulp extracts of nine banana varieties is depicted in Table 5.3.1.1. The total reduced glutathione content in banana pulp extracts ranged between 4.48 - 10.55 mg g^{-1} fresh tissues. Rasthali banana exhibit highest content of total reduced glutathione among other varieties. On the other hand Red banana showed least activity. The vitamin A levels varied from 0.050 – 0.835 mg g^{-1} fresh tissue. The highest vitamin A content recorded in Rasthali banana compared to other varieties and least amount recorded in Monthan banana.

5.3.1.2 Vitamin C and Vitamin E

Vitamin C or ascorbic acid one of the most important vitamin that is supplied by fruits and vegetables. It is a major soluble antioxidant within the body (Sies *et al.*, 1995). It lowers the blood pressure and cholesterol levels.

Vitamin E is a fat-soluble vitamin and one of a number of nutrients called antioxidants. Other well-known antioxidants are vitamin C and beta-carotene. Antioxidants are nutrients that block some of the damage caused by toxic by-products released when the body transforms food into energy or fights off infection.

Banana varieties	Vitamin C	Vitamin E
Kadali	3.27 ^{d} ±0.230	10.11 ^{e} ±0.110
Karpooravalli	4.03 ^{f} ±0.016	**13.73** ^{h} ±0.172
Nendran	4.29 ^{g} ±0.068	8.09 ^{c} ±0.160
Monthan	1.67 ^{c} ±0.052	11.84 ^{f} ±0.142
Pachanadan	3.79 ^{c} ±0.137	12.99 ^{g} ±0.344
Poovan	**4.69** ^{h} ±0.085	7.83 ^{b} ±0.170
Rasthali	0.88 ^{b} ±0.013	6.84 ^{a} ±0.104
Red	4.26 ^{g} ±0.070	10.18 ^{e} ±0.165
Robusta	0.71 ^{a} ±0.007	8.79 ^{d} ±0.152

Table 5.14 Vitamin C and Vitamin E contents in pulp extracts of nine varieties of banana.

Values represent mean ± SD of 3 replicates.

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

Units: Ascorbic acid – mg g^{-1} fresh tissue.

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

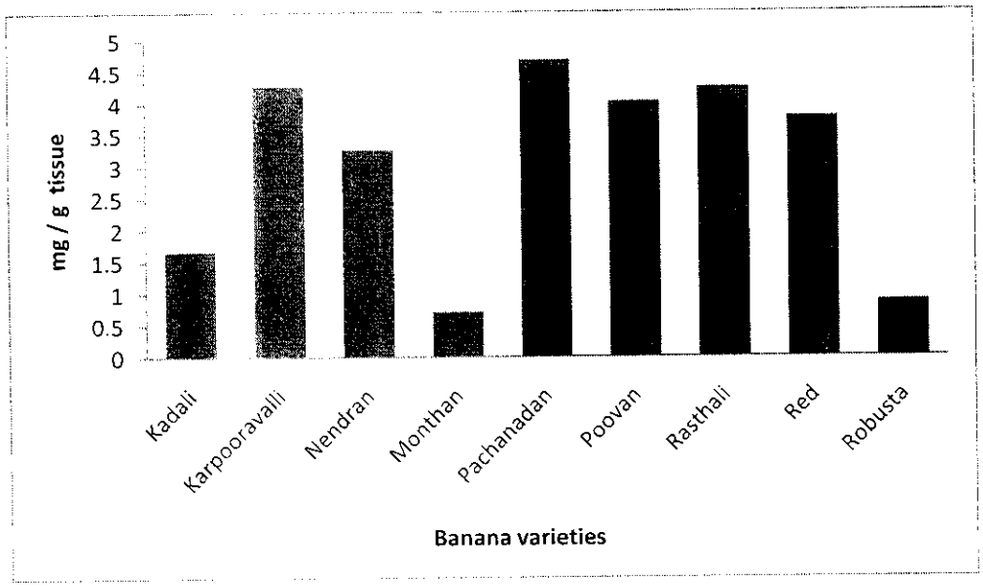


Figure 5.18 Vitamin C content in pulp extracts of nine varieties of banana.

