In vitro antioxidant potential and antimicrobial studies of peel extract in local varieties of banana

A PROJECT REPORT

Submitted by

SATHYAPRIYA.B

SHRISAKTHI.S

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ANNA UNIVERSITY: CHENNAI 600 025

ANNA UNIVERSITY: CHENNAI 600 025

BONAFIDE CERTIFICATE

Certified that this project report "***In vitro* antioxidant potential and antimicrobial studies of peel extract in local varieties of banana**" is the bonafide work of "**Sathyapriya.B, Shrisakthi.S**" who carried out the project work under my supervision.

SIGNATURE

DR. S. SADASIVAM

Professor and Head

Department of Biotechnology

Kumaraguru College of Technology

Coimbatore- 641006

SIGNATURE

DR.R.BASKAR

Associate Professor

Department of Biotechnology

Kumaraguru College of Technology

Coimbatore- 641006

CERTIFICATE OF EVALUATION

OLLEGE : Kumaraguru College of Technology

RANCH : Biotechnology

EMESTER : Eighth Semester

NAME OF THE STUDENTS	TITLE OF THE PROJECT	NAME OF THE SUPERVISOR WITH DESIGNATION
SATHYA PRIYA.B (71206214036) SHRI SAKTHI.S (71206214039)	*In vitro* antioxidant potential and antimicrobial studies of peel extract in local varieties of banana	**Dr. R. BASKAR** Associate Professor

The report of the project work submitted by the above students in partial fulfillment for the award of Bachelor of Technology degree in Biotechnology of Anna University was evaluated and confirmed to be a report of the work done by the above students. It was submitted for the viva voce examination on

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B. Sathya Priya

[B.SATHYAPRIYA]

S. Shrisakthi

[S.SHRISAKTHI]

DEDICATED TO

OUR BELOVED

PARENTS

ABSTRACT

ABSTRACT

Free radicals are produced in the body either naturally or on the exposure of radiation, cigarette smoke, etc. and can be implicated in many diseases like cancer, atherosclerosis, arthritis, Parkinson's disease, Alzheimer's disease , aging and other age-related diseases. Antioxidants are well known to be playing a role in reducing the risk of succumbing to early disease, such as heart disease, diabetes, arthritis and some cancers. Although the body produces antioxidants of its own in its defense against free radical damage, it seems to benefit from the extra antioxidants provided in the diet, especially from fruits, whole grains, and vegetables. Banana should be considered to be a good source of natural antioxidant for foods and functional food source against cancer and heart disease. The banana peel could be a potential source of antioxidant and antimicrobial activities. This project is aimed to evaluate and compare antioxidant and antimicrobial activities of peel extract in nine local varieties of banana viz., Poovan, Nendran, karpooravali, Kadali, Red banana, Pachainadan, Rasthali, Monthan. These extracts were subjected to *in vitro* free radical scavenging assays like DPPH scavenging assay, ABTS cation radical scavenging assay, nitric oxide scavenging assay, total antioxidant capacity assay and hydroxyl radical scavenging assay. Lipid peroxidation assay using goat liver was also carried out. The results were analyzed statistically and found that rasthali and monthan peel show significantly high antioxidant activity and rasthali show significantly high antimicrobial activity as compared to other

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ABBERVIATIONS

µg	microgram
µl	microliter
µm	microgram
ABTS	2, 2,-azinobis (3-ethylbenzoline-6- sulfonic acid)
ANOVA	Analysis of Variance
DMRT	Duncun's Multiple Range Test
DNA	Deoxyribose Nucleic Acid
DPPH	1, 1-diphenyl -2-picryl hydrazyl
EDTA	Ethylene Diamine Tetra Acetic acid
$FeCl_3$	Ferric Chloride
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

ROOH	Organic hydroperoxide
ROS	Reactive Oxygen Species
SOD	Superoxide dismutase
TBA	Thiobarbituric acid
TCA	Trichloro Acetic acid

INTRODUCTION

INTRODUCTION

Free radicals are reactive oxygen species that are unstable in nature and react quickly with other compounds, trying to capture the needed electron to gain stability. Free radicals attack the nearest stable molecule and the attacked molecule now becomes free radicals, thus initiating the chain reaction. These are atomic or molecular species with unpaired electrons. They are highly reactive and are normally derived during metabolism. Other environmental factors pollution, radiation, cigarette smoke, herbicides can also spawn free radicals.

Reactive oxygen species (ROS) are reactive molecules that contain the oxygen atom. They are very small molecules that include oxygen ions and peroxides and can be either organic or inorganic. ROS includes superoxide radicals, hydroxyl radicals, and hydrogen peroxide that are often generated as by -products of biological reactions or from exogenous factors. Though they have important roles in cell signaling, 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.

Oxygen, an essential element for life, can also be a reason for the destruction of tissue and/or impair its ability to function normally (Kehrer., 1993). The formation of oxygen radicals could be the reason for the damaging effects of oxygen. A class of enzymes called superoxide dismutase (SOD) is responsible for the catalytic removal of superoxide free radical (Lee *et al.*, 2001). An average person has around 10,000-20,000 free radicals attacking each body cell every day. In some cases, ROS are produced specifically to serve essential biological functions, whereas in other cases, they are the by-products of metabolic processes (Shigenga *et al.*, 1994). Highly reactive free radicals are

vascular diseases, neurodegenerative disorders, and inflammation (Stadtman., 1992;).

Antioxidants are defined as the substance that when present in low concentrations compared to those of an oxidizable substrate significantly delays or prevents oxidation of that substance (Halliwell and Gutteridge, 1989). For the *in vivo* situation, the concept of antioxidants includes antioxidant enzymes, iron binding and transport proteins and other compounds affecting signal transduction and gene expression (Gutteridge., 1989).

In case of foods and beverages, antioxidants are related to the protection of specific oxidation substrates or the formation of specific oxidation. Synergism, antagonism, co-antioxidants and oxidation retarders are the other useful concepts related to antioxidants. Synergism can be defined as the phenomenon in which a number of compounds, when present together in the same system, have a more pronounced effect than if they were alone. Antagonism can be defined likewise by substituting "more" with "less", whereas co antioxidants may be defined by substituting "more" with "same". The compounds that reduce the rate of oxidation without showing a distinct lag phase of oxidation are retarders of oxidation. Antioxidant action is measured as a decrease in over-all rate of oxidation and as the length of the lag phase.

Antioxidants are divided into two classes: preventive antioxidants and chain breaking antioxidants. Preventive antioxidants inhibit oxidation by reducing the rate of chain initiation. Preventive antioxidants convert the hydroperoxides to molecular products that are not potential sources of free radicals (Burton *et al.*, 1985). Most biological preventive antioxidants are also peroxide decomposers. Antioxidants can also be manufactured synthetically. These belong to the class of synthetic antioxidants. The main disadvantage with these antioxidants is their side effect when taken *in vivo* (Chen *et al.*, 1992).

the antioxidant components of fruits and vegetables contribute in the defense effect. Epidemiological studies and intervention trials on prevention of diseases such as cancer and cardiovascular disease in people have shown the positive effects of taking antioxidant supplements (Enstrom *et al.*, 1992; Rimm *et al.*, 1993).

Fruits and vegetables contain many different antioxidant and antimicrobial components. The majority of the antioxidant capacity of a fruit or vegetable may be from compounds such as vitamin C, vitamin E or β carotene. Banana, a tropical fruit protects itself from the oxidative stress caused by strong sunshine and high temperature by producing large amounts of antioxidants (Kananazawa, and Sakakibara, 2000).

Banana is one of the rare fruits which satisfy the definition of a good food i.e., one that contains an ample proportion of nutritive constituents which are easily digested and absorbed, while available at reasonable cost. It is one of the most easily assimilated fruits. From the nutritional point of view, banana has a calorific value ranging from 67to 137 calories per 100 g and is closely comparable with potatoes but digested more easily. It is relatively cheap. The average composition of banana fruit is as follows according to (Gopalan *et al.*1971) : Moisture-70.0% ; Phosphorus-290ppm; Carbohydrate-27.0%; Calcium 80.0ppm; Crude fibre-0.5%; Iron-6.0ppm; Protein-1.2%; Carotene-0.5ppm; Fat-0.3%; Riboflavin-0.5ppm; Ash-0.9%; Niacin-7ppm; Ascorbic acid-120.0ppm.

From an environmental perspective, it is vital that plant by-products produced by the agro-food industry be reused. The main by-product of the banana processing industry is the peel, which represents approximately 30% of the fruit. This by-product constitutes an environmental problem, because it contains large quantities of nitrogen and phosphorus and its high water content

Banana peel is rich in phytochemical compounds, mainly antioxidants. The total amount of phenolic compounds in banana (*Musa acuminata* Colla AAA) peel ranges from 0.90 to 3.0 g/100 g. Someya *et al.* (2002) identified gallocatechin at a concentration of 160 mg/100 g DW. Ripe banana peel also contains other compounds, such as the anthocyanins delphinidin and cyaniding , and catecholamines (Kanazawa & Sakakibara, 2000). Furthermore, carotenoids, such as β-carotene, α-carotene and different xanthophylls, have been identified in banana peel in the range of 300–400 lg lutein equivalents/100 g , as well as sterols and triterpenes, such as b-sitosterol, stigmasterol, campesterol, cycloeucalenol, cycloartenol, and 24-methylene cycloartanol. To date, only Someya *et al.* (2002) have evaluated the antioxidant activity in banana peel, measured as the effect on lipid autoxidation, in relation to its gallocatechin content.

Banana nutritional facts revealed that it contain abundant amounts of proteins, carbohydrates and fibres. It consists of sugars like glucose, lactose, maltose, galactose, sucrose, fructose, and starch. Banana is also rich in vitamins like vitamin A, vitamin C and vitamin E, Riboflavin, Thiamine, niacin, folic acid, vitamin B12 and minerals like iron, cacium, potassium, magnesium, phosphorus, copper, zinc and fluride. There are also essential amino acids like tryptophan, lysine, leucine, threonine, arginine, glycine etc., present in bananas. Bananas are well known to contain various antioxidant compounds such as gallocatechin and dopamine and the peel of banana is a potential source of antioxidant and antimicrobial activities. Banana peel contained large amounts of dopamine and L-dopa, catecholamines with a significant antioxidant activity. However, ascorbic acid, tocopherols or phytosterols were not detected in the different extracts. The antioxidant activity of banana peel extracts from different cultivars was similar. However, the impact of extraction time or temperature should be studied in greater depth.

This project is aimed to evaluate and compare antioxidant and antimicrobial activities of peel extract in nine local varieties of banana viz., Poovan, Nendran, Karpooravali, Kadali, Red, Pachainadan, Rasdali, Monthan, and Robusta.

OBJECTIVES:

- To determine and compare the antioxidant potential of local varieties of banana using different *in vitro* antioxidant assays.
- To study the antimicrobial properties in local varieties of banana peel.

LITERATURE REVIEW

2.0 LITERATURE REVIEW

2.1 Free radicals

Free radicals can be defined as chemical species possessing an unpaired electron, which is formed by homolytic cleavage of a covalent bond of a molecule by the loss of a single electron from a normal molecule or by the addition of a single electron to a normal molecule. Free radicals are a group of active molecules with unpaired electrons that react with biological 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. A number of antioxidant activity assays have been published, and its antioxidant activity is often expressed as Trolox Equivalent.

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

The oxygen and hydrogen peroxide so formed may lead to the formation of the most reactive $OH^{\cdot}$. 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). 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

DNA (Park, 1992).

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

2.2 Terminology and chemistry

Chemically, every compound, including oxygen that can accept electrons is an oxidant or oxidizing agent. In contrast, a substance that donates electrons is a reductant or reducing agent. In general, a chemical reaction in which a substance gains electrons is defined as a reduction. Oxidation is a process in which a loss of electrons occurs. When a reductant donates its electrons, it causes another substance to be reduced, and, when an oxidant accepts electrons, it causes another substance to be oxidized. In biology, a reducing agent acts via donation of electrons, usually by donation of hydrogen or removal of oxygen. An oxidation process is always accompanied by a reduction process in which there is usually a loss of oxygen, while in an oxidation process there is a gain in oxygen. Such reactions, called redox reactions, are the basis for numerous biochemical pathways and cellular chemistry, biosynthesis, and regulation. They are also important for understanding biological oxidation and radical/antioxidant effects.

While reductant and oxidant are chemical terms, in biological environments they should be termed antioxidant and pro-oxidant, respectively. There are many examples of the biological importance of pro-oxidants. In

radical group, often incorrectly called free-radical (the term is not accurate, because a radical is always free), contains compounds such as nitric oxide radical ($NO^{\cdot}$), superoxide ion radical ($O_2^{\cdot}$), hydroxyl radical ($OH^{\cdot}$), peroxyl ($ROO^{\cdot}$) and alkoxyl radicals ($RO^{\cdot}$), and one form of singlet oxygen .

Radical and non radical oxygen metabolites.

Name	**Symbol**
Oxygen (bi-radical)	O_2
Superoxide ion	O_2^{-}
Hydroxyl	$OH^{\cdot}$
Peroxyl	$ROO^{\cdot}$
Alkoxyl	$RO^{\cdot}$
Nitric oxide	$NO^{\cdot}$

Non radical oxygen derivatives

Hydrogen peroxide	H_2O_2
(Organic peroxide)	ROOH
Hypochlorous acid	HOCL
Ozone	O_3
Aldehydes	HCOR
Singlet oxygen	1O2
Peroxynitrite	ONOOH

2.3 Role of Pro-oxidants in free radical formation

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

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

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

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

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

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

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

2.4 Biological significance of Free radicals

2.4.1 Positive roles of free radicals

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

2.5 Reactive Oxygen Species

2.5.1 Superoxide Ion Radical ($O_2^{\cdot-}/HO_2^{\cdot}$)

This species possesses different properties depending on the environment and pH. Due to its pKa of 4.8, superoxide can exist in the form of either O_2 or, at low pH, hydroperoxyl (HO_2). The latter can more easily penetrate biological membranes than the charged form. Hydroperoxyl can therefore be considered an important species, although under physiological pH most of the superoxide is in the charged form. In a hydrophilic environment, both the O_2 and HO_2 can act as reducing agents capable, for example, of reducing ferric (Fe_3) ions to ferrous (Fe_2) ions; however, the reducing capacity of HO_2 is higher. In organic solvents the solubility of O_2 is higher, and its ability to act as a reducing agent is increased. It also acts as a powerful nucleophile, capable of attacking positively charged centers, and as an oxidizing agent that can react with compounds

2.5.2 Hydroxyl Radical *($OH^{\cdot}$)*

The reactivity of hydroxyl radicals is extremely high. In contrast to superoxide radicals that are considered relatively stable and have constant, relatively low reaction rates with biological components, hydroxyl radicals are short-lived species possessing high affinity toward other molecules $OH^{\cdot}$ is a powerful oxidizing agent that can react at a high rate with most organic and inorganic molecules in the cell, including DNA, proteins, lipids, amino acids, sugars, and metals. The 3 main chemical reactions of hydroxyl radicals include hydrogen abstraction, addition, and electron transfer. OH is considered the most reactive radical in biological systems; due to its high reactivity, it interacts at the site of its production with the molecules closely surrounding it.

2.5.3 Hydrogen Peroxide *(H_2O_2)*

The result of the dismutation of superoxide radicals is the production of H_2O_2. There are some enzymes that can produce H_2O_2 directly or indirectly. Although H_2O_2 molecules are considered reactive oxygen metabolites, they are not radical by definition; they can, however, cause damage to the cell at a relatively low concentration (10 *l* M). They are freely dissolved in aqueous solution and can easily penetrate biological membranes. Their deleterious chemical effects can be divided into the categories of direct activity, originating from their oxidizing properties, and indirect activity in which they serve as a source for more deleterious species, such as $OH^{\cdot}$ or HClO, which is discussed later. Direct activities of H_2O_2 include degradation of haem proteins; release of iron; inactivation of enzymes; and oxidation of DNA, lipids, SH groups, and keto acids.

2.5.4 Nitric Oxide ($NO^{\cdot}$), Peroxynitrite ($ONOO^{-}$), and other Members of the Family

reaction, catalyzed by the group of enzymes called nitric oxide synthase(NOS)s, L-arginine is converted to nitric oxide and L-citrulline. Three types of the enzyme exist: neuronal NOS, endothelial NOS (eNOS), and inducible NOS (iNOS). One-electron oxidation results in the production of nitrosonium cation (NO), while one-electron reduction leads to nitroxyl anion, which can undergo further reactions, such as interacting with NO to yield N_2O and OH. The half-life of the nitric oxide radicals depends on the square of the radical concentration. NO can react with a variety of radicals and substances. For example, it can react with H_2O_2 and HClO to yield a line of derivatives such as N_2O_3, NO_2, and NO_3. One of the most important reactions under physiological

2.6 Sources of ROS

The cell is exposed to a large variety of ROS and RNS from both exogenous and endogenous sources. The former include, first, exposure to di-oxygen, which, although a nonreactive biradical, can independently cause oxidation and damage to proteins and enzymes, exemplified by inhibition of aconitase and fumerase in the Krebs cycle and glutamate decarboxylase, which results in decreased *c* aminobutyric acid in the brain. The toxicity of the oxygen molecule itself is sometimes beneficial and used as a therapeutic aid as, for example, in hyperbaric oxygen therapy. Ozone (O_3) is essential in scavenging deleterious UV-C irradiation and extremely important with its presence in the upper atmosphere. On the other hand, it acts as a damaging species to biological tissues. Ozone is not a radical like oxygen, is characterized by its sharp odor, can damage lungs, and can serve as a powerful oxidizing agent that can oxidize biological components directly. Exposure of living organisms to ionizing and non ionizing irradiation constitutes another major exogenous source of ROS.

