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COMPARISON OF ANTIOXIDANT POTENTIAL IN DIFFERENT PARTS OF *Musa* species

PROJECT REPORT

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PROJECT WORK -PHASE II

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This is to certify that the project entitled **COMPARISON OF ANTIOXIDANT POTENTIAL IN DIFFERENT PARTS OF *Musa species*** is the bonafide record of project work done by **P.KALAISELVI Register No: 0820203003** of M.Tech during the year 2009-2010.

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DECLARATION

I affirm that the project work titled **Comparison of antioxidant potential in different parts of *Musa species*** being submitted in partial fulfilment for the award of **M.Tech(Biotechnology)** is the original work carried out by me. It has not formed the part of any other project work submitted for award of any degree or diploma, either in this or any other University.

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Antioxidants play a very important role in the defense against the damage caused by free radicals in the body. 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 and can also be held responsible for aging and other age-related diseases. The body can produce antioxidants to aid in its defense against free radical damage. But in most cases dietary supplement of antioxidants is recommended. Fruits and their parts are a very good source of antioxidants and other nutrients. Though their potency as antioxidants has been studied, comparative analysis is yet to be performed. This project aims to evaluate and compare antioxidant activity of the three parts of banana varieties namely, *Rasthali* leaf, *Rasthali* stem, *Rasthali* flower, *Karpooravalli* leaf, *Karpooravalli* stem, *Karpooravalli* flower. Ethanolic extraction was performed for the parts of banana varieties and the extracts were subjected to *in vitro* free radical scavenging assays, enzymatic, non- enzymatic and phytochemical components assays. Total antioxidant assay was also carried out to initially confirm the antioxidant potential. The results were obtained for the parts of *Musa spp* and they were found to possess significant antioxidant potential with leaf part having the highest activity comparatively.

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ABBREVIATIONS

ANOVA	Analysis of Variance
CAT	Catalase
CDNB	1-Chloro-2,4-dinitro phenyl hydrazine
DMRT	Duncun's Multiple Range Test
DNA	Deoxy Nucleic acid
DPPH	1, 1-diphenyl -2-picryl hydrazyl
CDNB	1-Chloro-2,4-dinitro phenyl hydrazine
EDTA	Ethylene Diamine Tetra Acetic acid
$FeCl_3$	Ferric Chloride
G6PD	Glucose-6-phosphate dehydrogenase
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Total Reduced Glutathione
GSSG	Oxidized Glutathione
GST	Glutathione-S-Transferase
H_2O_2	Hydrogen Peroxide
H_2SO_4	Sulphuric acid
K.F	*Karpoooravalli* flower
K.L	*Karpoooravalli* leaf
K.S	*Karpooravalli* stem
LDL	Low Density Lipoprotein
LPO	Lipid peroxidation
MDA	Malondialdehyde
mg	Milligram
Min	Minute
Ml	Milliliter
NO	Nitric oxide
O_2	Oxygen
OFR	Oxygen-Free Radicals

ROM	Reactive Oxygen Metabolites
ROS	Reactive Oxygen Species
R.F	*Rasthali* flower
R.L	*Rasthali* leaf
R.S	*Rasthali* stem
SOD	Superoxide dismutase
TBA	Thiobarbituric acid

INTRODUCTION

1. INTRODUCTION

A free radical is any atom or molecule that has a single unpaired electron in an outer shell. While a few free radicals such as melanin are not chemically reactive, most biologically-relevant free radicals are highly reactive. For most biological structures, free radical damage is closely associated with oxidative damage. Antioxidants are reducing agents, and limit oxidative damage to biological structures by passivating free radicals.

Free-radical damage occurs on an atomic level. Molecules are made of atoms, and a single atom is made up of protons, neutrons, and electrons. Electrons are always found in pairs. However, when oxygen molecules are involved in a chemical reaction, they can lose one of their electrons. This oxygen molecule that now only has one electron is called a free radical. With only one electron, the oxygen molecule must quickly find another electron, and it does this by taking the electron from another molecule. When that molecule in turn loses one of its electrons, it too must seek out another, in a continuing reaction. Molecules attempting to repair themselves in this way trigger a cascading event called "free-radical damage." (Bagchi and Puri,1998)

- Stable molecules are made up of a nucleus and paired electrons spinning around the nucleus.
- Some molecules have only one electron spinning around its nucleus making them unstable. This unstable molecule is now becomes a free radical.
- In order to stablize itself, this molecule steals an electron from a second molecule which now becomes unstable and is now a free radical. This process goes on over and over again.