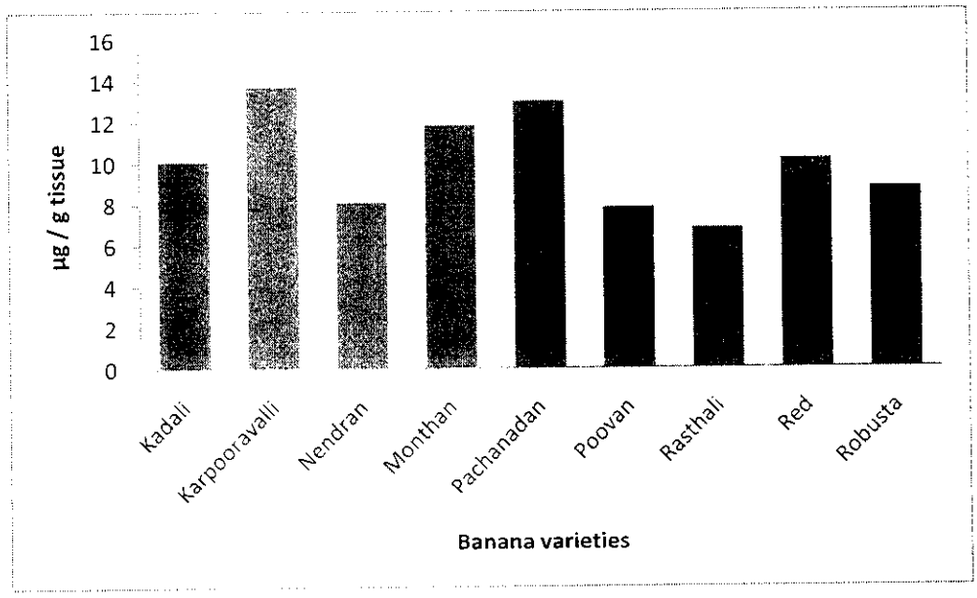


Figure 5.19 Vitamin E content in pulp extracts of nine varieties of banana.

The vitamin C and vitamin E content in pulp extracts of nine varieties of banana is furnished in Table 5.14. The vitamin C content from pulp extract of nine varieties of banana varied from 0.71-4.69 mg g^{-1} fresh tissue. The highest Vitamin C content was present in Poovan banana and on the other hand least content was found in Robusta.

The Vitamin E content in pulp extracts of nine varieties of banana ranged from 6.84-13.73µgg^{-1} fresh tissue. The highest Vitamin E content was recorded in Karpooravalli banana and least activity was found in Rasthali banana.

5.4 PHASE IV

5.4.1 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 properties of flavonoids. The number of flavonoid derivatives is more than 4000 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.

Banana varieties	Total phenols	Flavonoids	Alkaloids
Kadali	**1.34^{i} ±0.007**	**5.65^{e}±0.364**	0.029^{a} ±0.006
Karpooravalli	1.06 ^{h} ±0.005	0.86 ^{a}±0.018	0.081 ^{f} ±0.006
Nendran	0.26 ^{c} ±0.008	2.67 ^{c}±0.102	0.068 ^{dc} ±0.007
Monthan	0.06 ^{b} ±0.005	3.83 ^{d} ±0.077	0.038 ^{ab} ±0.007
Pachanadan	0.65 ^{f} ±0.006	3.52 ^{d} ±0.088	0.063 ^{cd} ±0.005
Poovan	0.22 ^{d}±0.010	8.59 ^{f}±0.018	**0.084 ^{f}±0.005**
Rasthali	1.02 ^{g}±0.006	1.61 ^{b} ±0.014	0.076 ^{ef} ±0.008
Red	0.08 ^{c} ±0.005	0.85 ^{a}±0.013	0.044 ^{b}±0.006
Robusta	0.04^{a} ±0.002	1.77^{b} ±0.018	0.056^{c} ±0.006

Table 5.15 Total Phenolic, Flavonoid and Alkaloid contents in pulp extracts of nine varieties of banana varieties

Values represent mean ± SD of 3 replicates.

Means followed 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.

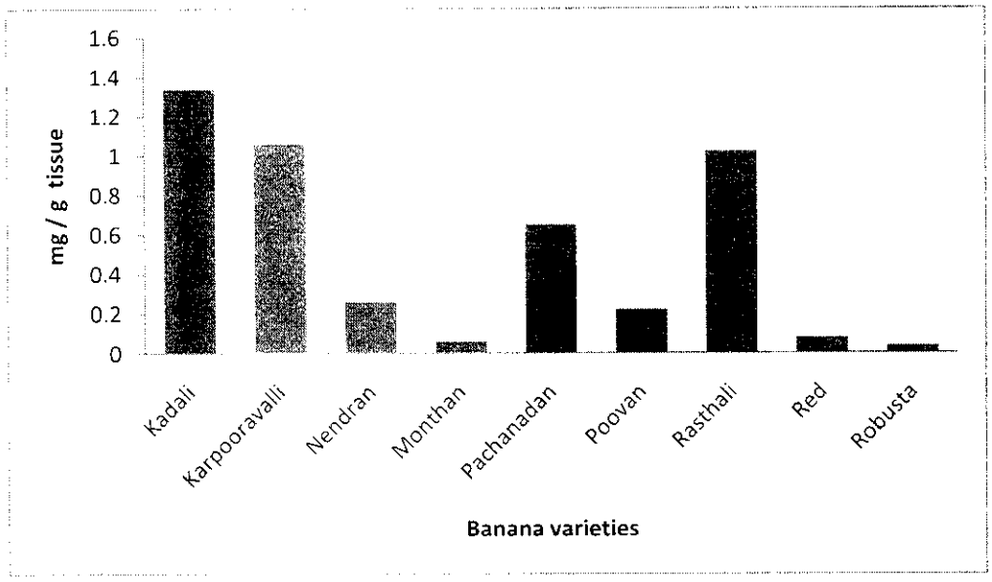


Figure 5.20 Total phenol content in pulp extracts of nine varieties of banana.