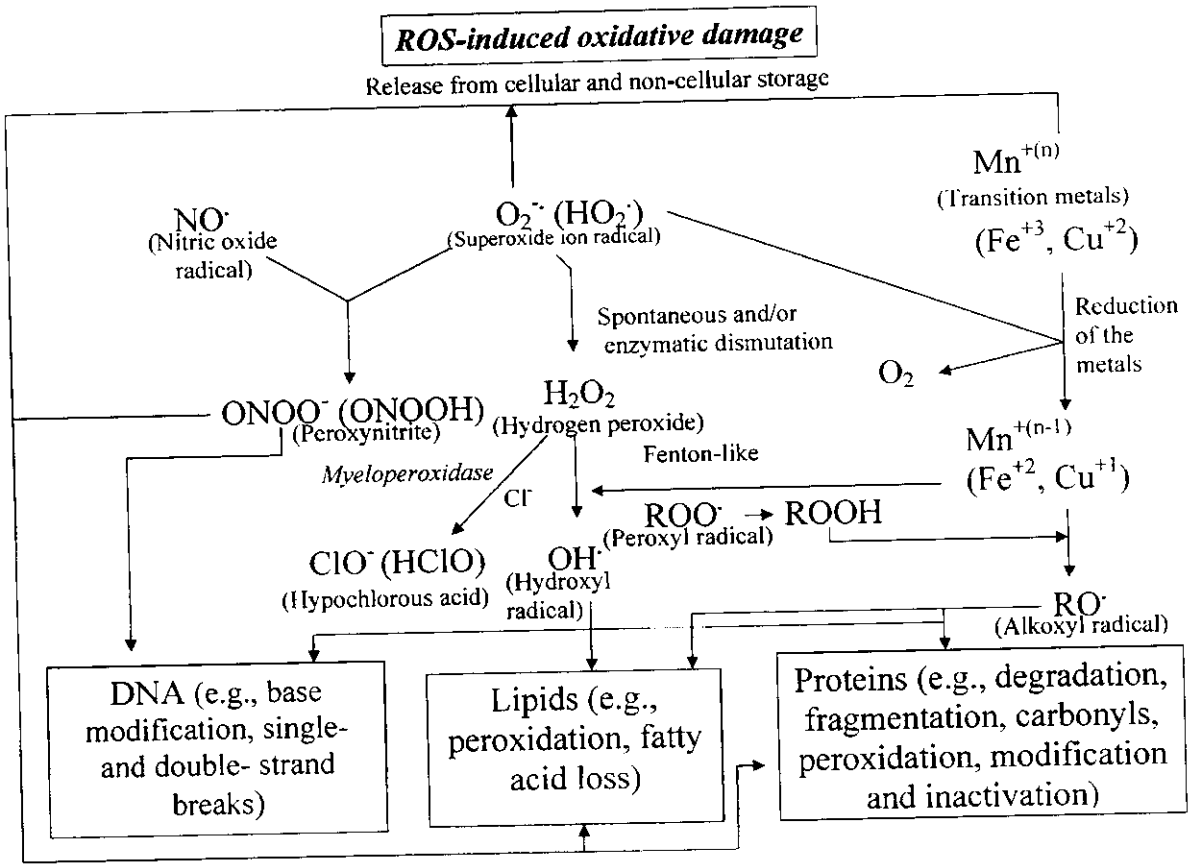
Exposure of the cell to *c* -irradiation results in the production of a whole range of radical and non radical species from ionization of intracellular

400 nm) can indirectly produce a variety of ROS including $^1O_2$, H_2O_2, and O_2 radicals; hemolytic cleavage of H_2O_2 by UV radiation yields OH radicals. Air pollutants such as car exhaust, cigarette smoke, and industrial contaminants encompassing many types of NO derivatives constitute major sources of ROS that attack and damage the organism either by direct interaction with skin or following inhalation into the lung. Drugs are also a major source of ROS. There are drugs, such as belomycinem and adreamicine, whose mechanism of activity is mediated via production of ROS, those like nitroglycerine that are NO donors, and those that produce ROS indirectly. Narcotic drugs and anesthetizing gases are considered major contributors to the production of ROS. A large variety of xenobiotics (eg, toxins, pesticides, and herbicides such as paraquat) and chemicals (eg,mustard gas, alcohol) produce ROS as a by-product of their metabolism in vivo. The invasion of pathogens, bacteria, and viruses might result in the production of many ROS species by direct release from the invaders or an endogenous response induced by phagocytes and neutrophils. One of the major sources of oxidants is food, for a large portion of the food we consume is oxidized to a large degree and contains different kinds of oxidants such as peroxides, aldehydes, oxidized fatty acids, and transition metals. Food debris that reaches the intestinal tract places an enormous oxidative pressure on the intestinal-tract mucosa. Although the exposure of the organism to ROS is extremely high from exogenous sources, the exposure to endogenous sources is much more important and extensive, because it is a continuous process during the life span of every cell in the organism. The reduction of oxygen to water in the mitochondria for ATP production occurs through the donation of 4 electrons to oxygen to produce water. During this process several major oxygen derivatives are formed. In many cases, there is a leakage of ROS from the mitochondria into the intracellular environment. The mitochondrion serves as the major organelle responsible for ROS production and many events

impaired and its membrane integrity damaged. Enzymes comprise another endogenous source of ROS. While most enzymes produce ROS as a by-product of their activity, exemplified by the formation of superoxide radicals by xanthine oxidase, there are some enzymes designed to produce ROS, such as nitric oxide synthase that yields NO radicals, those that produce H2O2, and those responsible for hydroxylation.

Numerous pathologies and disease states serve as sources for the continuous production of ROS. More than 200 clinical disorders have been described in the literature in which ROS were important for the initiation stage of a disease or produced during its course. ROS may be important initiators and mediators in many types of cancer, heart diseases, endothelial dysfunction , atherosclerosis and other cardiovascular disorders, inflammation and chronic inflammation, burns, intestinal tract diseases, brain degenerative impairments, diabetes, eye diseases, and ischemic and postischemic (eg, damage to skin, heart, brain, kidney, liver, and intestinal tract) pathologies. In several normal conditions ROS are produced and play a role in the pathogenesis of the physiological condition. These are exemplified during the aging process where ROS production significantly increases as a result of impaired mitochondrial function and in the early stages of embryonic development. Other pathological disorders, which are associated with impaired metal metabolism, such as hemochromatosis, Wilson disease, and thalassemia, in which iron is deposited in many organs, are known to increase significantly the concentration of ROS.

Figure 2.6.1 ROS- induced oxidative damage



2.7 Free radicals and Human Disease

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

Free radicals may also be involved in Parkinson's disease, senile and

adical syndrome, the iron storage disease hemochromatosis, is typically associated with a constellation of free radical related symptoms including movement disorder, psychosis, skin pigmentary melanin abnormalities, deafness, arthritis and diabetes mellitus. (Ames, 1983; Ames, 1998)

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

- Damage to DNA
- Oxidations of polyunsaturated fatty acids in lipids
- Oxidations of amino acids in proteins
- Oxidatively inactivate specific enzymes by oxidation of co-factors
- Oxidative stress due to free radicals contributes to tissue injury following irradiation and hyperoxia. It is linked to cardiovascular disease, since oxidation of LDL in the vascular endothelium is a precursor to plaque formation. Oxidative stress also plays a very important role in ischemic cascade due to oxygen reperfusion injury following hypoxia. This cascade includes roles both in stroke and heart attacks.
- Transition metals also produce free radicals and are hence responsible for many diseases. For example, chronic manganism is a classic pro-oxidant disease (Puglia and Powell, 1984). Another disease associated with the chronic presence of a pro-oxidant transition-series metal is hemochromatosis associated with elevated iron levels. Similarly, Wilson's disease is associated with elevated tissue levels of copper. Such syndromes tend to be associated with a common symptomology. This typically includes the various combinations of psychosis, dyskinesia, pigmentary abnormalities, fibrosis, deafness, diabetes and arthritis (James *et al.*, 2003). Wilson's disease and striatal iron have similarly been linked to human Parkinsonism.

include oxygen itself, nitrofurantoins, and bleomycin, which produce pulmonary fibrosis. Radical generating agents such as iron and copper are also associated with liver fibrosis and fibrotic changes in other organs such as the heart. The induction of vitreous scarring by interocular iron or copper is also well known, as is the association of homocystinuria with fibrotic lesions of the arteries.

2.8 Defense mechanisms of the cell against oxidative stress

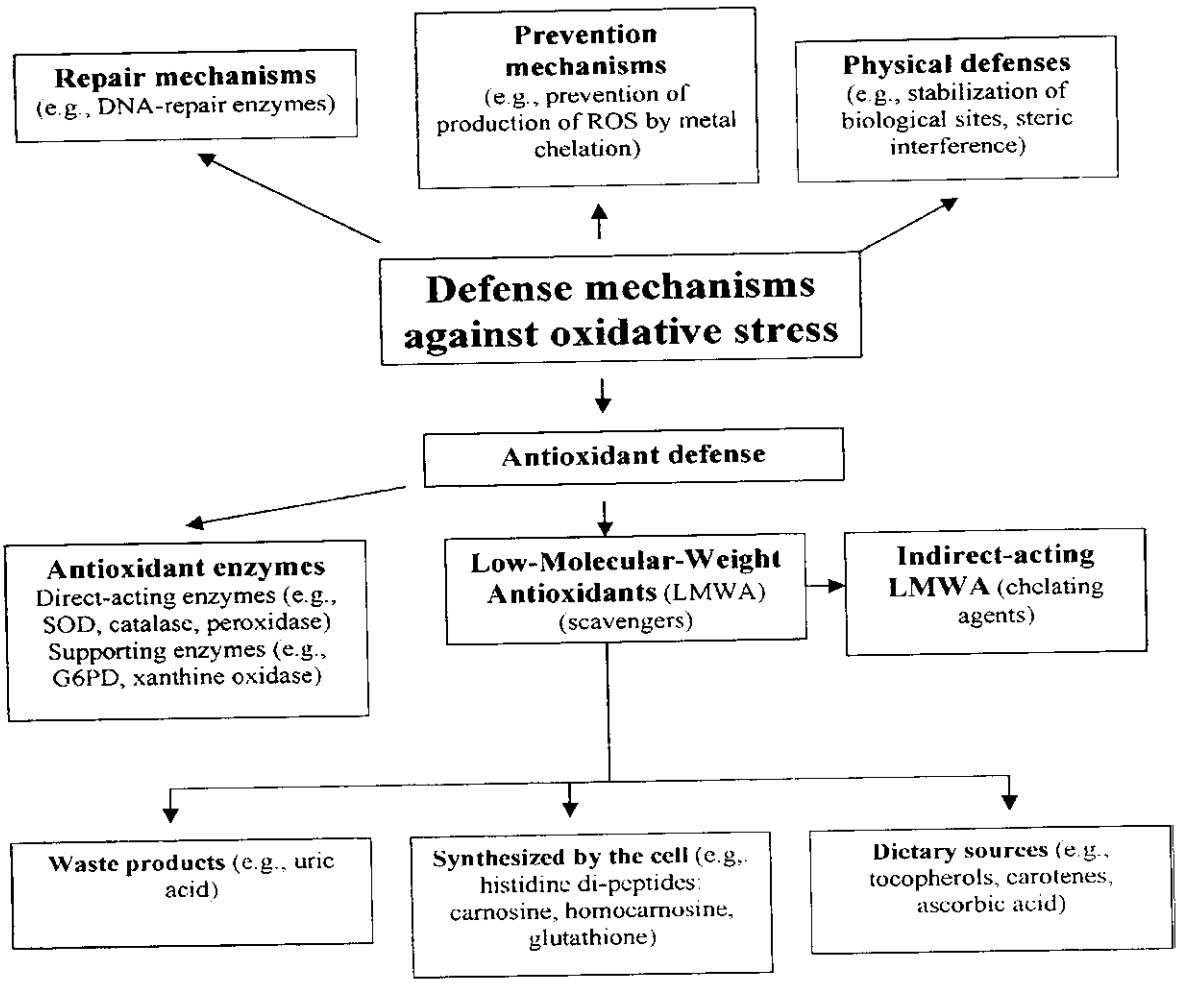
Continuous exposure to various types of oxidative stress from numerous sources has led the cell and the entire organism to develop defense mechanisms for protection against reactive metabolites. Indirect approaches may involve control of the endogenous production of ROS by, for example, altering the activity of enzymes, which indirectly produce oxygen metabolites; one such enzyme is xanthine oxidase. An efficient repair system, one of the most important methods for the organism to cope with oxidative damage, consists of enzymes and small molecules that can efficiently repair an oxidative-damage site on macromolecules.

The DNA repair system, for example, can identify a DNA-oxidized adduct [eg., 8 hydroxy-2-deoxyguanosine , thiamine glycol, and apurinic and apyramidenic (AP) sites], remove it, and incorporate an undamaged base. Molecules that can donate hydrogen atoms to damaged molecules are also considered repair compounds; one such example is the donation of a hydrogen atom by ascorbate or tocopherol to a fatty acid radical that was previously attacked by a radical and lost its hydrogen. Physical defense of biological sites such as membranes is also an important mechanism allowing the cell to cope with oxidative stress. Compounds such as tocopherols can provide enhanced stability to cellular membranes, and steric interference can prevent ROS from approaching the target.

Among the various defense mechanisms, the one involving antioxidants s extremely important due to its direct removal of pro-oxidants and the variety of compounds that can act as antioxidants and ensure maximum protection for biological sites. This system apparently developed throughout the evolutionary process, perhaps in response to the changing concentration of oxygen in the atmosphere. The uniqueness of this system is its direct interaction with ROS of various kinds and its provision of protection for biological targets.

The system contains 2 major groups—antioxidant enzymes and low-molecular-weight antioxidants (LMWA). The enzyme-containing group is composed of direct-acting proteins, such as SOD; the proteins in this family differ in their structure and cofactors. Cu-Zn SOD is an enzyme of molecular mass of approximately 32,000; contains 2 subunits, each of which possesses an active site; and is widely distributed in eukaryotic cells localized in the cytoplasm, while Mn-SOD, a protein of about 40,000, can be found in prokaryotic cells and eukaryotic mitochondria. Other types of SOD exist, such as extracellular SOD (EC-SOD) and Fe-SOD in plants. These enzymes possess different structures, molecular masses, and reaction rate constants. The enzyme activity itself, first discovered by McCord and Fridovich in 1969, is capable of enhancing the spontaneous dismutation of superoxide radicals to H_2O_2. There are significant changes among the rate constants of the various SODs depending on pH and site of activity. The end product of the dismutation reaction—H_2O_2—can be removed by the activity of the enzyme catalase and members of the peroxidase family including glutathione peroxidase.

2.8.1 Defense mechanisms of the cell against oxidative stress



2.9 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

Antioxidants have also been suggested to have well defined role as preservatives. These have been defined by the US Food and Drug Administration (FDA) as substance used to preserve food by retarding deterioration, rancidity or discoloration caused by oxidation. Lipid peroxidation is an important deteriorative reaction of foods during processing and storage. Toxic substance formed by lipid peroxidation may lead to adverse effects such as carcinogenesis, cell DNA mutagenesis and aging. Antioxidants therefore, according to their mode of action, have also been classified as the compounds that terminate the free radical chain in lipid peroxidation by donating electrons or hydrogen to fat containing a free radical and to the formation of a complex between the chain and a free radical. Antioxidants stop the reactions by contributing hydrogen from the phenolic hydroxyl hydrogen from the phenolic hydroxyl groups, themselves forming stable free radicals that do not initiate or propagate further oxidation of lipids (free radical terminators). Some of the important synthetic antioxidants of this class are butylated hydroxyanisole (BHA), butylated hydroxy toluene, terbutyl hydroquinone (TBHQ), propyl gallate (PG) and tocopherols.

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

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. A number of plant and medicinal mushrooms constituents have been recognized to have positive effects when tested against the oxygen reactive compounds. Foods rich in

known of which are Parkinson's and Alzheimer's diseases, inflammation and problem caused by cell and cutaneous aging (Shahidi and Wanasundara, 1992).

2.9.1 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.

2.10 Antioxidant capacity assays

2.10.1 DPPH radical scavenging assay

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.

2.10.2 Trolox Equivalent Antioxidant Capacity Assay

In this improved version, $ABTS^{\cdot}$, the oxidant is generated by persulfate oxidation of 2, 2-azinobis (3-ethylbenzoline-6- sulfonic acid) - ($ABTS^{2-}$). 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

30°C. The difference of the absorbance reading is plotted versus the antioxidant concentrations to give a straight line

2.10.3 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.

2.10.4 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

2.10.5 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.

2.10.6 β-carotene bleaching 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 acidemulsion.

2.11 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

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

2.12 Antioxidants from dietary sources

2.12.1 Importance of Antioxidant rich dietary sources

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

2.12.2 Natural Sources of antioxidants

Many people these days take antioxidants to counter the detrimental effect of free radicals. In fact, artichokes are among the best sources of antioxidants. Berries in general are among the foods, which are the most antioxidant-dense. Caretonoids are antioxidants that are in carrots, apricots and

high in antioxidants. Apart from natural sources supplements can go a long way in increasing the antioxidant content in the body.

2.13 Banana as a source of antioxidants

2.13.1 Taxonomy and classification of banana

Bananas are a large, monocotyledonous herb belong to the Musaceae family of the order Zingiberales. The genus *Musa* is comprised of all edible cultivars that was further divided into four sections, *Eumusa, Rhodochlamys, Australimusa* and *Callimusa.* Among the four sections, *Eumusa* is the largest and most widespread geographically. It has given rise to the great majority of the edible bananas including the edible bananas which are of primary interest in this study. Besides its edible fruits, *Eumusa* section also produces several minor fibres (e.g.from *Musa basjoo*) and vegetables derived from parts of the plant other than the fruit. The section *Australimusa* also yields edible fruits from the Fei'I bananas grown in the Pacific. However its distribution and variability is lesser than the *Eumusa*. It also contains *Musa textilis* (Abaca) which produces the commercial value Manila hemp. The other two sections of *Musa*, *Rhodochlamus* and *Callimusa* are only appreciated for their ornamental properties.

The edible bananas cultivars are mostly derived from two wild species of genus *Musa* (section Eumusa) namely *Musa acuminata* and *Musa balbisiana* (Valmayor *et al.*, 1990). *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 *acuminata* and *balbisiana*, diploid or triploid derivative and whether they are diploid, triploid or tetraploid hybrids of two wild species (Valmayor *et al.*, 1990). Hence, a classification system was

morphological characters, the differences between *Musa acuminate* and *Musa balbisiana* (Espino *et al.*, 1992). It is also necessary to determine the ploidy of a clone before it can be satisfactorily classified. Ploidy and relative contribution of the two species to a given banana cultivar is specified with a shorthand lettering system. Haploid contribution of *Musa acuminata* and *Musa balbisiana* are designated as A and B, respectively. Basically, all edible banana cultivars can be classified into six groups which are AA, BB, AAA, AAB, ABB, and ABBB. They are respectively diploid, triploid and tetraploid. However, most of them are triploid.