Chain reactions involving free radicals can usually be divided in to three distinct processes: initiation, propagation, and termination. Initiation reactions are those that result in a net increase in the number of free radicals. They may involve the formation of free radicals from stable species. They may involve reactions of free radicals with stable species to form more free radicals. Propagation reactions are those reactions involving free radicals in which the total number of free radicals remains same. Termination reactions are those reactions resulting in a net decrease in the number of free radicals. Typically two free radicals combine to form a more stable species.

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 *et al.*, 1984).

Their destructive effects on proteins may play a role in the causation of cataracts. Free radical damage to DNA is also implicated in the causation of cancer and its effect on LDL cholesterol is very likely responsible for heart disease. In fact, the theory associating free radicals with the aging process has also gained widespread acceptance.

Some internally generated sources of free radicals are:

- Mitochondria
- Phagocytes
- Xanthine oxidase
- Reactions involving iron and other transition metals
- Arachidonate pathways
- Peroxisomes
- Inflammation
- Ischaemia/reperfusion.

Some externally generated sources of free radicals are:

- Cigarette smoke
- Environmental pollutants
- Radiation
- Ultraviolet light
- Certain drugs, pesticides, anaesthetics and industrial solvents
- Ozone.

If free radicals are not inactivated, their chemical reactivity can damage all cellular

Although oxidation reactions are critical for life, they also result in cell damage which, research shows manifests in ageing. Other research is on the investigation on the role of antioxidants in the treatment of many diseases like cancer, Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. Animals maintain complex systems of multiple types of antioxidants, such as Vitamin A, Beta Carotene and Vitamin E. (Ajay Sharma *et al.*,2007)

Antioxidants are compounds capable of slowing or preventing the oxidation of other molecules. They are also known as free radical scavengers of the body for their ability to terminate chain oxidation reactions in two ways – by removing radical intermediates and by getting oxidised themselves. (Amalia Ubeda *et al.*, 1993)

There are several enzyme systems within the body that scavenge free radicals, the principle micronutrient (vitamin) antioxidants are Vitamin E, Beta Carotene, and Vitamin C. Different Antioxidants work in different ways. Beta carotene plays a major role in protein synthesis, when it is converted to fat-soluble Vitamin A. It helps in maintaining healthy skin. Vitamin C prevents skin damage and helps build collagen, the fibrous protein that gives skin elasticity. Vitamin E protects the body tissues from damage by harmful free radicals. Apart from vitamins, selenium, a trace metal is also required for proper functioning of the body's antioxidant enzyme systems.

An antioxidant rich diet, which is a diet rich in fruits and vegetables may significantly lower the risk of a first stroke. Vitamin B or folic acid may also protect against stroke. Reserach findings suggest that higher levels of folate in the body reduce the risk of stroke. Reasearchers summarise that this could be because of folate appears to reduce levels of homocysteine, an amino acid strongly linked to an increased risk of coronary artery disease, stroke, and Alzheimer's disease. Other studies report that people with diets rich in carotenoids, including beta carotene and lycopene, have lowered risk of stroke.

Flavonoids found in both black and green tea, dark chocolate, onions, red wine or red grape juice, and apples, appear to have qualities that protect the heart. In a 2003 study, it was found that people who consumed the most flavonoids in foods had a 20% lower risk of getting heart disease compared to those who did not consume so many flavenoids.

Phytochemicals like isothiocyanates and sulforaphane, found in cruciferous vegetables,

the maximum cruciferous vegetables had a 24% lower risk of breast cancer compared to women who consumed significantly lower amounts. Resveratrol has also been found have tumor-suppressing properties.