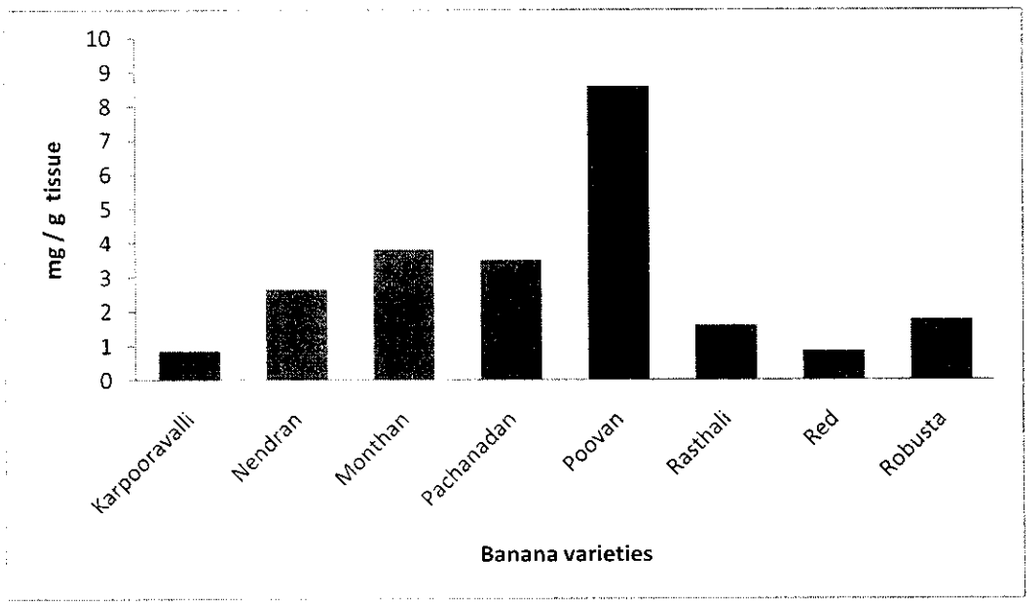


Figure 5.21 Total flavonoid content in pulp extracts of nine varieties of banana.

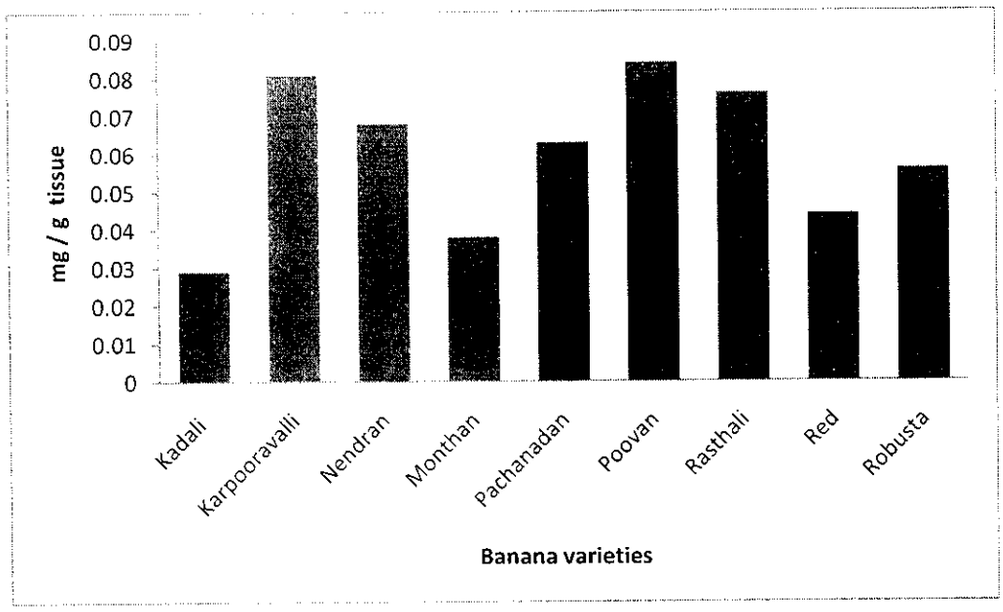


Figure 5.22 Alkaloid content in pulp extracts of nine varieties of banana.

The phytochemicals like total phenols, flavonoids and alkaloids contents were depicted in Table 5.15. The total phenol content among the nine varieties of banana varied from 0.04-1.34 mgg^{-1} fresh tissue. The highest amount of total phenol was recorded in Kadali banana least content was found in Robusta banana.

The flavonoid content from pulp extracts of nine varieties of banana ranged from 0.85–5.65 mg g^{-1} fresh tissue. The highest flavonoid content was found in Kadali and least content was found in Red banana. The alkaloid contents varied from 0.029 – 0.084 mg g^{-1} fresh tissue. Among the nine varieties of banana extracts, Poovan banana showed highest alkaloid content and Kadali banana exhibit least content.

Carotenoids 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.