Figure 2.13.1 Poovan-Musa spp Cv. Poovan[AAB].

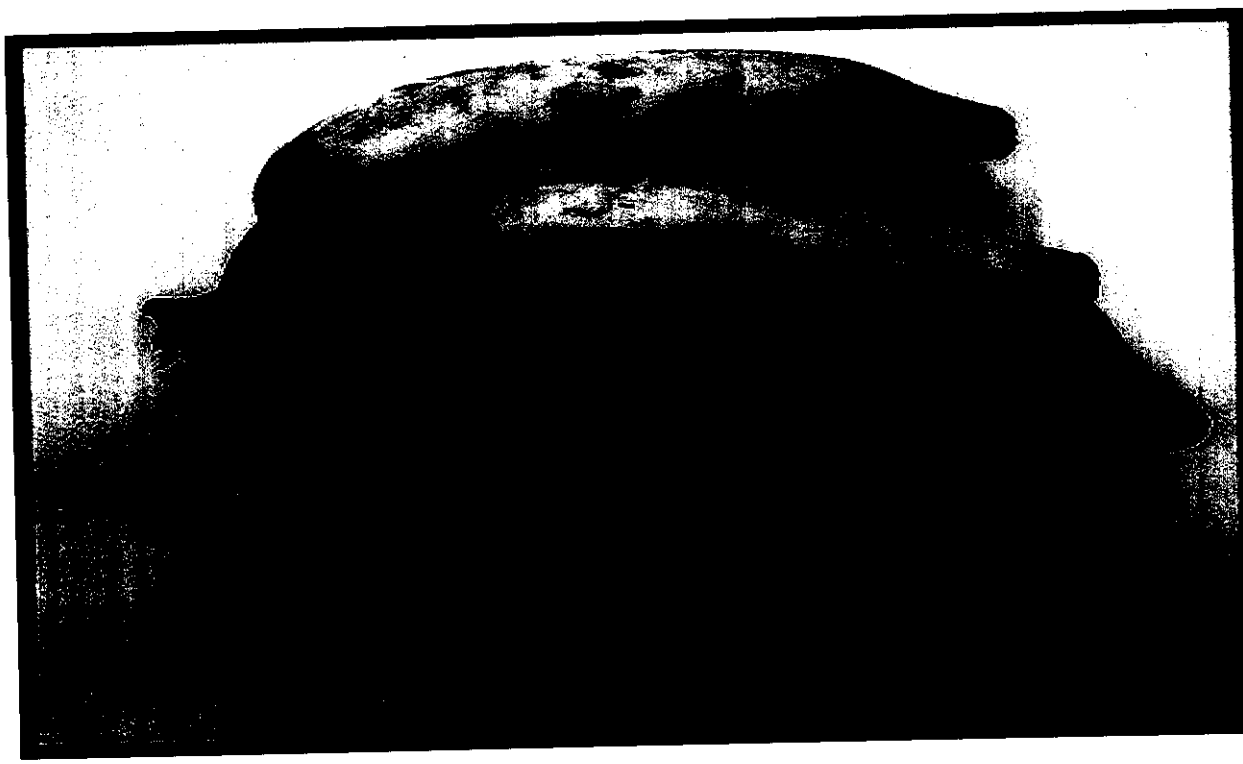


Figure 2.13.2 Pachinadan- Musa spp Cv. Pachinadan [AAA].

Figure 2.13.3 Red- Musa spp Cv . Red [AAA].

Figure 2.13.4 Nendran- Musa spp Cv. Nendran [AAB].

Figure 2.13.5 Rasthali- Musa spp Cv. Rasthali [AAB].

Figure 2.13.6 Karpooravalli-Musa spp Cv. Karpooravalli[ABB].

Figure 2.13.7 Monthan-Musa spp Cv. Monthan[ABB].

Figure 2.13.8 Robusta-Musa spp Cv. Robusta[AAA].

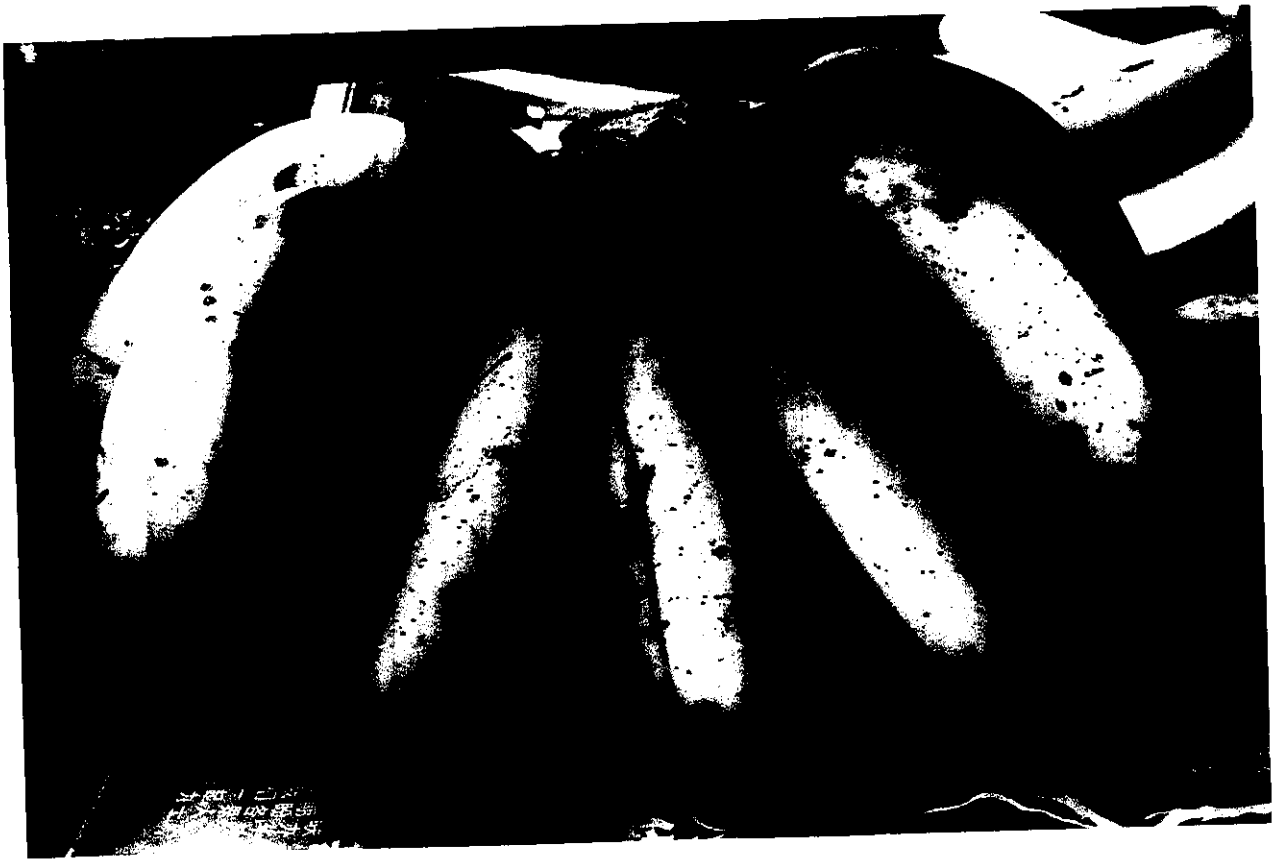


Figure 2.13.9 Kadali- Musa spp Cv. Kadali[AB].

2.13.2 Nutritional and therapeutic values of banana:

Bananas contain about 74% water, 23% carbohydrates, 1% proteins, 0.5% fat, and 2.6% fiber (these values vary between different banana cultivars, degree of ripeness and growing conditions). In an unripe banana the carbohydrates are mostly starches. In the process of ripenening the starches are converted to sugars; a fully ripe banana has only 1-2% starch.

Besides being a good source of energy, banana is a rich source of potassium, and hence is highly recommended for patients suffering from high blood pressure.It is claimed that bananas have beneficial effect in the treatment of intestinal disorders, including diarrhea. Bananas are unusual in that they work for constipation too. They contain mucilaginous bulking substances and are easy to digest.

Banana has earned the status of high nutritive fruit. It has a unique combination of energy value, tissue-binding elements, proteins, vitamins, and minerals. Apart from being a nutritious food, banana fruit is already proven as possessing many curative properties because of its various kinds of vitamins, minerals, fibres, and carbohydrates.

Banana first emerged in the medical literature as a cure for ulcer in the early 1930 reported the ability of banana in reducing gastric ulcers and proved that bananas are useful for person with peptic ulcer. The ability of banana in reducing or curing the ulcers is due to its soft texture, smoothness and high in fiber content. Fiber helps to restore normal bowel function without the ill effect of a laxative. In overacidity cases, banana helps to neutralize the gastric juices and reduces the irritation by coating the lining of the stomach potassium which is a vital mineral for controlling body's fluid balance, present in substantial amount in banana fruit. Hence, banana is recommended for patient with low

pressure. Potassium is also required for normalizing the heart rhythm and transfer of oxygen to the brain. Meanwhile, banana also is beneficial for the anemic patient because of its iron content.Iron can stimulate the production of hemoglobin in the blood which is essential in cases ofanemia.(Bogert *et al* .,1942)

As an excellent source of B vitamins, banana can help in calming the nervous system. Moreover, its vitamin B6 also performs a role in regulating blood glucose level which is related to the mood condition. Therefore its role in normalizing blood glucose level can also help to avoid morning sickness and hangover. Also, vitamin C, A as well as B6 and B12 found in banana might help the body recovers from the effect of nicotine withdrawal. Because of its low lipid level but high in energy value, banana is recommended for obese and geriatric patient. Besides, banana is also valuable in the treatment of kidney disorder such as uraemia and nephritis due to its low protein and salt content. Interestingly, banana has a type of protein called tryptophan even though its protein level is low. Tryptophan can stabilize depression state when the body converts it into serotonin. Data regarding nutritional value of banana clearly shows banana is among the healthiest fruit and is natural remedy for many illnesses.

Apart of the fruit, several other parts of the banana plant possess medicinal properties.The young unfolded leaves are used against chest pains and as a cool dressing for an inflamed or blistered skin. On the other hand, the sap exuding from the base of the cut trunk is used for urethral injection against gonorrhoea, dysentery and diarrhea, to stop the loss of hair and to stimulate hair growth. Not only that, the juice of the root is febrifuge and restorative and in powder form, the juice is used in anaemia cases and general weakness and malnutrition (Espino *et al.*, 1992).

Table 2.13.2.1 Nutritional values of banana

Nutrient	Banana/1 fruit
Calories	109
Protein(g)	1
Carbohydrates(g)	28
Dietary fiber(g)	2.8
Total fiber(g)	0.6
Saturated fat(g)	0.2
Mono unsaturated fat(g)	0.1
Poly unsaturated fat(g)	0.1
Cholesterol(mg)	0
Potassium(mg)	467
Sodium(mg)	1
Calcium(mg)	9.2
Magnesium(mg)	44.1

Some of the specific diseases known to be cured by banana are:Anaemia:

Blood Pressure: Banana is extremely high in potassium yet low in salt, making it the perfect food for helping to beat blood pressure. Even the US Food and Drug Administration has just allowed the banana industry to make official claims for the ability of banana to lower the risk of blood pressure and stroke.

Brain Power: A study on 200 students at an English school showed that eating bananas

at breakfast, break and lunch improved their brainpower. Research has shown that the potassium-packed fruit can assist learning by making pupils more alert.

Constipation: High in fibre, including bananas in the diet can help restore normal bowel action, helping to overcome the problem without resorting to laxatives.

Depression: According to a recent survey undertaken amongst people suffering from depression, many felt much better after eating a banana. This is because bananas contain tryptophan, a type of protein that the body converts into serotonin known to make you relax, improve your mood and generally make you feel happier.

Hangovers: One of the quickest ways of curing a hangover is to make a banana milkshake, sweetened with honey. The banana calms the stomach and, with the help of the honey, builds up depleted blood sugar levels, while the milk soothes and re-hydrates your system.

Heartburn: Bananas have a natural antacid effect in the body so if you suffer from heartburn, try eating a banana for soothing relief.

Morning Sickness: Snacking on bananas between meals helps to keep blood sugar levels up and avoid morning sickness.

Mosquito bites: Before reaching for the insect bite cream, try rubbing the affected area with the inside of a banana skin. Many people find it amazingly successful at reducing swelling and irritation.

Nerves: Bananas are high in B vitamins that help calm the nervous system.

Overweight and at work: Studies at the Institute of Psychology in Austria found pressure at work leads to gorging on comfort food like chocolate and crisps. Looking at 5,000 hospital patients, researchers found the most obese were more likely to be in high-pressure jobs. The report concluded that, to avoid panic-induced food cravings, we need to control our blood sugar levels by snacking on high carbohydrate foods such as bananas every two hours to keep levels steady.

PMS: It is recommended that eating a banana works much better in PMS than popping in the pills. The vitamin B6 it contains regulates blood glucose levels, which can positively affect your mood.

Seasonal Affective Disorder (SAD): Bananas can help SAD sufferers because they contain the natural mood enhancer, trypotophan.

Smoking: Bananas can also help people trying to give up smoking, as the high levels of Vitamin C, A1, B6, B12 they contain, as well as the potassium and magnesium found in them, help the body recover from the effects of nicotine withdrawal.

Stress: Potassium is a vital mineral, which helps normalize the heartbeat, sends oxygen to the brain and regulates your body s water-balance. When we are stressed, our metabolic rate rises, thereby reducing our potassium levels. These can be re-balanced with the help of a high-potassiumbanana snack.

Medicine, eating bananas as part of a regular diet can cut the risk of death by strokes by as much as 40%!

Temperature control: Many other cultures see bananas as a cooling fruit that can lower both the physical and emotional temperature of expectant mothers. In Thailand, for example, pregnant women eat bananas to ensure their baby is born with a cool temperature.

Ulcers: The banana is used as the dietary food against intestinal disorders because of its soft texture and smoothness. It is the only raw fruit that can be eaten without distress in over-chronic ulcer cases. It also neutralizes over-acidity and reduces irritation by coating the lining of the stomach.

Banana peel contains lutein, an antioxidant from carotenoid family which provides nutritional support to the eyes. Lutein can also be found in green leafy vegetables, some fruits and corn. In its clinical tests, the research team exposed two groups of retina cells-one a control group and the other group soaked in a solution of banana peel extract-to strong light six hours a day for two days. A the end of the experiment the control group of retina cells had died while the group soaked in banana peel had regenerated and suffered no damage.

Taiwan researchers have discovered that banana peel extract can ease depression and protect the retina. A research team from Taichung's Chung Shan Medical University found after two years of research that banana peel is rich in serotonin, which is vital to balancing moods. The team also found that banana peel extract could protect the retina damage caused by light because it can cause retina cells to regenerate.

The appreciable high content of potassium in the peel signifies that if the peel is taken, it will help in the regulation of body fluids and maintained normal

reported for the fruit. Iron carries oxygen to the cells and is necessary for the production of energy, synthesis of collagen and the proper functioning of the immune system. Its low concentration implies that banana peel will be an idyllic source of iron since its excess is implicated in abnormal functioning of the immune system, cell growth and the heart. Manganese known to aid formation of skeletal and cartilage was also found to be high . Manganese dearth is scarce but could affect glucose tolerance, normal reproductive, skeletal and cartilage formation. Banana peels are good sources of nutrients, carbohydrates and fibre.

The study of the anti-nutrient content of the peel indicates generally low values except saponins. This means that if the peels are properly processed could be good source of feed for livestock. Antifungal and antibiotic principles are found in the peel and pulp of fully ripe bananas.

More than just a tasty fruit, the skin of a banana contains esterified fatty acids, which can be used as a skin lotion. Natives rub banana peels on their skin problems; which is supported by modern research, which says that banana peels used on red, scaly patches of psoriasis will provide a natural relief. Exorex lotion is a patented lotion created from the isolation of the esterified fatty acids and is sold commercially.

Ethyl acetate and water soluble fractions of green banana peel displayed high antimicrobial and antioxidant activity. Most of the compounds isolated from green peel ß-sitosterol, malic acid, 12-hydroxystrearic acid and succinic acid, which showed significant antibacterial activities and low antioxidant activities. While, those compounds isolated from water soluble extracts glycoside and monosaccharide components displayed significant antioxidant and low antimicrobial activity (Kanazawa, Shakakibara., 2000).

The antibiotic acts against Mycobacteria. A fungicide in the peel and pulp

pulp. The first two elevate blood pressure; serotonin inhibits gastric secretion and stimulates the smooth muscle of the intestines.

There is a great deal of antioxidant activity in banana peel and it could be a very inexpensive source of extracts rich in bioactive compounds, as previously suggested by Someya et al. (2002). Extracting banana peel with acetone:water was not only very efficient but also produced extracts with high antioxidant capacity, as confirmed by various model systems. This may be due to variation in the quality and quantity of phenolic compounds and other bioactive compounds present in the different extracts, such as catecholamines and anthocyanins (ascorbic acid, tocopherols and were not detected). The antioxidant activities of banana peel extracts obtained from different cultivars (''Grande Naine" and ''Gruesa") were similar. However, the impact of other extraction conditions, such as time or temperature, should be studied in greater depth.

MATERIALS AND METHODS

3.0. MATERIALS AND METHODS

3.1. Chemicals used

1,1-Diphenyl-2-Picryl hydrazyl (DPPH), methanol, Ammonium persulfate, 2,2 azobis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS), acetone, sodium nitroprusside, sulphanilamide, napthyl ethylene diamine dihydrochloride, o-phosphoric acid, deoxyribose, Ethylene diamine tetraacteic acid (EDTA), ferric chloride, trichloro acetic acid (TCA), thiobarbituric acid (TBA), ferrous sulphate, acetic acid, sulfuric acid, ammonium molybdate, disodium hydrogen phosphate, potassium ferricyanide, hydrogen peroxide, sodium nitrate etc.