A polyphenol antioxidant is a type of antioxidant containing a polyphenolic substructure. In human health, these compounds, numbering over 4,000 distinct species, are thought to be instrumental in combating oxidative stress, a process associated with some neurodegenerative diseases and some cardiovascular diseases.

The main source of polyphenol antioxidants is nutritional, since they are found in a wide array of phytonutrient-bearing foods. For example, most legumes; fruits such as apples, blackberries, blueberries, cantaloupe, cherries, cranberries, grapes, pears, plums, raspberries, and strawberries; and vegetables such as broccoli, cabbage, celery, onion and parsley are rich in polyphenol antioxidants. Red wine, green coffee, chocolate, green tea, coffee, olive oil, bee pollen (honey) and many grains are alternative source.

α-tocopherol, found in green parts of plants scavenges lipid peroxy radicals through the concerted action of other antioxidants (Kiffin *et al.,* 2006, Hare *et al.*, 1998).Further, tocopherols were also known to protect lipids and other membrane components by physically quenching and chemically reacting with O_2 in chloroplasts, thus protecting the structure and function of PSII. Researchers reported a two-fold increase in α-tocopherol in turf grass under water stress (Kiddle *et al.*, 2003).

In plant cells, the most important reducing substrate for H_2O_2 removal is ascorbic acid. α-tocopherols (vitamin E) are lipophilic antioxidants synthesized by all plants. α-Tocopherols interact with the polyunsaturated acyl groups of lipids, stabilize membranes, and scavenge and quench various reactive oxygen species (ROS) and lipid soluble byproducts of oxidative stress (Fath *et al.,* 2002, Cvetkovska *et al.,* 2005).

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.

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.

OBJECTIVES

- To evaluate and compare the *in vitro* antioxidant potential in different parts of banana *Musa spp.*
- To determine and compare enzymatic and non enzymatic antioxidants in different parts of *Musa spp.*
- To estimate and compare the phytochemical composition in different parts of *Musa spp.*

REVIEW OF LITERATURE

2. LITERATURE REVIEW

2.1. Free Radicals

Molecules are made up of one or more atomic nuclei surrounded by orbiting electrons. The electrons are arranged in orbitals, depending upon their respective distances from these nuclei. In most molecules, the electrons within each orbital are paired with another electron that's spinning in the opposite direction. (The concept of "spin," distinct from the concept of electrical charge, is less of a literal description of the electron's actual behavior than an analogy that helps us visualize this unseen world in familiar terms.) The paired electrons keep the molecule relatively stable (at a lower energy state) and thereby less reactive. When one or more electrons, especially within the outer orbital, is/are unpaired with respect to spin, the molecule becomes relatively unstable (at a higher energy state) and consequently more reactive with other molecules.

A free radical is a molecule with one or more unpaired electrons in its outer orbital. Many of these molecular species are oxygen (and sometimes nitrogen) centered. Indeed, the molecular oxygen we breathe is a free radical. These highly unstable molecules tend to react rapidly with adjacent molecules, donating, abstracting, or even sharing their outer orbital electron(s). This reaction not only changes the adjacent, target molecule, sometimes in profound ways, but often passes the unpaired electron along to the target, generating a second free radical or other ROS, which can then go on to react with a new target. In fact, much of the high reactivity of ROS is due to their generation of such molecular chain reactions, effectively amplifying their effects many fold.

The changes wrought on adjacent molecular targets can vary in magnitude, but because many of the components of the living cell are particularly susceptible to free radical injury, the molecular chain reactions can have substantial effects on the structure and function of living tissue. As a consequence, natural selection has driven the evolution of a number of intracellular defense mechanisms to neutralize or control the potentially destructive reactivity of ROS. These include molecules that react preferentially with ROS without passing that reactivity along. Some of these are simple molecules like vitamins E and C, while some are enzymes like superoxide dismutase (SOD) and catalase, which catalyze such electron-quenching reactions. Often these compounds are referred to collectively as free radical

2.2. Examples of Chemical Qualities and Reactivities of Some ROS

2.2.1. Superoxide Ion Radical ($O^{\cdot}_2{}^{-}$ /$HO^{\cdot}_2$)

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 $O^{\cdot}_2{}^{-}$ and $HO_2^{\cdot}$ can act as reducing agents capable, for example, of reducing ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions; however, the reducing capacity of $HO^{\cdot}_2$ is higher. In organic solvents the solubility of $O^{\cdot}_2$ 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 capable of donating H^+ (eg, ascorbate and tocopherol). The most important reaction of superoxide radicals is dismutation.