Banana varieties	Total Carotenoids	Lycopene
Kadali	3.40[a] ±0.070	5.00[a] ±0.152
Karpooravalli	3.71 [b] ±0.070	2.00 [a] ±0.251
Nendran	**30.79 [i] ±0.090**	33.00 [c] ±2
Monthan	5.55[c] ±0.05	23.00 [bc] ±2
Pachanadan	16.07 [g] ±0.101	13 .00[ab] ±2
Poovan	20.09 [h] ±0.2	**67.00 [d] ±2.51**
Rasthali	14.56 [f] ±0.122	21.00 [b] ±2.51
Red	12.00 [e] ±0.2	23.00 [bc] ±2.51
Robusta	8.58[d] ±0.070	3.00[a] ±0.251

Table 5.16 Total Carotenoids and Lycopene contents in pulp extracts of nine varieties of Banana

Values represent mean ± SD of 3 replicates.

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

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

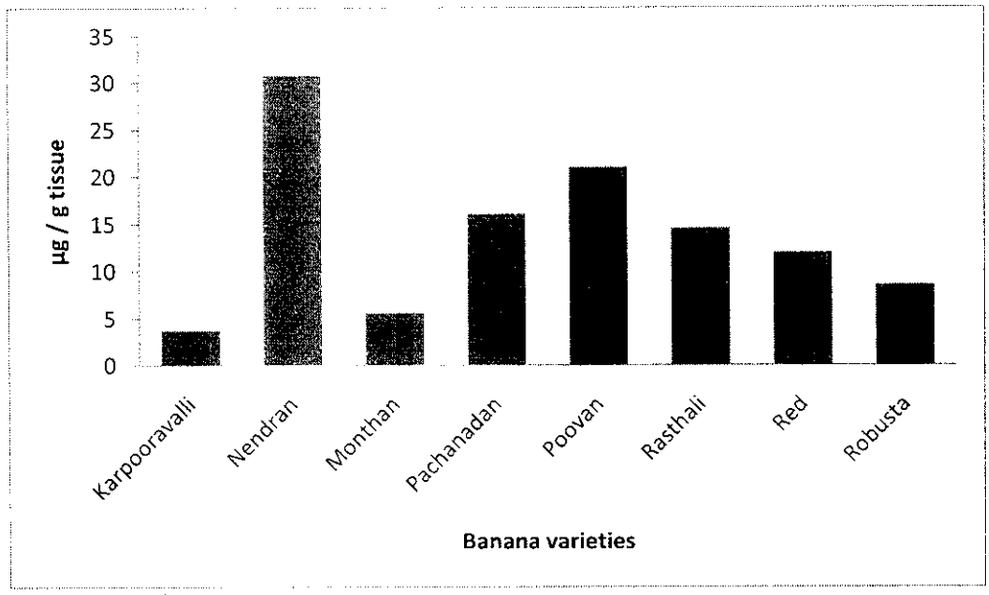


Figure 5.23 Total carotenoid contents in pulp extracts of nine varieties of banana.

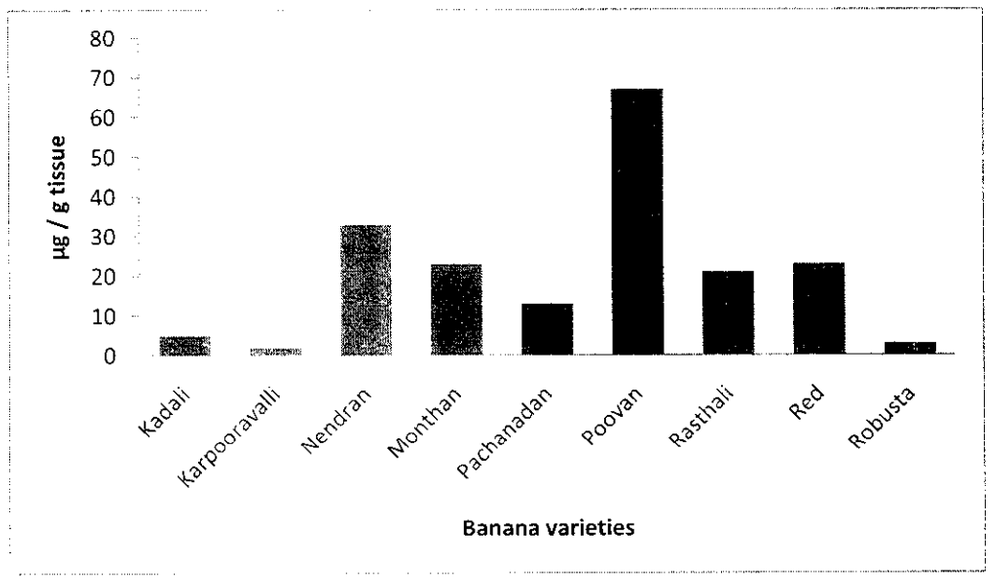


Figure 5.24 Lycopene content in pulp extracts of nine varieties of banana

The content of total carotenoids and lycopene present in banana pulp extracts were depicted in Table 5.16. Total carotenoid and lycopene contents varied in the range of 3.40- 30.79 and 2.0 - 67 μg g^{-1} fresh tissue respectively. Among all the nine varieties of banana highest total carotenoid and lycopene contents were recorded in Poovan and Nendran bananas respectively and least amount was recorded in Kadali and Karpooravalli respectively.