All reagents used were of the analytical grade.

3.2. Banana specimens used

The following bananas varieties that were available commercially from the market were used:

1) Kadali – Musa sp. Cv. Kadali [AB],
2) Karpooravalli – Musa sp. Cv. Karpooravalli [ABB]
3) Monthan – Musa sp. Cv. Monthan [ABB]
4) Nendran – Musa sp. Cv. Nendran [AAB]
5) Poovan – Musa sp. Cv. Poovan [AAB]
6) Pachainadan – Musa sp. Cv. Pachainadan [AAA]
7) Rasdali – Musa sp. Cv. Rasdali [AAB]
8) Red – Musa sp. Cv. Red [AAA]
9) Robusta – Musa sp. Cv. Robusta [AAA].

The aforementioned banana specimens was authenticated by Dr.T.S.Balamohan, Professor and Head, Faculty of Horticulture , Tamil Nadu Agricultural University, Coimbatore.

3.3. Methods

3.3.1. Phase I

Different methods were used to estimate the free radical scavenging activity of the various varieties of banana peel extracts by *in vitro* antioxidant capacity assays.

3.3.1.1. Preparation of the banana peel extracts:

10 gram of shade dried and powdered sample, mixed with 100 ml ethanol. Then it was kept in a orbital shaker for 24 hrs at 37°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.

3.3.1.2. Determination of Total antioxidant capacity

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

95°C for 90 minutes. The tubes were then cooled to room temperature and the absorbance was measured at 695 nm 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; Umamaheshwari, Chatterjee) 2008).

3.3.1.2 Determination of DPPH radical scavenging activity.

(Ajay Sharma *et al.*, 2007).

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 changes from purple to yellow after reduction, which is quantified by the decrease of absorbance at 517nm.

Reagents

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

Procedure

The banana peel extracts were dissolved in ethanol. A solution of DPPH in methanol (0.004%)was prepared freshly. 4ml 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

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

3.3.1.2 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.

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 upto 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

3.3.1.4 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 chromophore is formed due to the diazotization of nitrite ions with sulfanilamide and subsequent coupling with naphthyl ethylene diamine. This is measured at 546 nm

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.

3.3.1.5 Determination of Hydroxyl radical scavenging activity

(Umamaheshwari, Chatterjeel., 2008).

Principle

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

Reagents

1) 2-deoxyribose(28mM in 20mM KH_2PO_4 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 1.04mM 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 Chatterjee., 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.

3.3.1.6 Determination of superoxide radical scavenging activity

Reagents

Phosphate buffer

Riboflavin

EDTA

NBT

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.

3.3.1.7 Determination of Lipid peroxidation inhibition activity

(Okhawa *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

Reagents

Phosphate buffer saline(pH 7.4)

0.07M Ferrous sulphate

20% acetic acid (pH 3.5)

0.8%TBA in 1.1% SDS

20% TCA

Butan-1-ol

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 cheesecloth 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 (1-10mg/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. About 5ml of butan-1-ol was added to each tube. The solution was mixed well and centrifuged at 3000 rpm for 10 min. The upper layer was withdrawn and used to read the absorbance at 532nm. The control contained PBS instead of the sample. The percentage inhibition was calculated.

3.3.1.8 Determination of Ferric Reducing Antioxidant Potential

(Benzie and Strain., 1996)

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 (1-10mg/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.

3.3.1.9 Determination of β Carotene bleaching activity

(Mi-Yae *et al.*, 2002)

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 (4.8ml) of this emulsion are transferred into different test-tubes containing 0.2ml of different concentrations of the sample (1-5mg/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

3.3.2 Phase II

3.3.2.1 Extract preparation

10 gram of shade dried and powdered sample, mixed with 100ml ethanol. Then it was kept in a orbital shaker for 24hrs at 37°C, after which the solution was filtered and the filtrate was dried completely. The dried extract was then

3.3.2.2 Stock preparation

100 mg of ethanolic banana peel extract diluted in 1 ml of autoclaved distilled water.

The following microorganism strains were used:

- *Staphylcoccus aureus,*
- *Bacillus subtilis*
- *Escherichia coli.*

3.3.2.3 Inoculum Preparation

Bacterial Inoculum

The inoculum was prepared using gram positive and gram-negative bacterial from a 24 hours old culture. With a sterile loop, the tops of four to five colonies were transferred to a tube containing 5mL of Nutrient broth. The tube was incubated at 35 °C for 24 hours. The turbidity of the culture suspension was adjusted with broth or a sterile saline solution (1%).

Test Medium

The well diffusion methods is performed using nutrient agar medium.

3.3.2.4 Antimicrobial Susceptibility Testing

Well Diffusion Method

The well diffusion test was performed using Nutrient agar medium, as per the procedure described by Magaldi *et al* (2004) and the medium was autoclaved at 121°C for 15minutes then was immediately cooled to 50-55°C in a water bath after removing it from the autoclave. The cooled medium was poured into sterile petri plates to a uniform depth of 4mm; this is equivalent to

density of the inoculums, a sterile cotton swab was dipped into the standardized bacterial suspension or inoculated with 1mL of the organism suspension. The sterile swab was used on the surface of the Nutrient agar medium to ensure an even distribution of the inoculums. The plates were undisturbed for 3 to 5 minutes to ensure absorption of excess moisture. Sterilized 9mm cork borer was used to make agar wells, 100 µL of the diluted sample stock solutions were placed into each wells. The plates were incubated at 35- 37 °C for 24 hours.

The percentage (%) of inhibition was calculated by using the formula.

% of inhibition =I (Diameter of the inhibition zone in mm) /90 (Diameter of the petri-plates in mm) x 100

3.4 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

4.0 RESULTS AND DISCUSSION

Reactive oxygen species (ROS) and other radicals are involved in a variety of biological phenomena, such as mutation, carcinogenesis degenerative and other diseases, inflammation, aging, and development. ROS are well recognized for playing a dual role as deleterious and beneficial species.

Due to the increased prevalence of chronic degenerative diseases, people are more aware of the food consumption. Nutritionalists believe that the degeneration of cell occurred from the reaction of free radicals is one of the important factor related to chronic diseases.

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

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

Antioxidants are those substances that when present in low concentrations compared to those of an oxidizable substrate significantly delays

organism and therefore, a number of studies have evaluated the effect of various antioxidants *in vitro* and *in vivo* studies.

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. The peel could be a potential source of antioxidant and antimicrobial activities. A Chinese study showed that eating a single banana made cholesterol in the blood less likely to cause damage to the arteries. The prevailing view is that oxidised LDL plays a major role in the formation of plaques leading to heart disease, so anything that reduces this risk is good news. A second study from Korea revealed that the flavonoid compounds in bananas prevented oxidative stress-induced neurotoxicity. The authors suggested that the protection of nerve cells by the flavonoids could reduce the risk of Alzheimer's Disease. The third study was conducted in India on rats and showed that when they received banana flavonoids, their body increased the amount of natural antioxidants their body could make. They had higher levels of superoxide dismutase and catalase, two powerful natural antioxidants that the body uses to protect itself from free radicals.

Banana peel is rich in phytochemical compounds, mainly antioxidants. The total amount of phenolic compounds in banana (*Musa acuminata* Colla AAA) peel ranges from 0.90 to 3.0 g/100 g DW identified gallocatechin at a concentration of 160 mg/100 g DW. Ripe banana peel also contains other compounds, such as the anthocyanins delphinidin and cyaniding, and catecholamines. Furthermore, carotenoids, such as b-carotene, a-carotene and different xanthophylls, have been identified in banana peel in the range of 300–400 lg lutein equivalents/100 g, as well as sterols and triterpenes, such as b-sitosterol, stigmasterol, campesterol, cycloeucalenol, cycloartenol, and 24-

antioxidant activity in banana peel, measured as the effect on lipid autoxidation, in relation to its gallocatechin content.

Potential applications for banana peel depend on its chemical composition. Banana peel is rich in dietary fibre (50% on a dry matter (DW) basis), proteins (7% DW), essential amino acids, polyunsaturated fatty acids and potassium. Attempts at the practical utilization of banana by-products include the production of biomass, protein, ethanol, methane, pectins and enzymes Banana peel has also been used as food for livestock or as an adsorbent for water purification.

Phase I

4.1. Free radical scavenging assays

4.1.1. Total antioxidant capacity assay

The total antioxidant activities of various banana peel extracts are depicted in Table 4.1.1.1. The total antioxidant assay gives an estimate of the overall antioxidant potential of the banana peel. There is a formation of phosphomolybdenum complex, the intensity of which indicates the potential of the peel as a scavenger of free radicals. The total antioxidant capacity of banana peel extracts was expressed as number of equivalents of ascorbic acid.

Table 4.1.1.1 Total antioxidant activity of ethanolic extracts of banana peel varieties expressed as ascorbic acid equivalents.

Extract	Ascorbicacid equivalent(AAE) mM g^{-1}
Kadali	$4.89^{d} \pm 0.33$
Karpooravali	$3.49^{b} \pm 0.02$
Monthan	$4.73^{d} \pm 0.06$
Nendran	$2.64^{a} \pm 0.06$
Poovan	$3.59^{b} \pm 0.03$
Pachainadan	$\mathbf{5.85^{e}} \pm 0.11$
Rasdali	$3.39^{b} \pm 0.09$
Red	$4.04^{c} \pm 0.09$
Robusta	$4.79^{d} \pm 0.11$

Values represent mean ± SD of 3 replicates.

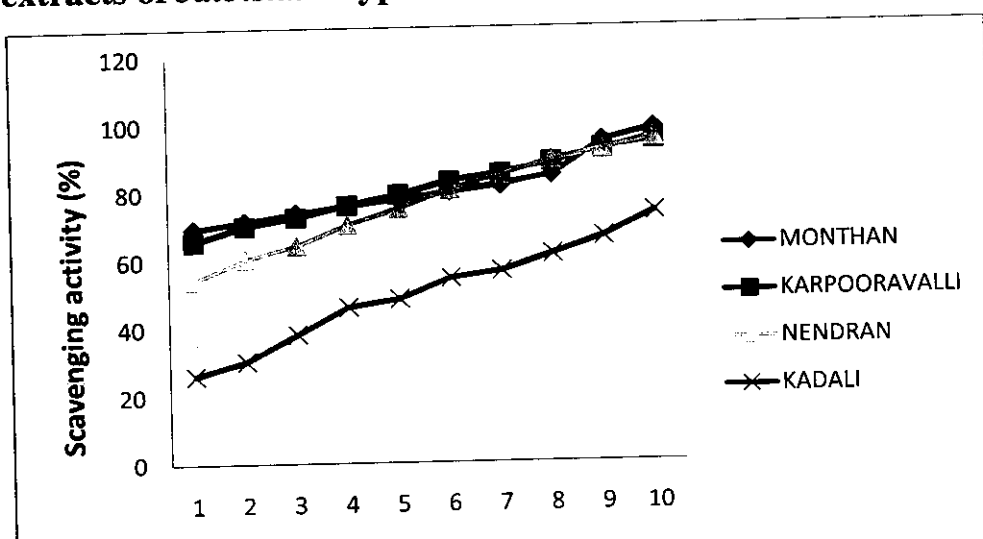
Different peel extracts exhibited various degrees of antioxidant capacity .The ethanolic extract of Pachainadan showed higher activity in the range of 5.85 mM g^{-1} in comparison to other varieties of banana peel, whereas the ethanolic extract of Nendran showed least activity.

Similar studies by Gonzalez-Montelongo., *et al* (2010) showed the total antioxidant activity activity of banana peel extract under different solvent and incubation condition.

4.1.2. DPPH radical scavenging activity

DPPH is nitrogen centered free radical that shows strong absorbance at 517 nm. Deep violet coloured methanolic DPPH solution changes to yellow colour in presence of DPPH radical scavengers. DPPH radical accepts an electron or hydrogen radical to become a stable diamagnetic molecule. 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).

Figure 4.1.2.1 Scavenging activity (%) on DPPH radical by ethanolic extracts of *balbisiana* type of banana varieties.



DPPH radical scavenging activity (%) on ethanolic extract of nine varieties of banana peel

Sample concentration(mg/ml)										EC_{50} (mg/ml)
1	2	3	4	5	6	7	8	9	10	
2^{g}±1.84	30.41^{f}±1.94	38.22^{d}±2.97	46.17^{d}±1.65	48.51^{e}±1.01	54.45^{e}±1.22	56.51^{g}±1.47	61.33^{g}±0.82	66.41^{f}±1.57	73.58^{d}±1.55	**5.80**
8^{f}±1.33	35.54^{e}±1.63	40.84^{d}±1.84	46.48^{d}±1.64	50.74^{e}±0.75	53.28^{e}±2.06	57.74^{g}±0.96	65.85^{f}±1.94	77.01^{e}±2.04	77.10^{c}±3.09	6.47
6^{a}±0.99	71.55^{a}±1.47	73.88^{a}±1.84	75.88^{a}±0.94	77.80^{c}±1.11	79.95^{b}±1.01	81.66^{cd}±1.46	84.54^{d}±1.08	94.68^{ab}±1.59	**98.19^{a}±1.33**	6.39
3^{d}±2.55	60.50^{c}±1.55	64.42^{b}±3.42	70.40^{b}±1.55	75.06^{c}±2.13	80.43^{b}±2.55	84.55^{c}±1.79	89.30^{ab}±1.48	92.15^{bc}±1.40	95.78^{a}±1.63	6.28
5^{c}±2.94	63.59^{b}±3.17	73.19^{a}±2.51	78.45^{a}±2.0	83.63^{a}±2.05	87.77^{a}±1.79	91.84^{a}±1.79	91.93^{a}±1.72	95.57^{a}±1.45	97.74^{a}±1.18	6.60
9^{e}±2.48	47.05^{d}±2.45	53.07^{c}±2.28	58.03^{c}±1.39	64.82^{d}±1.39	71.16^{c}±1.46	77.36^{e}±1.51	86.68^{bcd}±3.14	89.98^{cd}±4.11	97.93^{a}±1.82	**5.80**
4^{e}±2.03	47.64^{d}±1.46	53.22^{c}±1.51	56.90^{c}±1.93	61.95^{d}±1.68	65.25^{d}±1.49	70.54^{f}±1.39	76.94^{c}±1.05	78.89^{c}±1.69	88.00^{b}±2.6	6.07
3^{b}±1.20	70.28^{a}±1.52	72.69^{a}±2.06	76.15^{a}±1.08	79.15^{b}±0.98	82.98^{b}±1.09	85.35^{b}±0.65	88.71^{bc}±1.56	92.49^{abc}±2.16	94.93^{a}±0.99	6.64
2^{cd}±2.05	57.96^{c}±1.49	64.82^{b}±1.51	70.6^{b}±1.84	76.14^{c}±1.66	81.22^{b}±2.01	81.45^{d}±1	86.00^{cd}±1.44	88.17^{d}±1.81	90.10^{b}±1	6.44
10	20	30	40	50	60	70	80	90	100	
48.43	55.58	64.67	73.55	79.05	84.56	87.04	90.56	93.45	96.45	

mean ± SD of 3 replicates.

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

Figure 4.1.2.2 Scavenging activity (%) on DPPH radical by ethanolic extracts *acuminate* type of banana varities.

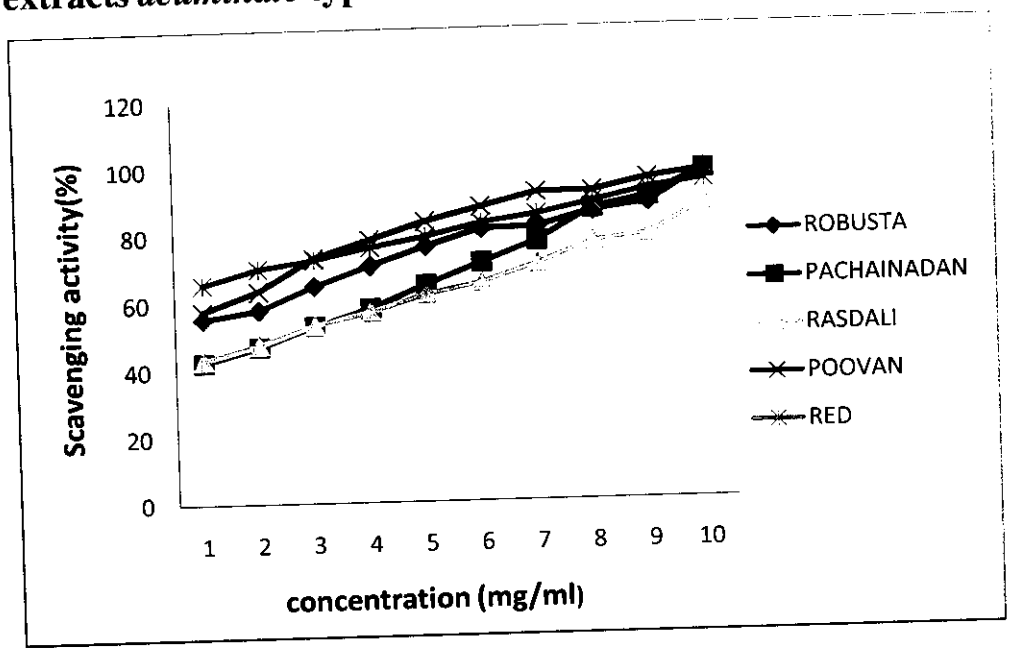
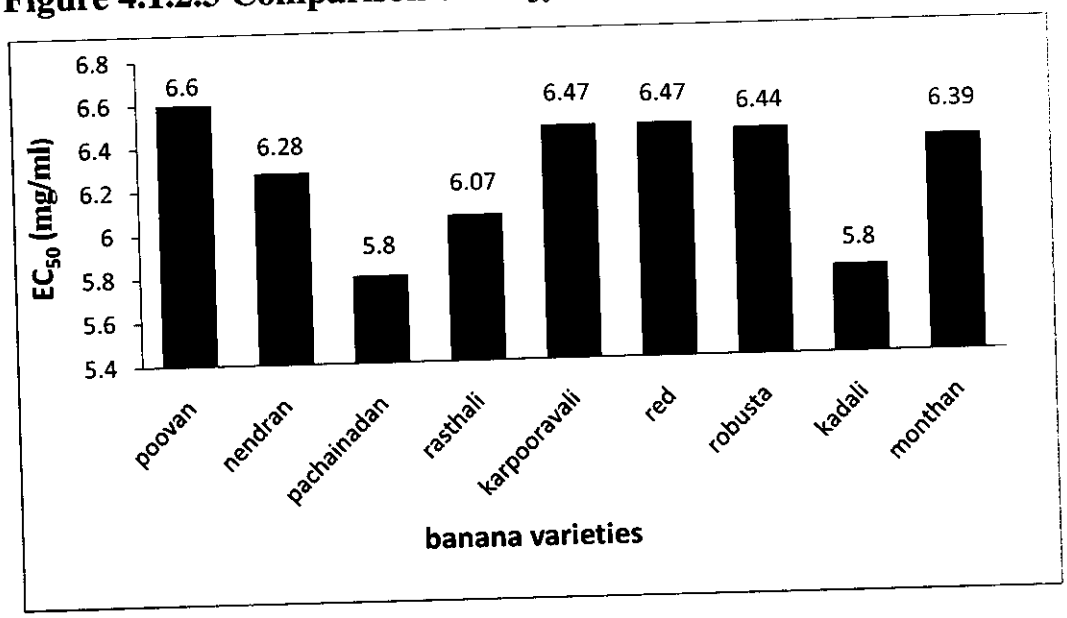


Figure 4.1.2.3 Comparison of EC_{50} values of DPPH inhibition activity



Free radical scavenging potential of the ethanolic extracts of local varieties of banana peel is shown in Table 4.1.2.1 which increases with the increase in concentration.