2.2.2. Hydroxyl Radical ($OH^{\cdot}$)

The reactivity of hydroxyl radicals is extremely high (Bielski *et al.*, 1995, Halliwell *et al.*, 1999). 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. $OH^{\cdot}$ 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.

2.2.3. 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 produceH_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 μM). 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. 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.

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

The nitric oxide-superoxide radical ($NO^{\cdot}$) is produced by the oxidation of

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 (NO^-), which can undergo further reactions, such as interacting with $NO^{\cdot}$ to yield N_2O and $OH^{\cdot}$. The half-life of the nitric oxide radicals depends on the square of the radical concentration(Ron Kohen *et al.*, 2002).

$NO^{\cdot}$ 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, NO2 , and NO_3 . One of the most important reactions under physiological conditions is that of superoxide and nitric oxide radicals resulting in peroxynitrite. This reaction helps to maintain the balance of superoxide radicals and other ROS and is also important in redox regulation. The protonated form of peroxynitrite (ONOOH) is a powerful oxidizing agent that might cause depletion of sulphhydryl (-SH) groups and oxidation of many molecules causing damage similar to that observed when $OH^{\cdot}$ is involved. It can also cause DNA damage such as breaks, protein oxidation, and nitration of aromatic amino acid residues in proteins (eg, 3-nitrosotyrosine),

2.2.5. The Role of Transition Metals

In 1894, Fenton described the interaction between Fe^{+2} and H_2O_2 .This Fenton reaction, was found to produce hydroxyl radicals, which explain its oxidizing power (Haber *et al.*, 1934). This reaction was recognized as one of the most important in the explanation of oxidative damage that occurs in biological environments. Transition metals can participate in the chemistry of radicals and convert relatively stable oxidants into powerful radicals. Among the various transition metals, copper and especially iron are most abundant, present in relatively high concentrations, and are major players in the Fenton reaction (Fenton, 1894) and the metal-mediated Haber-Weiss reaction. The metal ions participating in this reaction are those bound to the surface of proteins, DNA, and other macromolecules or chelators. Ascorbic acid can reduce ferric ions to ferrous ions, enabling it to undergo the Fenton reaction and produce hydroxyl radicals for hydroxylating aromatic compounds. This transformation is the Udenfriend reaction.