Tannins are polyphenolics that make cranberries and pomegranates bitter. Tannins along with Vitamin C, helps to build and strengthen collagen. Tannins prevent urinary tract infection by preventing bacteria from adhering to the walls. Mainly sugars and starches together constituting one of the three principal types of nutrients used as energy sources(calories) by the body. Carbohydrates can also be defined chemically as neutral compounds of carbon, hydrogen and oxygen.

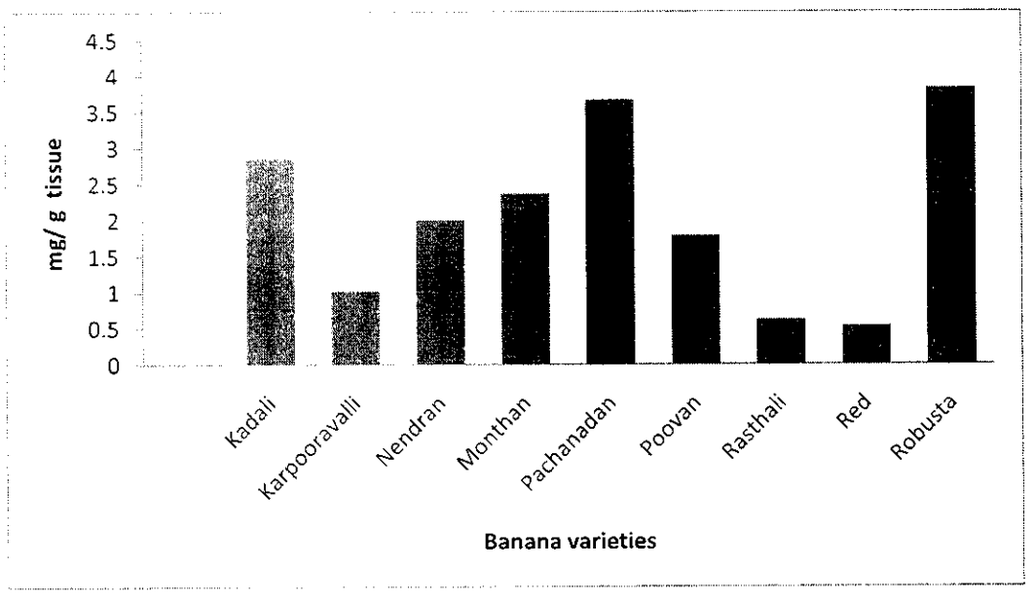


Figure 5.25 Tannin content in pulp extracts of nine varieties of banana

Banana varieties	Tannins	Total sugars
Kadali	2.85^{g} ±0.040	1.02^{c}±0.009
Karpooravalli	1.02^{c} ±0.070	1.34^{f} ±0.065
Nendran	2.00^{e} ±0.076	1.06^{d} ±0.015
Monthan	2.37^{f} ±0.051	**1.43**^{g} ±0.058
Pachanadan	3.67^{h} ±0.085	1.23^{e} ±0.011
Poovan	1.79^{d} ±0.070	0.95^{b} ±0.011
Rasthali	0.62^{b}±0.050	1.34^{f}±0.015
Red	0.53^{a} ±0.003	1.43^{g} ±0.012
Robusta	**3.83**^{i} ±0.080	0.74^{a} ±0.011

Table 5.17 Tannins and total sugars content in pulp extract of nine varieties of banana

Values represent mean ± SD of 3 replicates.

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

Units: Tannins – mg g^{-1} fresh tissue.

Total sugars – mg g^{-1} fresh tissue.