At 1-10 mg ml^{-1}, the ethanolic extract of Kadali, Karpooravalli, Monthan, Pachainadan, Rasdali, Red and Robusta showed the

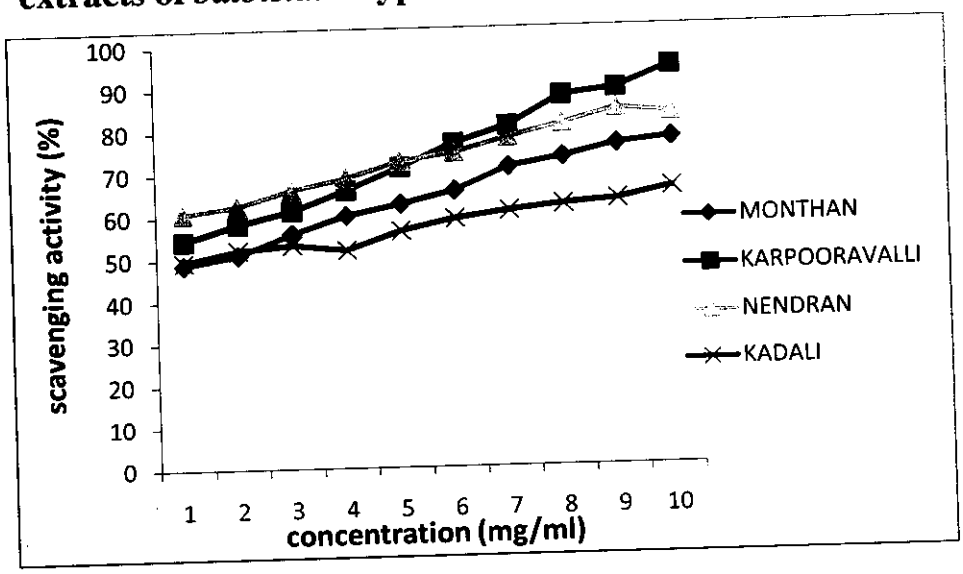
95.78%, 57.75-97.74%, 42.59-97.73%, 43.34-88.00%, 65.73-94.93%, and 55.52-90.10% respectively. However, at 10mg ml^{-1}, Mondhan peel extract exhibited highest DPPH scavenging activity. With respect to the EC_{50} value, Pachainadan and Kadali showed least value, which represent highest antioxidant activity. Statistically, the scavenging activity of antioxidants was effective in the order of Mondhan > Pachainadan > Poovan > Nendran > Red > Robusta > Rasdali > Karpooravalli > Kadali.

Studies by Gonzalez-Montelongo et al (2010) showed the scavenging activity of banana peel extract on DPPH radical under different solvent and different incubation conditions.

4.1.3. ABTS cation radical scavenging activity

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

Figure 4.1.3. Scavenging activity (%) on ABTS radical by ethanolic extracts of *balbisiana* type of banana varieties.



TS cation radical scavenging activity (%) of ethanolic extract of nine varieties of banana peel

Sample concentration(mg/ml)										EC_{50} (mg/ml)
1	2	3	4	5	6	7	8	9	10	
^{f}±1.87	52.08^{e}±1.56	53.23^{f}±1.45	51.86^{f}±0.34	56.23^{f}±1.56	58.90^{e}±1.45	60.61^{e}±0.69	61.96^{f}±1.76	62.92^{e}±0.67	65.58^{d}±1.95	6.56
^{e}±2.09	58.24^{d}±1.98	61.26^{e}±1.56	65.96^{b}±1.98	71.19^{d}±2.55	76.63^{c}±1.90	80.55^{c}±0.87	87.33^{bc}±1.50	89.07^{b}±0.49	94.16^{a}±0.47	6.17
^{f}±2.76	51.01^{e}±1.34	55.90^{f}±1.09	59.98^{e}±2.34	62.56^{e}±1.65	65.46^{d}±1.04	70.89^{d}±0.65	73.06^{e}±1.89	75.94^{d}±0.38	77.23^{c}±1.56	**6.33**
^{d}±1.98	62.55^{d}±1.98	66.12^{d}±1.30	68.77^{cd}±1.96	72.49^{d}±1.37	76.19^{c}±1.50	78.77^{c}±1.09	82.30^{d}±1.06	89.37^{b}±1.49	93.78^{a}±0.96	6.57
^{bc}±1.85	73.83^{b}±2.03	77.01^{b}±1.37	79.53^{b}±0.45	82.10^{ab}±1.90	85.03^{b}±1.70	87.50^{b}±1.45	90.27^{ab}±1.34	93.95 ^{a}±1.38	95.02^{a}±0.45	6.56
^{c}±2.80	69.23^{c}±1.49	70.97^{c}±1.98	72.4^{c}±0.39	74.38^{cd}±1.34	77.76^{c}±1.21	80.82^{c}±0.95	84.21^{cd}±1.56	83.08^{c}±1.39	89.07^{b}±1.76	6.44
^{a}±1.56	82.92^{a}±1.78	85.32^{a}±1.34	86.58^{a}±1.49	88.77^{bc}±1.34	90.43^{a}±1.10	93.27^{a}±1.34	93.68^{a}±1.45	95.77^{a}±0.97	**97.66^{a}±0.23**	6.73
^{ef}±1.30	51.91^{e}±1.37	54.73^{f}±1.20	56.16^{ef}±1.89	58.97^{ef}±1.45	52.38^{dc}±1.21	65.14^{e}±1.49	70.68^{e}±1.67	74.08^{d}±0.99	80.92^{c}±0.45	5.11
^{b}±1.98	76.37^{b}±1.56	77.86^{b}±1.00	81.57^{b}±1.55	85.94^{a}±1.98	88.09^{ab}±1.06	89.16^{ab}±1.46	92.12^{ab}±1.94	95.32^{a}±1.45	96.41^{a}±1.56	6.71
10	20	30	40	50	60	70	60	90	100	
8.56	23.67	34.54	41.56	46.67	52.69	57.68	67.90	77.67	87.67	

mean ± SD of 3 replicates.

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

Figure 4.1.3.2 Scavenging activity (%) on ABTS radical by ethanolic extracts *acuminate* type of banana varities.

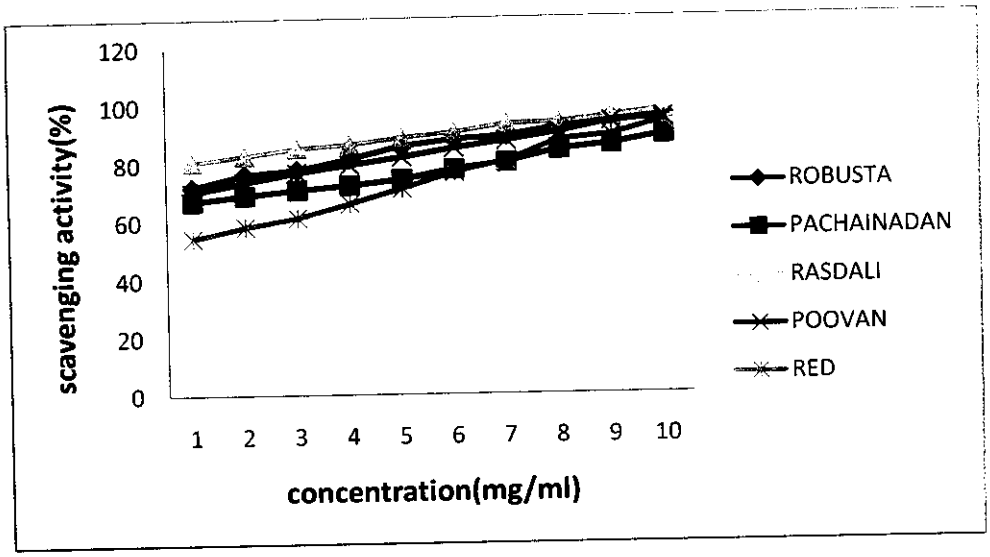
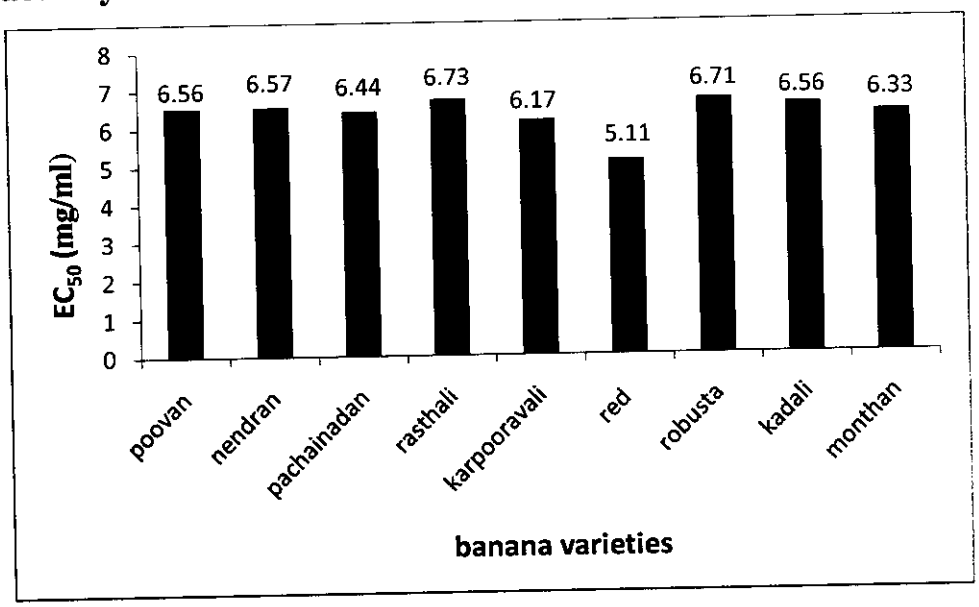


Figure 4.1.3.3 Comparison of EC_{50} values of ABTS radical scavenging activity



Free radical scavenging potential of the ethanolic extracts of local varieties of banana peel is shown in Table 4.1.3.1 which increases with the increase in concentration

At 1 -10 mg ml^{-1}, the ethanolic extract of the ethanolic extract of Kadali, Karpooravalli, Monthan, Nendran, Poovan, Pachainadan. Rasdali, Red and

48.82%-77.24%, 60.79%-93.78%, 70.43%-95.02%, 67.20%-89.07%., 80.72%-97.66%, 50.52%-80.92%,72.19%-96.41% respectively. However,at 10mg ml^{-1}, Rasthali peel extract exibit highest ABTS scavenging activity.with respect to the EC_{50} value of Red peel showed least value, which represent highest antioxidant activity. Statistically, the scavenging activity of antioxidants was effective in the order of Rasdali> Robusta> Poovan> Karpooravalli >Nendran > Pachainadam > Red> Monthan >Kadali.

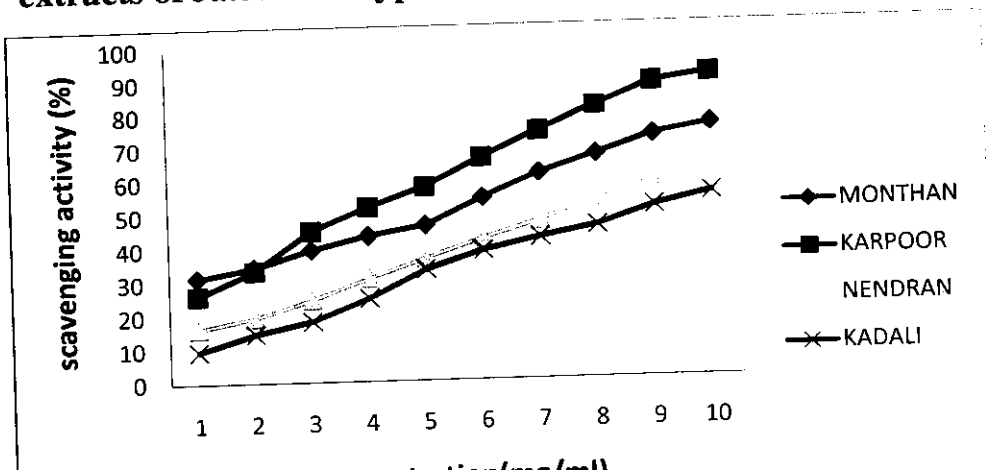
Similar studies by Okonogi et al (2007) determine the scavenging activity of banana peel extract on ABTS radical.

4.1.4. 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; 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 4.1.4.1 Scavenging activity (%) on nitric oxide radical by ethanolic extracts of *balbisiana* type of banana varieties.



ric oxide radical scavenging activity (%) on ethanolic extract of nine varieties of banana peel

Sample concentration(mg/ml)										EC_{50} (mg/ml)
1	2	3	4	5	6	7	8	9	10	
$.19^{de}$±2.12	37.67^{bcd}±1.19	62.47^{b}±1.79	64.59^{b}±2.05	67.90^{b}±1.56	70.30^{b}±1.56	72.06^{b}±1.45	76.47^{b}±1.90	79.58^{b}±0.65	85.02^{b}±0.93	6.54
$.51^{de}$±2.19	33.64^{d}±2.67	36.52^{d}±2.67	38.34^{f}±2.45	42.41^{e}±1.39	44.38^{f}±1.45	47.18^{e}±1.56	47.97^{f}±1.24	44.63^{f}±1.23	58.03^{de}±1.45	6.27
97^{bcd}±2.47	38.19^{bc}±2.17	41.88^{c}±2.34	43.55^{de}±1.85	46.57^{d}±0.67	51.06^{d}±0.99	53.93^{d}±1.67	56.37^{d}±1.96	58.63^{d}±1.67	60.75^{d}±0.68	**6.15**
$.30^{b}$±2.34	40.13^{b}±2.37	45.41^{c}±1.96	50.73^{c}±1.45	55.99^{c}±1.65	59.55^{c}±0.98	61.10^{c}±2.05	64.80^{c}±1.49	69.57^{c}±1.46	72.85^{c}±0.49	6.35
$.62^{bc}$±1.95	37.21^{bcd}±2.34	44.26^{c}±2.87	46.19^{d}±1.93	47.84^{d}±0.93	48.66^{de}±1.65	50.22^{de}±1.68	52.09^{e}±0.67	54.25^{e}±1.70	56.43^{e}±0.79	6.62
$.19^{e}$±2.45	34.40^{cd}±1.76	36.79^{d}±2.29	40.19^{ef}±1.68	42.47^{e}±1.78	44.29^{f}±1.94	48.98^{e}±1.49	51.59^{ef}±0.63	53.48^{e}±0.76	55.95^{e}±1.09	6.19
$.49^{f}$±2.33	11.77^{e}±1.98	15.35^{ae}±1.98	17.20^{g}±1.96	19.41^{f}±2.03	20.37^{g}±2.78	22.04^{f}±1.87	24.56^{g}±0.97	27.48^{g}±0.49	35.75^{f}±1.47	5.80
$.08^{cd}$±1.34	34.62^{cd}±2.43	37.72^{d}±0.94	39.4^{f}±1.67	41.75^{e}±1.79	$45,44^{ef}$±1.94	47.47^{e}±1.95	53.45^{de}±1.29	56.30^{de}±0.83	61.07^{d}±1.69	5.88
7.83^{a}±2.45	81.09^{a}±2.12	83.60^{a}±0.95	86.89^{a}±1.95	91.99^{a}±2.03	94.35a±1.43	95.77^{a}±0.69	95.79^{a}±1.73	97.35^{a}±0.80	**97.78^{a}±1.90**	6.87
10	20	30	40	50	60	70	60	90	100	
43.34	48.67	52.78	57.45	62.45	66.78	70.78	75.67	81.99	87.56	

mean ± SD of 3 replicates.

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

Figure 4.1.4.2 Scavenging activity (%) on nitric oxide radical by ethanolic extracts *acuminate* type of banana varities.