2.3. Sources of Free radicals

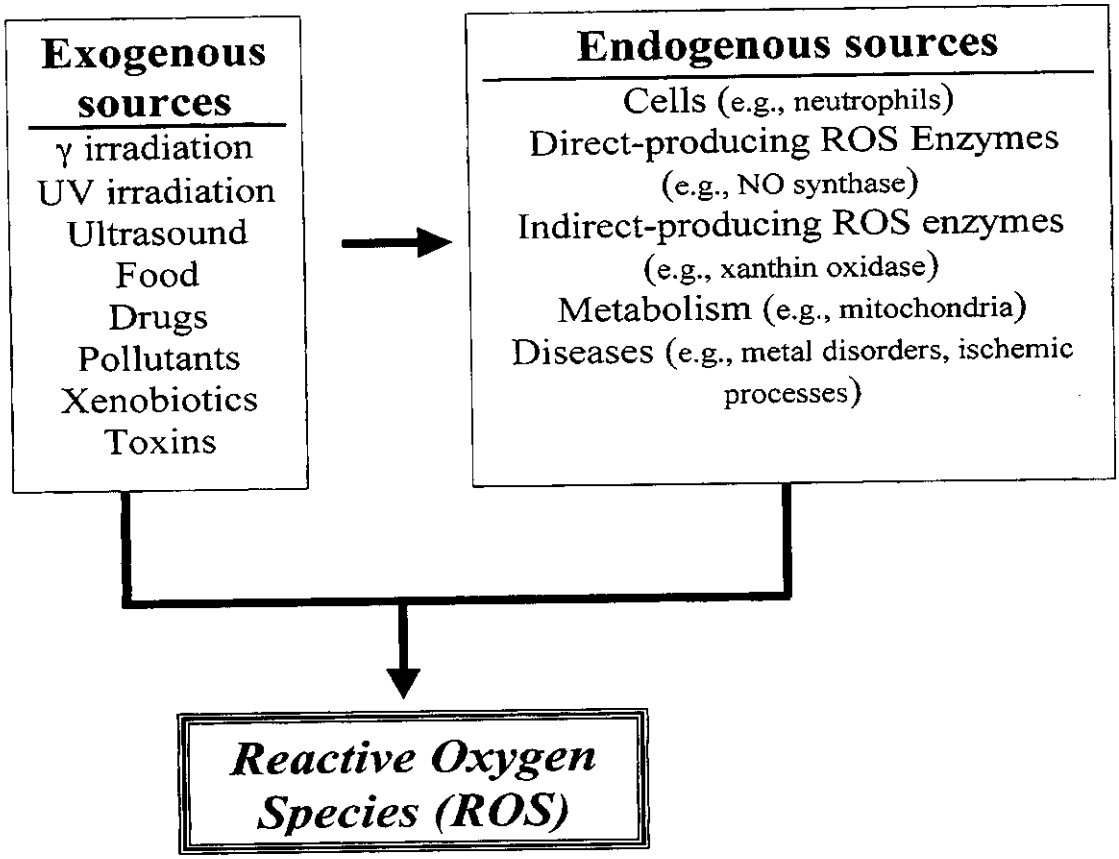


Figure.2.1. Exogenous and Endogenous Sources Of Reactive Oxygen Species (ROS).

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 (Roth *et al.*, 1994). Ozone (O_3) is essential in scavenging deleterious UV-C irradiation and extremely important with its presence in the upper atmosphere (Pari, *et al.*, 2000).

2.3.1. Specific targets of oxidative damage

2.3.1.1. Lipids

All cellular membranes are especially vulnerable to oxidation due to their high

peroxidation, occurs in 3 stages. The first stage, initiation, involves the attack of a reactive oxygen metabolite capable of abstracting a hydrogen atom from a methylene group in the lipid. The presence of a double bond adjacent the methylene group weakens the bond between the hydrogen and carbon atoms so that it can easily be removed from the molecule. Following hydrogen abstraction, the remaining fatty acid radical retains 1 electron and is stabilized by rearrangement of the molecular structure to form a conjugated diene. When oxygen is in sufficient concentration in the surroundings, the fatty acid radical will react with it to form ROO˙ during the propagation stage(Sies, 1997).