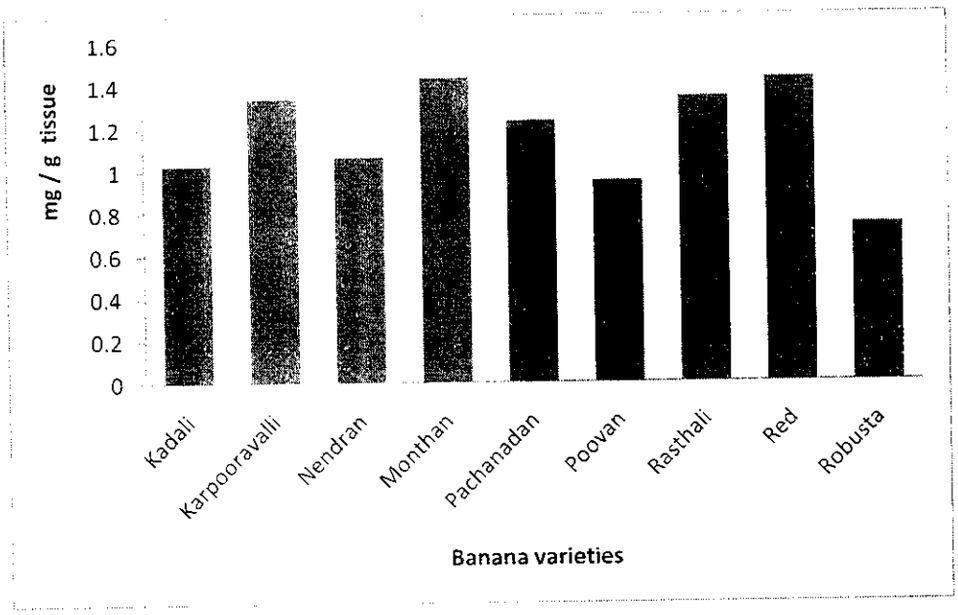


Figure 5.26 Total sugar content in pulp extracts of nine varieties of banana

Tannin and total sugar contents in pulp extracts of nine varieties of banana were depicted in Table 5.4.1.3. The quantities of the tannin and total sugars were varied between 0.53-3.83 and 0.741-1.436 mg g^{-1}fresh tissue. Among the nine varieties of banana, Robusta showed highest tannin content and least was found in Red banana. The highest amount of total sugar exhibited by Monthan banana and least content exhibited by Robusta banana.

5.5 PHASE V

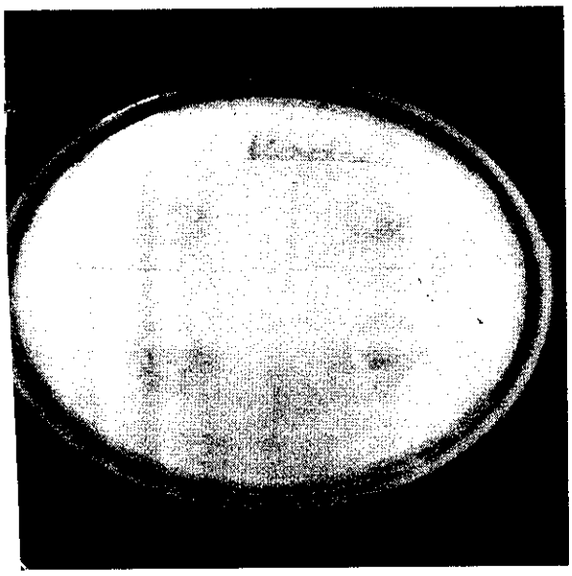
5.5.1 Antimicrobial study on the banana pulp extract

The well diffusion test was performed using Nutrient agar medium, as per the procedure described by (Magaldi *et al.*, 2004) in order to study the antimicrobial properties in the nine varieties of banana peel extracts. The following are the microbial strains used for the tests: *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeroginosa.*

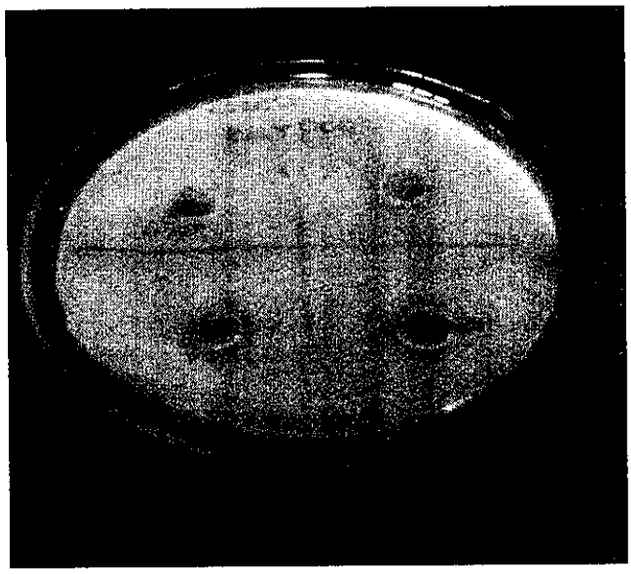
Banana extracts	***E.coli***	***Staphylococcus aureus***	***Pseudomonas aeroginosa***	***Bacillus subtilis***
Kadali	12mm	No inhibition	No inhibition	29mm
Karpooravalli	20mm	No inhibition	No inhibition	12mm
Nendran	19mm	No inhibition	13mm	28mm
Monthan	No inhibition	No inhibition	No inhibition	16mm
Pachanadan	No inhibition	No inhibition	No inhibition	30mm
Poovan	No inhibition	No inhibition	No inhibition	No inhibition
Rasthali	12mm	No inhibition	No inhibition	15mm
Red	No inhibition	No inhibition	No inhibition	10mm
Robusta	9mm	No inhibition	No inhibition	No inhibition