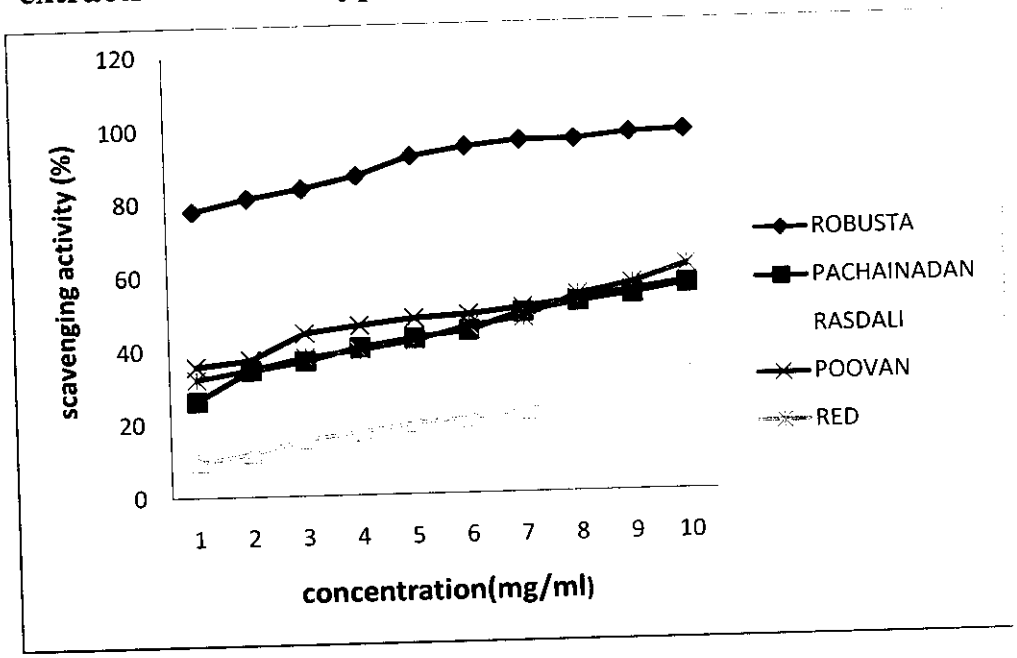
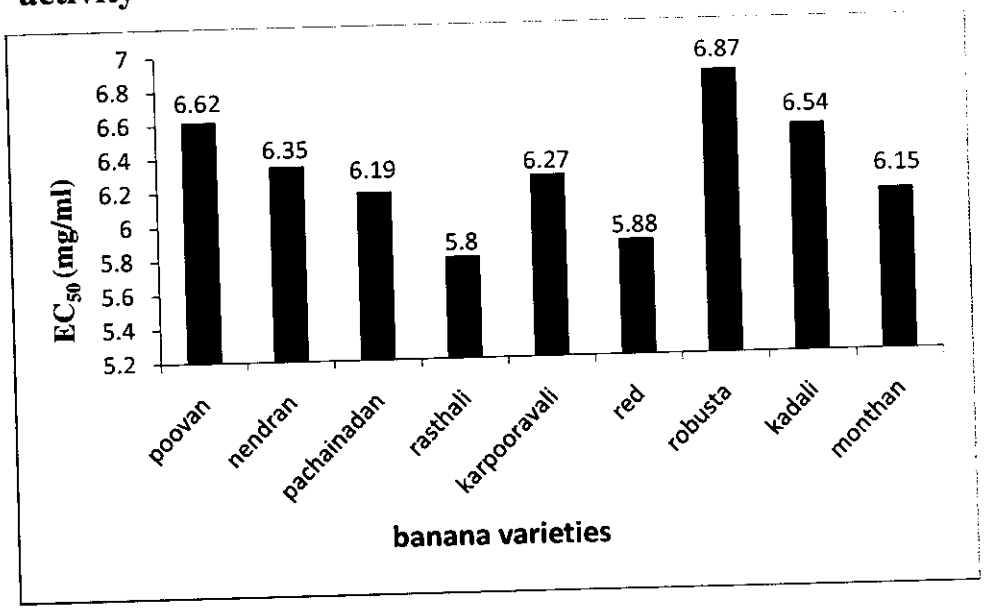


Figure 4.1.4.2 Comparison of EC_{50} values of Nitric oxide radical inhibition activity



Free radical scavenging potential of the ethanolic extracts of local varieties of banana peel is shown in Table 4.1.4.1 which increases with the increase in concentration

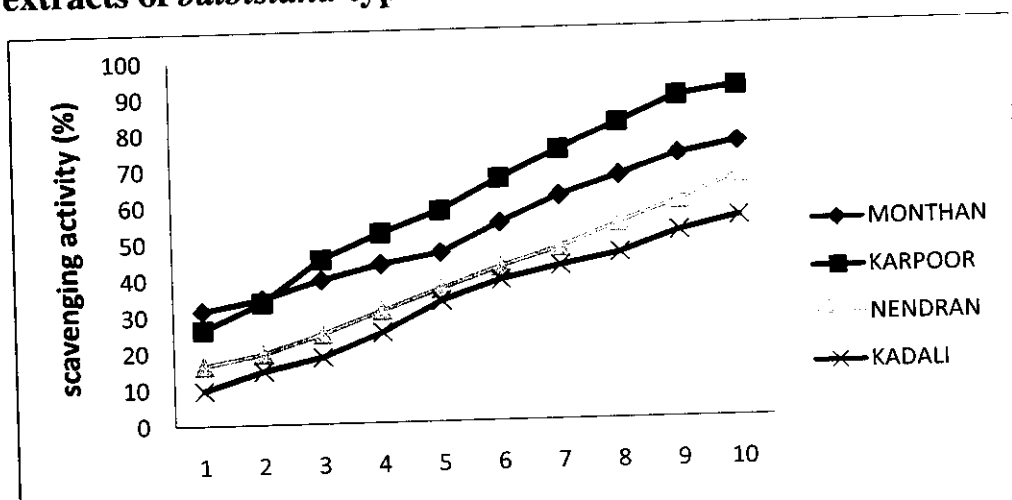
$mgml^{-1}$ the ethanolic extract of the ethanolic extract of

and Robusta shows the percentage inhibition of 29.19%-85.02%, 29.51%-58.03%, 32.97%-60.75%, 36.30%-72.85%, 35.62%-56.43%, 26.19%-55.95%, 9.49%-35.75%, 32.08%-61.07%, 77.83%-97.78% respectively. However, at 10mg ml^{-1}, robusta peel extract exibit highest nitric oxide scavenging activity. With respect to the EC_{50} value rasdali showed least value, which represent highest antioxidant activity Statistically, the scavenging activity of antioxidants was effective in the order of Robusta> Kathali> Nendran> Red> Mondan> Karpooravalli> Poovan> Pachainadan> Rasdali.

4.1.5. Hydroxyl radical scavenging activity

The ability of extracts to scavenge OH radical was assessed using the classic deoxyribose degradation assay described by Halliwell *et al* (2007). When EDTA chelated iron- (III) ions are incubated with reducing agent and H_2O_2 in the assay, OH radicals are generated in free solution that attack the deoxyribose substrate and fragmenting it into TBARS. The generated TBARS reflect the extent of generation of OH. Extent of hydroxyl radical scavenged was determined by the decrease in intensity of pink coloured chromophore in the form of EC_{50} values which was determined at 532nm. Lower EC_{50} value represents higher antioxidant activity.

Figure 4.1.5.1. Scavenging activity (%) on hydroxyl radical by ethanolic extracts of *balbisiana* type of banana varieties.



radical scavenging activity (%) on ethanolic extract of nine varieties of banana peel

Sample concentration(mg/ml)										EC_{50} (mg/ml)
1	2	3	4	5	6	7	8	9	10	
5^{f}±2.49	14.67^{f}±0.59	18.23^{f}±1.45	24.82^{f}±1.69	33.33^{f}±1.87	38.58^{f}±2.46	42.20^{g}±2.56	45.54^{f}±1.12	51.15^{g}±1.67	54.89^{f}±1.71	5.65
9^{b}±1.78	33.46^{b}±2.45	45.15^{a}±2.21	52.04^{a}±1.56	57.94^{ab}±1.56	66.44^{a}±2.50	74.11^{a}±1.69	81.42^{a}±1.05	88.52^{a}±1.39	90.87^{a}±1.42	5.57
9^{a}±1.90	34.43^{ab}±0.89	39.50^{b}±1.79	43.53^{b}±1.56	46.37^{c}±1.70	54.30^{c}±1.56	61.39^{c}±1.95	66.75^{c}±1.11	72.39^{d}±1.45	75.44^{c}±1.56	**5.37**
4^{c}±2.46	19.42^{e}±1.85	24.57^{e}±1.50	30.80^{e}±1.34	36.45^{e}±3.02	42.15^{e}±1.09	47.15^{f}±1.45	53.65^{e}±1.96	59.49^{f}±0.45	66.39^{e}±0.45	5.30
7^{c}±2.03	29.09^{c}±1.34	37.03^{c}±2.04	40.48^{c}±1.67	45.76^{c}±2.89	53.62^{cd}±2.40	61.57^{c}±2.48	69.05^{b}±1.34	77.25^{c}±1.40	80.37^{b}±1.23	5.20
8^{c}±2.67	29.50^{c}±0.67	35.66^{c}±1.76	39.28^{c}±2.04	44.36^{c}±1.69	51.39^{d}±2.01	54.38^{d}±2.34	60.06^{d}±0.95	64.33^{e}±1.95	71.83^{d}±1.14	5.62
7^{c}±1.69	24.46^{d}±2.08	31.77^{b}±0.56	45.19^{b}±1.06	57.11^{b}±1.96	63.43^{b}±1.49	71.55^{b}±1.97	82.51^{a}±1.43	88.91^{a}±0.87	**93.29^{a}±0.67**	5.64
7^{d}±1.50	36.51^{a}±0.68	44.46^{a}±0.96	53.16^{a}±1.56	59.59^{a}±1.69	67.86^{a}±1.98	72.01^{ab}±1.56	80.47^{a}±1.34	85.73^{b}±0.79	91.48^{a}±0.95	5.76
3^{e}±2.56	22.47^{d}±1.87	27.51^{d}±1.69	33.84^{d}±1.97	39.33^{d}±2.08	43.41^{e}±2.04	49.45^{e}±2.56	53.51^{e}±2.45	60.35^{f}±1.16	64.34^{e}±1.14	5.61
10	20	30	40	50	60	70	60	90	100	
9.20	14.45	21.78	28.67	33.27	38.63	44.56	50.67	58.67	65.78	

mean ± SD of 3 replicates.

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

Figure 4.1.5.2. Scavenging activity (%) on hydroxyl radical by ethanolic extracts *acuminate* type of banana varities.

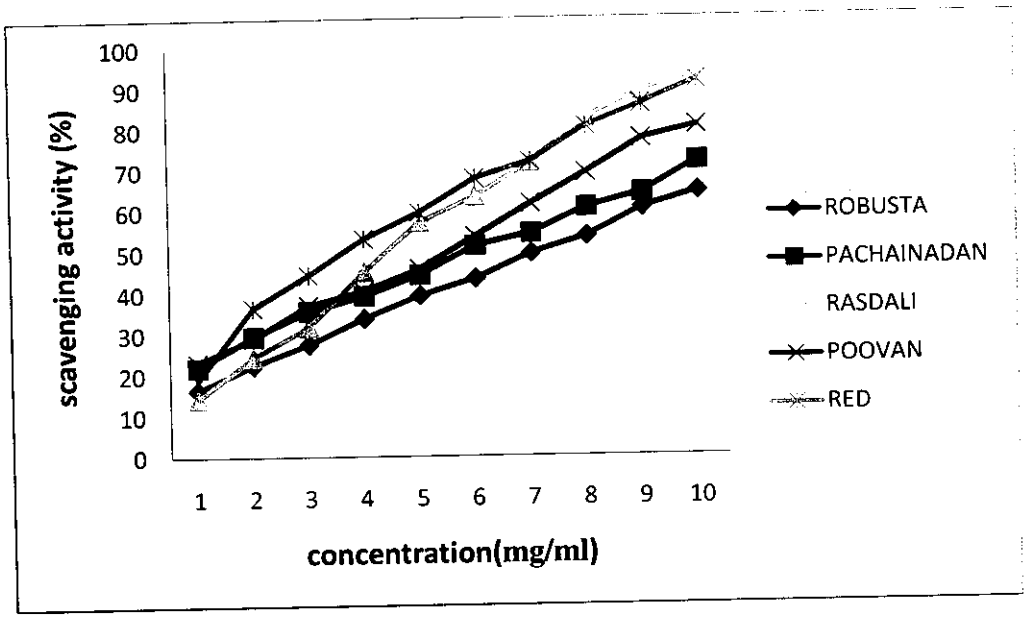
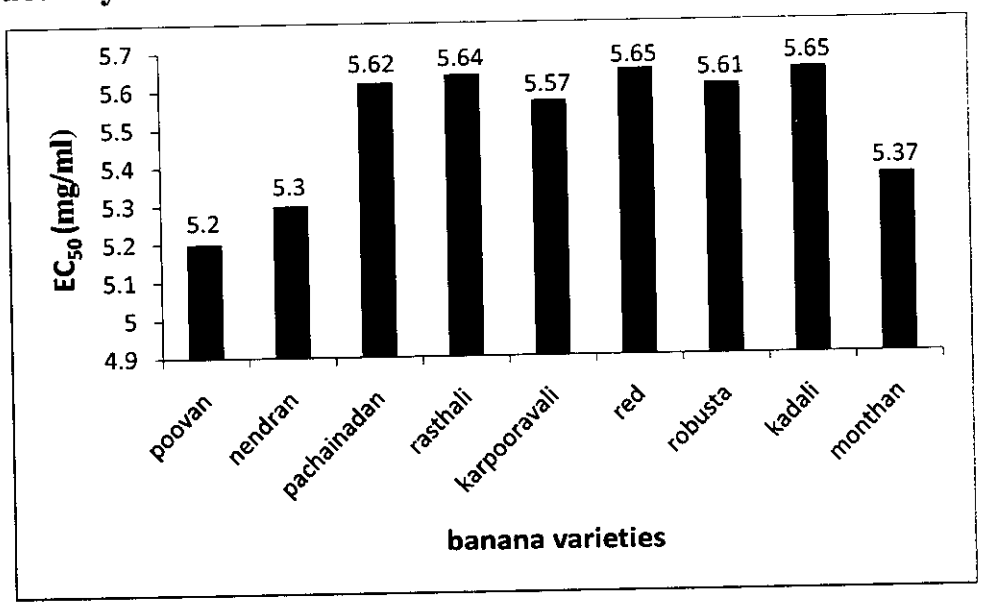


Figure 4.1.5.3 Comparison of EC_{50} values of hydroxyl radicals inhibition activity



Free radical scavenging potential of the ethanolic extracts of local varieties of banana peel is shown in Table 4.1.5.1 which increases with the increase in concentration

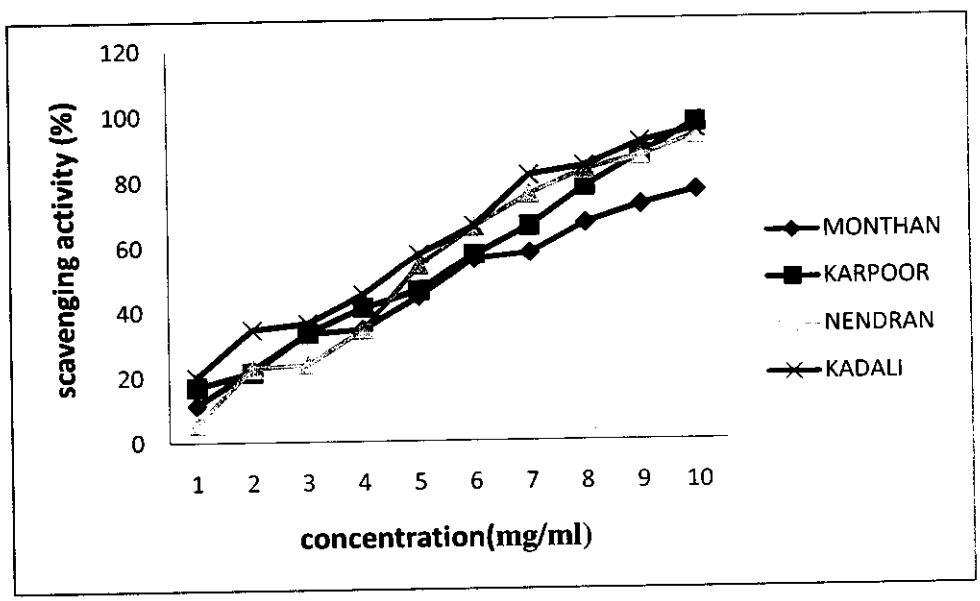
At 1-10 mg ml^{-1}, the ethanolic extract of the ethanolic extract of Kadali,

Robusta shows the percentage inhibition of 9.55%-54.89%, 26.19%-90.87%, 31.49%-75.44%, 16.34%-66.39%, 23.17%-80.37%, 21.98%-71.83%, 14.27%-93.29%,18.87%-91.48%, 16.43%-64.34% respectively. However, at 10mg ml^{-1}, Rasdali peel extract exibit highest hydroxy scavenging activity. With respect to the EC_{50} value Poovan showed least value, which represent highest antioxidant activity. Statistically, the scavenging activity of antioxidants was effective in the order of Rasdali> Red> karpooravali> Poovan> Monthan> Pachainadan> Nendran> Robusta >Kadali.

4.1.6 Superoxide radical scavenging activity

The superoxide radical scavenging assay is carried out to determine the ability of the banana peel 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 peel (Sakanaka and Tachibana 2006).

Figure 4.1.6.1. Scavenging activity (%) on superoxide radical by ethanolic extracts of *balbisiana* type of banana varieties.



peroxide radical scavenging activity (%) on ethanolic extract of nine varieties of banana peel

Sample concentration(mg/ml)										EC_{50} (mg/ml)
1	2	3	4	5	6	7	8	9	10	
08^{b}±0.93	34.53^{a}±1.35	36.34^{c}±2.01	45.15^{b}±2.00	57.51^{b}±1.27	66.28^{c}±1.05	81.55^{a}±1.87	84.22^{ab}±1.02	91.39^{b}±1.17	95.26^{bc}±0.89	5.42
03^{c}±1.75	21.37^{b}±1.49	33.44^{d}±1.70	41.09^{c}±0.95	46.37^{d}±2.94	57.34^{d}±1.14	66.16^{c}±3.17	78.00^{c}±1.45	87.66^{c}±0.87	97.34^{ab}±0.82	4.76
42^{d}±1.55	22.32^{b}±0.63	33.37^{d}±1.06	34.45^{e}±2.08	44.53^{de}±1.58	55.91^{de}±2.16	57.83^{d}±1.58	66.92^{d}±1.97	72.36^{e}±0.70	76.62^{f}±1.29	**5.34**
8^{e}±0.66	22.78^{b}±2.41	**23.77^{e}±1.84**	34.30^{e}±1.97	54.03^{c}±2.54	65.96^{c}±1.56	75.67^{b}±1.28	83.13^{b}±0.99	87.17^{cd}±1.69	93.31^{c}±1.11	5.42
27^{b}±1.99	22.35^{b}±1.11	34.22^{cd}±1.23	37.73^{d}±1.37	43.22^{e}±1.16	53.51^{e}±1.12	58.90^{d}±1.21	65.96^{d}±1.03	74.89^{e}±2.22	87.36^{d}±2.75	4.97
91^{b}±1.62	33.32^{a}±1.73	43.18^{b}±1.57	53.59^{a}±1.13	62.39^{a}±1.31	71.41^{b}±1.53	73.17^{d}±1.05	81.75^{b}±1.17	87.82^{c}±2.63	95.25^{bc}±1.01	5.98
87^{a}±1.44	34.40^{a}±1.29	44.87^{b}±1.59	55.26^{a}±1.49	56.40^{bc}±2.23	66.64^{c}±2.35	66.08^{c}±2.54	76.00^{c}±2.35	84.77^{d}±2.37	94.33^{c}±1.35	5.55
95^{a}±1.31	33.63^{a}±0.97	48.15^{a}±2.14	55.00^{a}±1.74	63.76^{a}±1.46	76.22^{a}±1.60	81.89^{a}±1.25	86.48^{a}±2.96	94.36^{a}±1.93	**99.22^{a}±1.10**	5.52
3^{e}±1.10	11.57^{c}±1.18	16.14^{f}±0.76	24.03^{f}±0.98	28.78^{f}±1.45	40.59^{f}±1.05	54.52^{e}±1.87	57.29^{e}±1.02	65.90^{f}±1.17	83.66^{e}±0.89	3.91
10	20	30	40	50	60	70	60	90	100	
34.34	42.89	51.65	56.65	62.56	68.23	75.45	82.78	86.34	98.45	

ean ± SD of 3 replicates.

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

Figure 4.1.6.2. Scavenging activity (%) on superoxide radical by ethanolic extracts *acuminate* type of banana varieties.

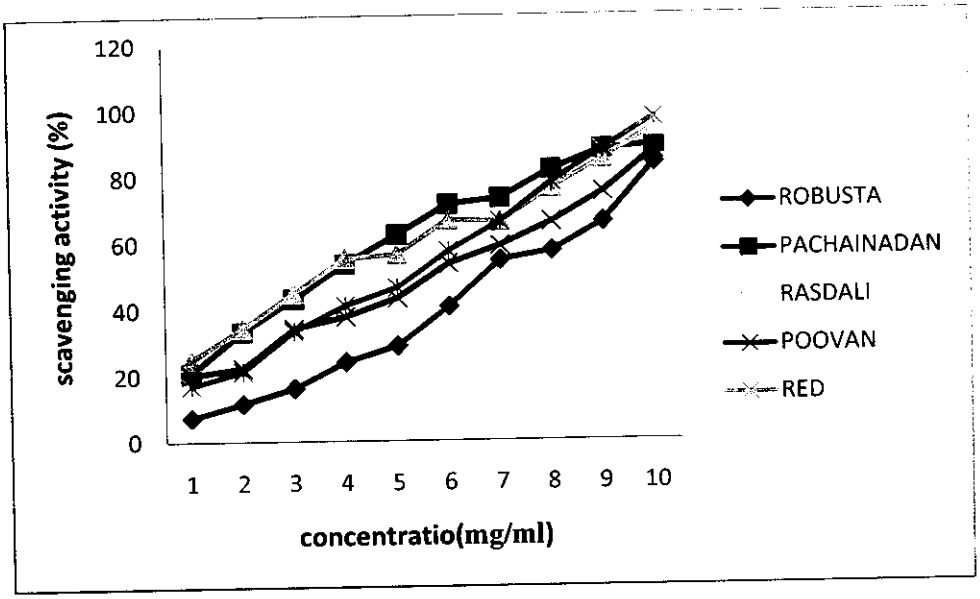
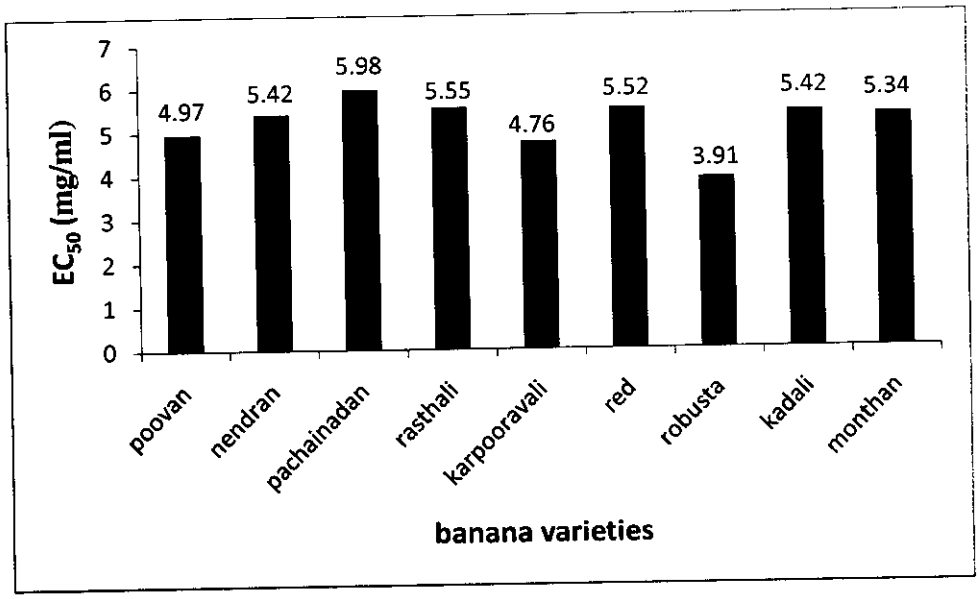


Figure 4.1.6.3 Comparison of EC_{50} values of superoxide radicals inhibition activity



Free radical scavenging potential of the ethanolic extracts of local varieties of banana peel is shown in Table 4.1.6.1 which increases with the increase in concentration

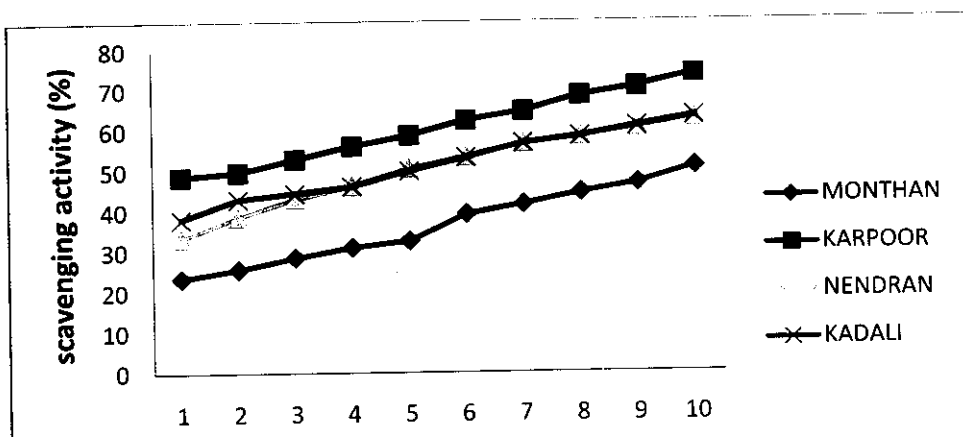
At 1 -10 mg ml^{-1}, the ethanolic extract of the ethanolic extract of

and Robusta shows the percentage inhibition of 20.08%-95.29%, 17.03%-97.34%, 11.42%-76.62%, 5.58%-93.31%, 20.27%-87.36%, 20.91%-95.25%, 24.87%-94.33%, 24.95%-99.22%, 7.33%-83.66% respectively. However, at 10mg ml^{-1}, red peel extract exibit highest super oxide scavenging activity. With respect to the EC_{50} value of Robusta showed least value, which represent highest antioxidant activity. Statistically, the scavenging activity of antioxidants was effective in the order of red > Karpooravali > Kadali > Pachainadan > Rasdali > Nendran >Poovan > Robusta > Monthan.

4.1.7. Inhibition of lipid peroxidation activity

Lipid peroxides, derived from polyunsaturated fattyacids, are unstable and decompose to form a complex series of compounds (Kulisic *et al* , 2006). These include reactive carbonyl compounds. The most abundant among them is malondialdehyde (MDA), one of the secondary lipid peroxidation products. These carbonyl products are responsible for DNA damage, generation of cancer and aging related diseases (Kulisic *et al* , 2006). Thus the decrease in the MDA levels in the presence of increased concentration of each extract indicates the role of extracts as antioxidants. TBARS assay was used to determine the anti-lipid peroxidation properties of the banana peel extracts.

Figure 4.1.7.1. Scavenging activity (%) on lipid peroxidation by ethanolic extracts of *balbisiana* type of banana varieties.



eroxidation inhibition (%) on ethanolic extract of nine varieties of banana peel

Sample concentration(mg/ml)										
1	2	3	4	5	6	7	8	9	10	EC_{50} (mg/ml)
.13^{b}±2.34	43.03^{h}±1.89	44.52^{c}±2.78	46.22^{c}±1.96	49.93^{c}±1.78	53.16^{cd}±0.78	56.73^{b}±0.98	59.33^{b}±1.06	60.74^{c}±1.78	63.20^{cd}±0.76	6.29
.70^{a}±1.89	49.70^{a}±1.78	52.94^{a}±2.45	56.16^{a}±2.11	58.74^{a}±1.65	62.39^{a}±1.79	64.57^{a}±1.79	68.48^{a}±1.96	70.54^{b}±1.95	73.70^{b}±0.56	6.33
.51^{e}±1.89	25.71^{i}±2.47	28.57^{i}±2.07	31.03^{i}±1.70	32.60^{g}±2.12	39.15^{g}±1.78	41.59^{f}±1.56	44.47^{e}±1.74	46.70^{e}±1.76	50.73^{f}±1.96	**5.56**
.35^{c}±1.95	38.66^{cd}±1.56	43.10^{c}±1.45	46.23^{c}±1.67	50.64^{c}±1.79	53.33^{cd}±1.11	56.62^{b}±1.73	58.42^{b}±0.89	60.41^{c}±0.67	62.59^{d}±1.67	6.43
.68^{e}±2.06	39.23^{c}±1.91	50.05^{b}±1.45	53.66^{b}±1.90	55.45^{b}±1.95	59.30^{b}±0.69	63.78^{a}±1.89	69.06^{a}±0.50	73.30^{a}±0.95	**76.30^{a}±0.65**	6.06
.35^{f}±2.78	22.59^{g}±2.58	30.45^{i}±1.67	47.38^{c}±1.89	50.88^{c}±2.05	53.72^{c}±1.49	55.55^{b}±1.59	60.00^{b}±1.86	60.46^{c}±0.67	65.29^{c}±1.03	6.56
.75^{d}±2.06	32.04^{e}±1.89	37.32^{e}±1.57	38.83^{e}±1.94	41.85^{e}±1.69	44.47^{e}±1.67	49.56^{d}±1.74	52.49^{c}±1.78	58.90^{c}±0.89	62.49^{d}±1.23	5.82
.67^{cd}±1.89	36.32^{d}±2.06	40.45^{d}±1.89	43.45^{d}±2.09	45.92^{d}±0.89	50.90^{d}±0.39	52.81^{c}±0.67	54.86^{c}±0.79	58.03^{c}±1.74	61.91^{d}±1.09	6.12
.66±^{f}1.95	23.02^{g}±1.97	28.57^{i}±1.95	32.87^{i}±1.76	36.65^{i}±0.79	42.72^{i}±0.96	46.90^{e}±1.59	47.94^{d}±1.89	50.93^{d}±1.86	55.70^{e}±1.00	5.76
10	20	30	40	50	60	70	60	90	100	
5.56	11.34	17.45	22.45	29.19	37.67	43.90	52.45	61.95	70.5	

mean ± SD of 3 replicates.

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

Figure 4.1.7.2. Scavenging activity (%) on lipid peroxidation by ethanolic extracts *acuminate* type of banana varities.

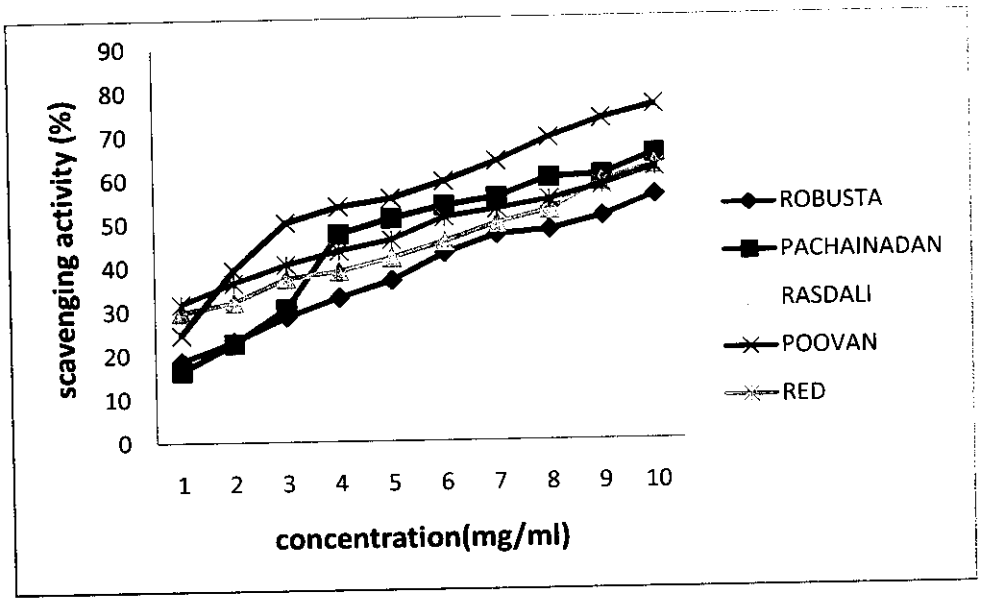
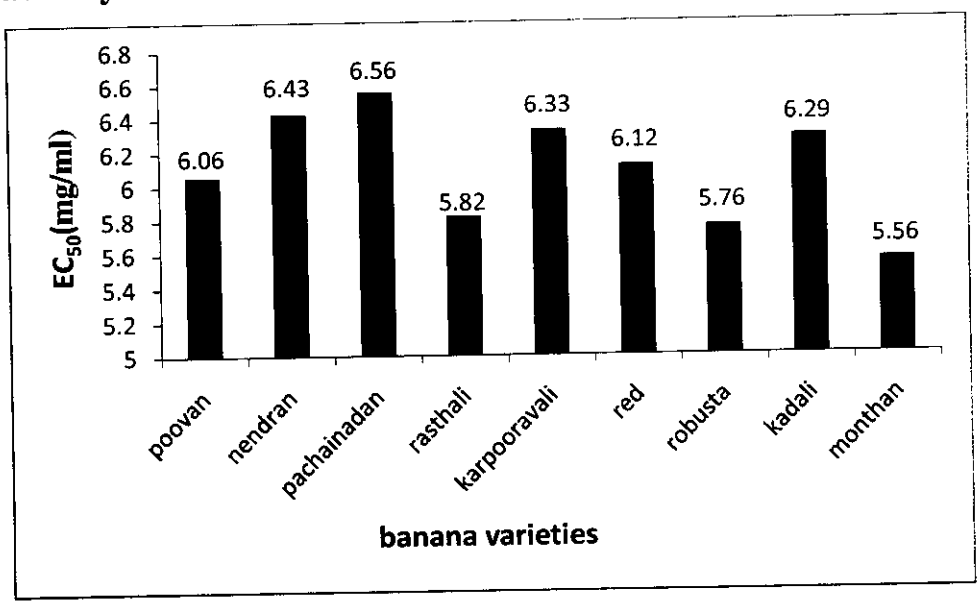


Figure 4.1.7.3 Comparison of EC_{50} values of lipid peroxidation inhibition activity



Free radical scavenging potential of the ethanolic extracts of local varieties of banana peel is shown in Table 4.1.7.1 which increases with the increase in concentration

At 1 -10 mg ml^{-1}, the ethanolic extract of the ethanolic extract of

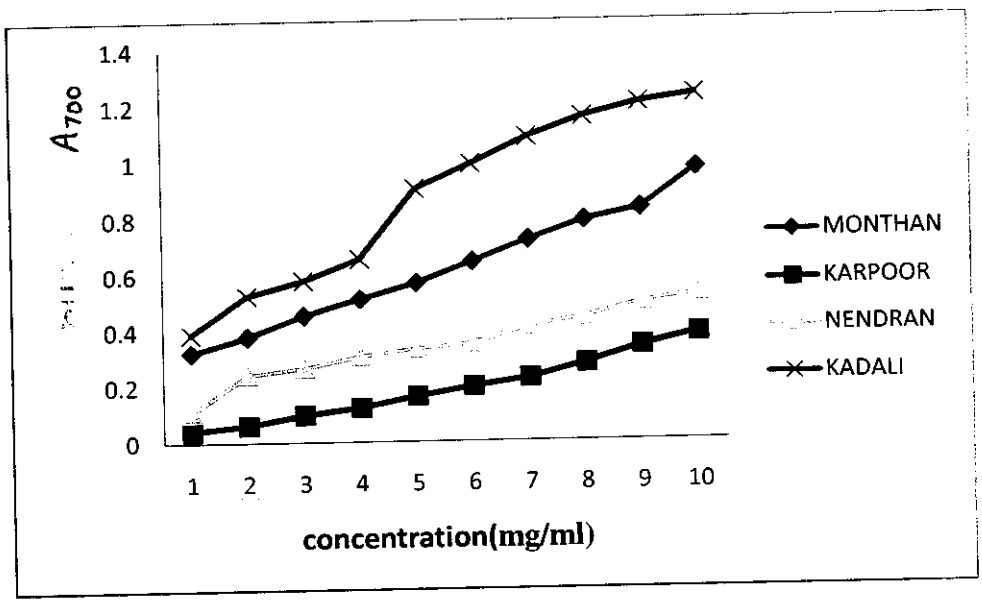
and Robusta shows the percentage inhibition of 38.13%-63.20%, 48.70%-73.70%, 23.51%-50.78%, 33.35%-62.59%, 24.68%-76.30%, 16.35%-65.29%, 29.75%-62.49%, 31.67%-61.91%, 18.66%-55.70% respectively. However, at 10mg ml^{-1}, poovan peel extract exibit highest lipid peroxidation inhibition activity. With respect to the EC_{50} value mondan showed least value, which represent highest antioxidant activity. Statistically, the scavenging activity of antioxidants was effective in the order of Poovan > Karpooravalli > Pachainadan > Kadali > nendran > Rasthali > Red > Robusta > Monthan.