Table 5.18 Antimicrobial study on the banana pulp extract

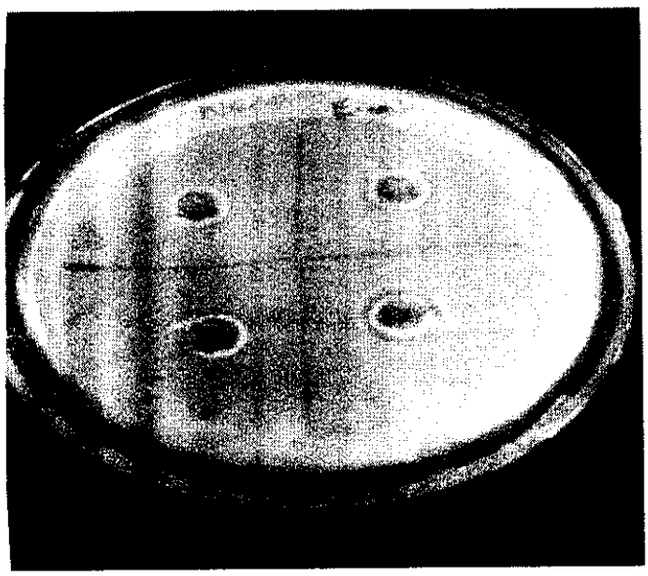
Among the nine varieties of banana, Kadali banana inhibited the strains of two organisms namely *E.coli* and *Bacillus subtilis*. There is appreciable antimicrobial activity in the Nendran banana against *Ecoli, Pseudomonas aeroginosa* and *Bacillus subtilis*. The pulp extract of Rasthali banana effectively inhibited two strains namely *E.coli* and *Bacillus subtilis*. Karpooravalli banana showed inhibition of growth against *E.coli* and *Bacillus subtilis*. There are two prime factors which essentially determines the anti bacterial activity. They are the solvents like ethanol/water used and the ripened/unripened stage of banana. In a study, it is observed that unripe banana showed antibacterial activity and the potency of unripe banana was enhanced by the type of solvent used, indicating that some active materials in these dissolve well in ethanol than in water. (Aibinu *et al*, 2007). The extracts of pulp and peel of ripe bananas effectively inhibited *Staphylococcus aureus, Bacillus subtilis, Escherichia coli*. Under the conditions employed the samples had potent inhibitory effects on the group of bacteria tested unripe banana (ethanolic extract) showed a high antimicrobial activity all test organisms with zone diameters ranging from 8mm (*E.coli*) to 31mm (*S.aureus*). In a preliminary survey conducted by a laboratory, approximately 40% of the extracts from 80 banana plants tested exhibited either antibacterial or antifungal activity or both. Karpooravalli banana showed inhibition of growth against *E.coli* and *Bacillus subtilis*.