Similar studies by (Someya *et al.*, 2002) determine the scavenging activity of lipid peroxidation inhibition.

4.1.8. 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 (Tenpe *et a*l, 2008)

Figure 4.1.8.1 Reducing power of ethanolic extracts of *balbisiana* type of banana varieties



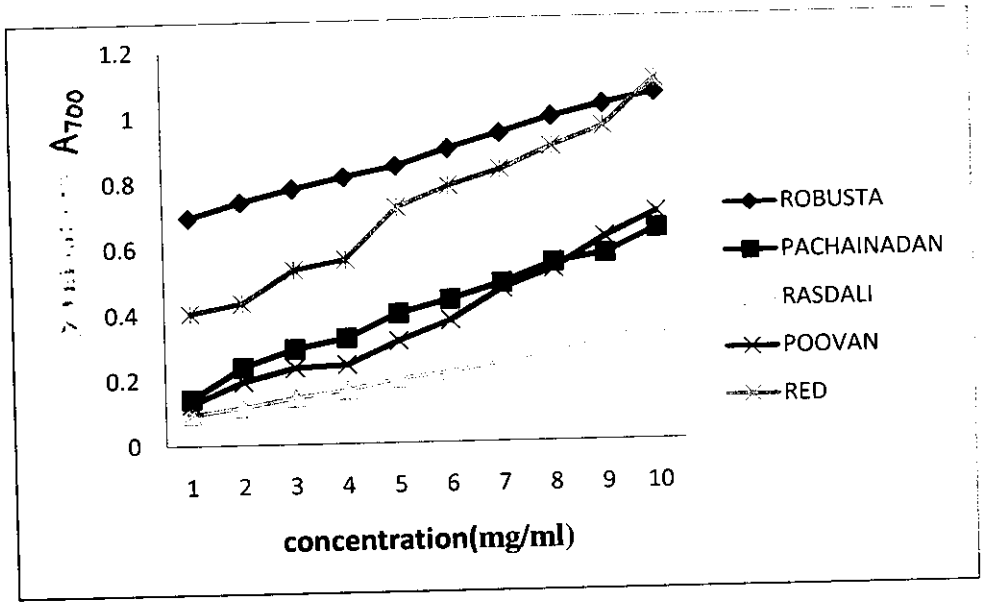
educing Antioxidant Potential activity on Stan

Sample concentration(mg/ml)									
1	2	3	4	5	6	7	8	9	10
39[b]±0.002	0.526[b]±0.00	0.581[b]±0.003	0.658[b]±0.002	0.905[a]±0.004	0.993[a]±0.001	1.087[a]±0.002	1.157[a]±0.003	1.207[a]±0.003	**1.235[a]±0.002**
42[g]±0.001	0.061[h]±0.002	0.097[h]±0.002	0.122[h]±0.001	0.162[h]±0.003	0.196[h]±0.005	0.226[g]±0.002	0.277[h]±0.002	0.339[h]±0.003	0.384[h]±0.002
22[c]±0.002	0.379[d]±0.001	0.457[d]±0.001	0.512[d]±0.001	0.570[d]±0.002	0.646[d]±0.004	0.722[d]±0.004	0.790[d]±0.002	0.831[d]±0.001	0.972[d]±0.002
08[f]±0.001	0.242[e]±0.003	0.265[f]±0.004	0.306[e]±0.001	0.332[f]±0.002	0.351[g]±0.003	0.411[f]±0.002	0.443[g]±0.001	0.494[g]±0.001	0.525[g]±0.002
26[dc]±0.001	0.193[f]±0.001	0.234[g]±0.001	0.241[f]±0.001	0.311[g]±0.001	0.372[f]±0.001	0.466[e]±0.004	0.524[f]±0.002	0.618[e]±0.001	0.692[e]±0.001
41[d]±0.001	0.237[e]±0.002	0.291[e]±0.002	0.322[e]±0.002	0.395[e]±0.002	0.435[e]±0.003	0.483[e]±0.001	0.543[e]±0.001	0.568[f]±0.004	0.642[f]±0.001
93[f]±0.004	0.114[g]±0.002	0.142[h]±0.001	0.163[g]±0.002	0.195[h]±0.002	0.213[h]±0.002	0.241[g]±0.001	0.275[h]±0.004	0.313[f]±0.003	0.329[f]±0.001
03[h]±0.002	0.432[e]±0.003	0.531[e]±0.001	0.560[e]±0.002	0.717[e]±0.002	0.780[e]±0.002	0.828[e]±0.001	0.897[e]±0.002	0.955[e]±0.004	1.096[b]±0.001
94[a]±0.004	0.741[a]±0.002	0.779[a]±0.001	0.813[a]±0.002	0.844[b]±0.001	0.895[b]±0.001	0.940[b]±0.004	0.987[b]±0.002	1.022[b]±0.005	1.053[c]±0.002
10	20	30	40	50	60	70	60	90	100
0.159	0.240	0.299	0.390	0.482	0.560	0.672	0.751	0.859	0.942

ean ± SD of 3 replicates.

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

Figure 4.1.8.2 Reducing power of ethanolic extracts of *acuminate* type of banana varieties.



The reducing power of ethanolic extracts of local banana varieties increases with the increase in concentration as depicted in the graph. The ethanolic extract of Kadali, Karpooravalli, Monthan, Nendran, Poovan, Pachainadan. Rasdali, Red and Robusta shows the Mean absorbance of 0.389-1.235, 0.042-0.384, 0.322-0.972, 0.108-0.525, 0.126-0.692, 0.141-0.642, 0.093-0.329, 0.403-1.096, 0.694-1.053 respectively. However, at 10mg ml^{-1}, Kadali peel extract exibited highest reducing power.

Similar observation was made by (Guo et al., 2003) which showed significant reducing power of banana peel.

4.1.9 β-carotene bleaching (BCB) activity

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 depends upon the amount of antioxidants present in the extract.

The BCB method is usually used to evaluate the antioxidant activity of

carotene bleaching (BCB) activity (%) on ethanolic extract of nine varieties of banana peel

Extract	Sample Concentration (mg/ml)					EC_{50} (mg/ml)
	1	2	3	4	5	
Kadali	$24.45^{c}\pm2.34$	$28.37^{c}\pm1.90$	$34.33^{dc}\pm3.09$	$40.34^{cd}\pm1.56$	$59.33^{c}\pm1.11$	**2.87**
Karpooravali	$22.00^{d}\pm2.56$	$29.35^{dc}\pm1.56$	33.55e±2.07	$42.27^{dc}\pm1.97$	$49.52^{e}\pm1.97$	3.08
Monthan	$45.54^{a}\pm1.94$	$52.21^{a}\pm1.78$	$57.52^{ab}\pm2.09$	$63.71^{a}\pm1.09$	**$70.77^{a}\pm2.65$**	3.39
Nendran	$47.78^{a}\pm1.34$	$53.00^{a}\pm1.45$	$59.61^{a}\pm1.89$	$62.56^{a}\pm1.87$	$66.96^{b}\pm1.04$	3.57
Poovan	$15.66^{e}\pm1.56$	$25.55^{f}\pm1.78$	$36.59^{d}\pm1.12$	$46.47^{c}\pm2.06$	$48.74^{e}\pm1.56$	3.32
Pachainadan	$24.89^{c}\pm1.09$	$31.49^{d}\pm1.84$	$35.86^{dc}\pm1.54$	$41.37^{e}\pm1.96$	$45.62^{f}\pm1.54$	3.34
Rasdali	$32.71^{b}\pm1.06$	$39.60^{b}\pm1.67$	$44.85^{c}\pm0.76$	$52.08^{b}\pm0.87$	$59.44^{c}\pm1.98$	3.28
Red	$45.52^{a}\pm0.99$	$51.09^{a}\pm1.94$	$55.51^{b}\pm1.98$	$61.81^{a}\pm0.45$	$66.60^{b}\pm0.89$	3.41
Robusta	$32.41^{b}\pm1.30$	36.38±1.95	$44.60^{c}\pm1.76$	$52.63^{b}\pm0.87$	$53.60^{d}\pm0.69$	3.42
Standard (µg/ml)	10	20	30	40	50	
Ascorbic acid	14.99	19.45	27.56	37.56	59.07	

Values represent mean ± SD of 3 replicates.

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

Figure 4.1.9.1 Scavenging activity (%) on β-carotene bleaching by ethanolic extracts of *balbisiana* type of banana varieties.

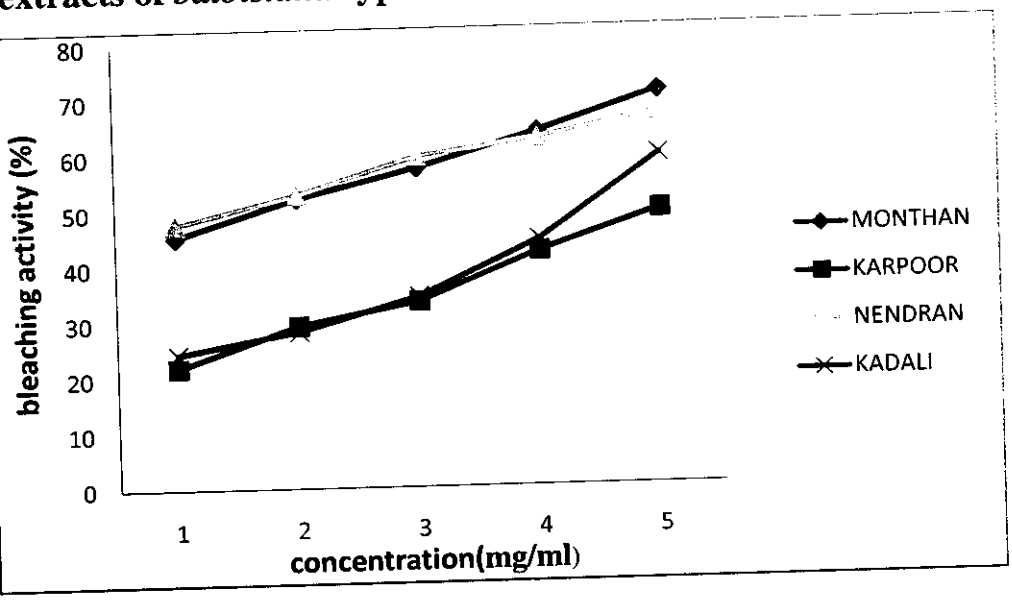


Figure 4.1.9.2 Scavenging activity (%) on β-carotene bleaching by ethanolic extracts *acuminate* type of banana varieties.

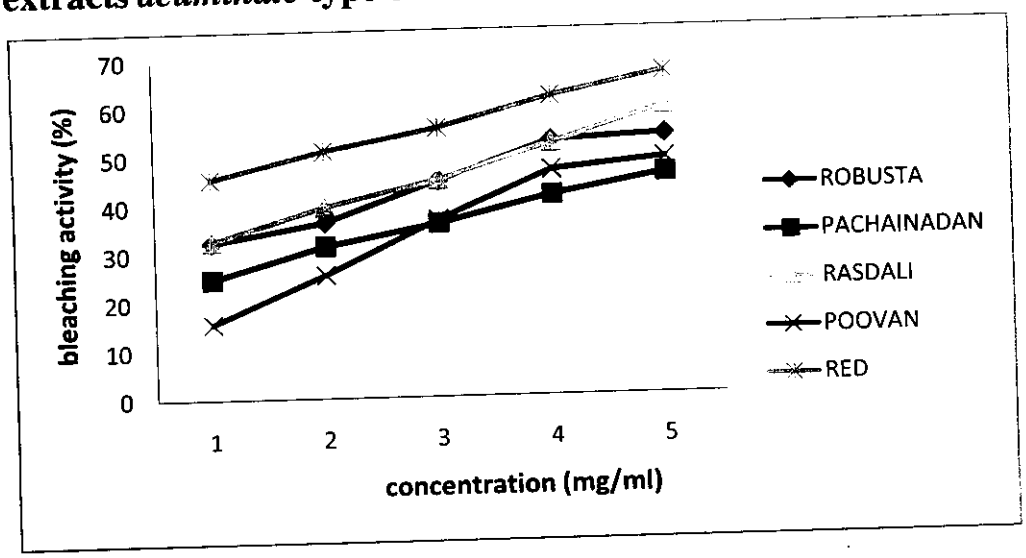
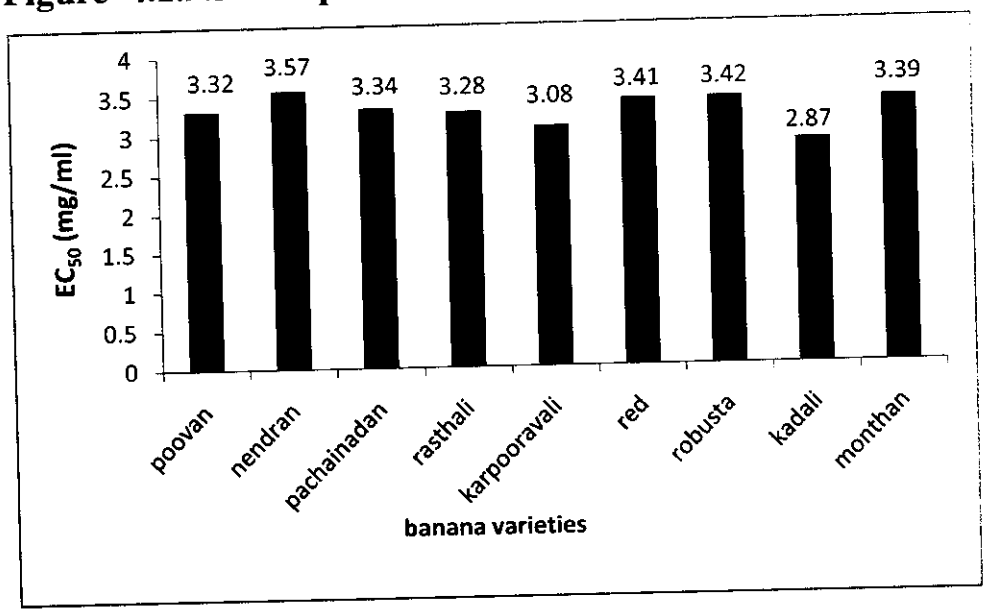


Figure 4.1.9.3 Comparison of EC_{50} values of β-carotene bleaching activity.



Free radical scavenging potential of the ethanolic extracts of local varieties of banana peel is shown in Table 4.1.9.1 which increases with the increase in concentration

At 1-5 mg ml^{-1}, the ethanolic extract of the ethanolic extract of Kadali, Karpooravalli, Monthan, Nendran, Poovan, Pachainadan. Rasdali, Red and Robusta show the percentage inhibition of 24.45%-59.33%, 22.00%-49.52%, 45.54%-70.77%, 47.78%-66.96%, 15.66%-48.74%, 24.89%-45.62%, 32.71%-59.44%, 45.52%-66.60%, 32.41%-53.60% respectively. However, at 5mg ml^{-1}, mondhan peel extract exibit highest activity. With respect to the EC_{50} value of kadali showed the least value, which represent highest antioxidant β-carotene bleaching activity.. Statistically, the scavenging activity of antioxidants was effective in the order of Mondhan > Nendran> Red > Rasdali > Kadali > Robusta > Karpooravali > Poovan > Pachainadan.

Studies by González-Montelongo et al (2010) showed the scavenging activity of banana peel extract on on β-carotene bleaching under different solvent and different incubation conditions.

PHASE II

ANTIMICROBIAL STUDY ON BANANA PEEL 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: *Staphylcoccus aureus, Bacillus subtilis, Escherichia coli.* There was appreciable antimicrobial activity in the peel of banana variety Rasdali, for the stains *Staphylcoccus aureus, Bacillus subtilis, Escherichia coli.* The peel of banana variety Nendram effectively inhibited the two stains namely: *Staphylcoccus aureus and Escherichia coli.* Where as, Kadali showing good antimicrobial activity against *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. Aibinu1182 Afr. J. Biotechnol.*et al*, (2007). The extracts of pulp and peel of ripe bananas effectively inhibited *Staphylcoccus 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).(Fagbemi *et al*, 2009).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. The banana skin has been referred to as nature's bacteria-proof wrapper and investigations show the

provided their toxicity and *in vivo* activity are favorable. The *in vitro* antibacterial activity of banana peel warrants further investigation to isolate, identify and characterise the active substances.

CONCLUSION

CONCLUSION

The human body has a mechanism for eliminating the free radicals. Substrates, which are the most important for this mechanism, are nutrients that come from human's diet. These subsrates are called as "antioxidants". The regular consumption of these dietary antioxidants reduces the risk of several diseases. The present work has been undertaken to evaluate and hence compare the antioxidant potential of nine varieties of banana viz.,Kadali, Karpooravali, Monthan, Nendran, Poovan, Pachainadan, Rasdali, Red, Robusta. The preliminary study carried out in our work has confirmed the significant free radical scavenging potential of the banana peel extracts and also the presence of natural antioxidants in the banana peel extracts. The free radical scavenging ability of the banana peel extracts was tested against various free radicals generated *in vitro* and the results obtained were analyzed statistically. On the whole, all the nine varieties of banana peel extracts exhibited a good antioxidant activity. Then, the peel extracts were analyzed for the presence of the anti microbial activity. A statistical analysis of ranks of the species with respect to free radical scavenging potential was carried out. The present study was mainly aimed at determining and comparing the antioxidant potential of local varieties of banana using different *in vitro* antioxidant assays and to study the antimicrobial properties in local varieties of banana peel. The future perspectives of the study will be 'isolate the bioactive compounds from banana peel and carry out studies in *in vivo* animal models.

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