E. coli – Kadali

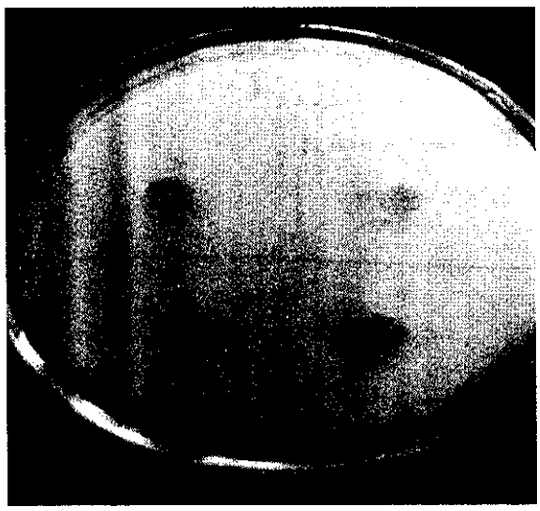


E.coli- Karpooravalli

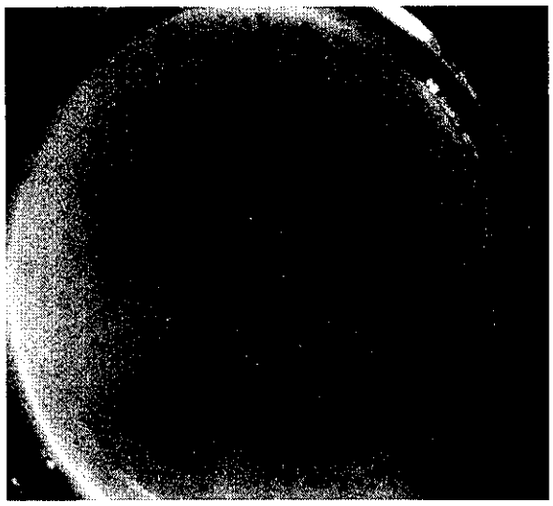


E.coli - Nendran

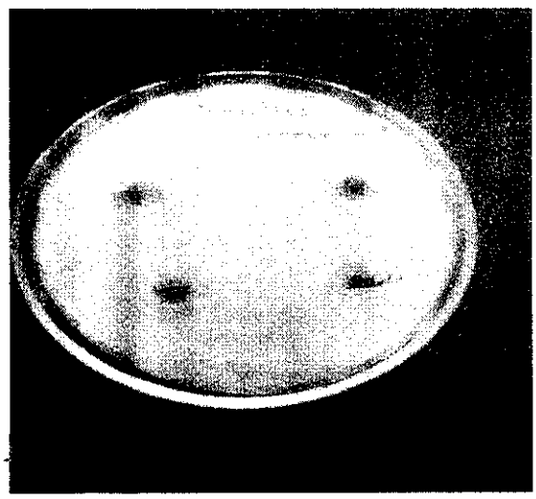
E.coli - Rasthali



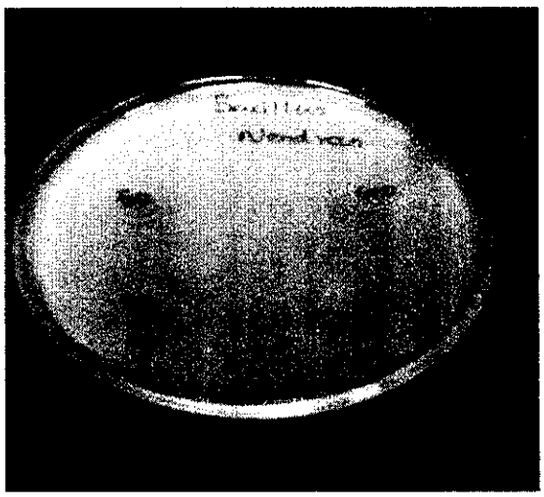
Pseudomonas- Nendran



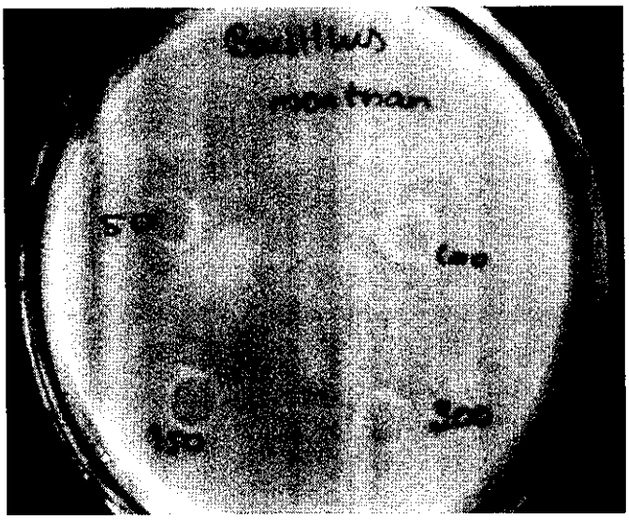
Bacillus- Kadali



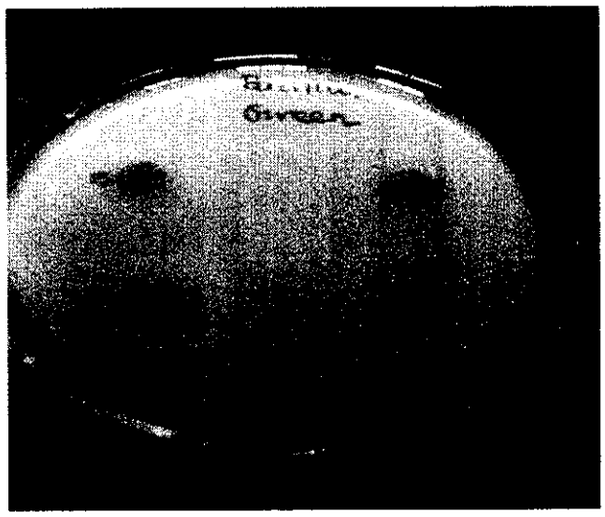
Bacillus – Karpooravalli



Bacillus - Nendran



Bacillus- Monthan



Bacillus- Pachanadan

Bacillus- Red banana

Bacillus -Rasthali

Conclusion

6. CONCLUSION

The human body has been naturally blessed with a number of disease combating compounds that are sensibly programmed to act instantaneously. It is during the deficit of these substances that our body becomes afflicted with various ailments that may subsequently turn chronic. To prevent this, it is often recommended that people should intake natural supplements of these substances and antioxidants take the priority lead considering its valuable functions in the body. The present work has been undertaken to evaluate and compare the antioxidant, phytochemical, antimicrobial potential of nine varieties of banana viz. Kadali, Karpooravalli, Monthan, Nendran, Pachainadan, Poovan, Rasthali, Red and Robusta. The preliminary study carried out in our work has confirmed the significant free radical scavenging potential of the banana pulp extracts. The free radical scavenging ability of the banana pulp extracts was tested against various free radicals generated *in vitro* . The pulp 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, tannins and total sugars etc. The potency of banana extracts against various bacterial growths was also tested. The results obtained were subjected to two- way ANOVA and the varieties were ranked according to their antioxidant and phytochemical content. All the nine banana varieties showed significant antioxidant and phytochemical content with Rasthali, Pachanadan, Poovan, and Robusta topping the list. Appreciable antimicrobial activity in the Nendran, Kadali, Karpooravalli, Rasthali pulp extracts for the *Pseudomonas aeroginosa* , *Bacillus subtilis, Escherichia coli* strains.

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