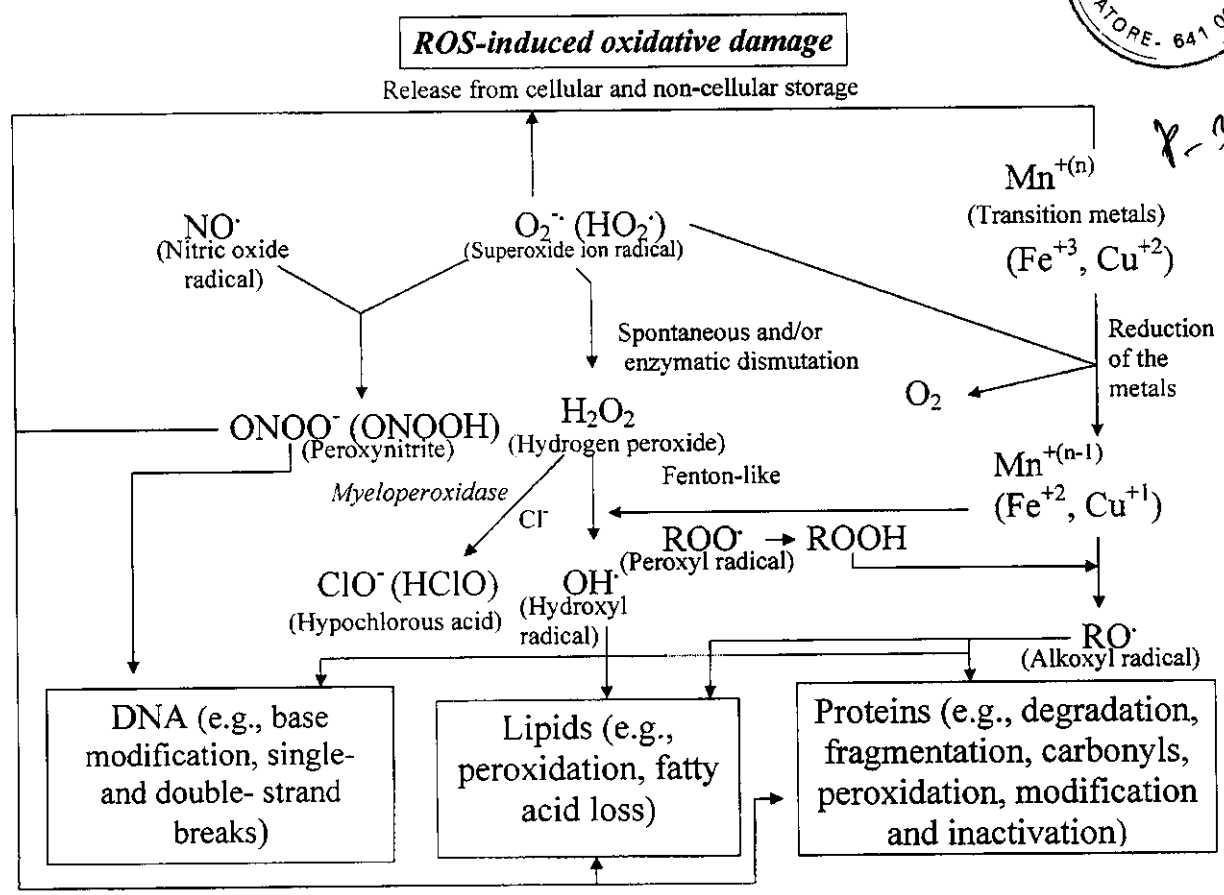


Figure 2.2. Reactive oxygen species (ROS)-induced oxidative damage

These radicals themselves are capable of abstracting another hydrogen atom from a neighboring fatty acid molecule, which leads again to the production of fatty acid radicals that undergo the same reactions—rearrangement and interaction with oxygen. The ROO˙ becomes a lipid hydroperoxide that can further decompose to an aldehyde or form cyclic

continue. A single initiation can lead to a chain reaction resulting in peroxidation of all the unsaturated lipid in the membrane. An antioxidant that can stop this process is therefore defined as a chain-breaking antioxidant. Fatty acids with no double bonds or with 1 double bond can undergo oxidation but not a chain lipid peroxidation process; for example, oleic acid with 18 carbon atoms and 1 double bond (18:1) cannot undergo the lipid peroxidation process. The last stage, chain termination, occurs following interaction of one ROO. with another radical or antioxidants.

2.3.2.2. Proteins

Proteins, also major constituents of membranes, can serve as possible targets for attack by ROS. Among the various ROS, the $OH^{\cdot}$, $RO^{\cdot}$, and nitrogen-reactive radicals predominantly cause protein damage. Hydrogen peroxide itself and superoxide radicals in physiological concentrations exert weak effects on proteins; those containing SH groups, however, can undergo oxidation following interaction withH_2O_2. Proteins can undergo direct and indirect damage following interaction with ROS, including peroxidation, damage to specific amino-acid residues, changes in their tertiary structure, degradation, and fragmentation (Grune *et al.*, 1997, Davis *et al.*, 1987).

The consequences of protein damage as a response mechanism to stress are loss of enzymatic activity, altered cellular functions such as energy production, interference with the creation of membrane potentials, and changes in the type and level of cellular proteins. Protein oxidation products are usually aldehydes, keto compounds, and carbonyls. One of the major adducts that can easily be detected and serve therefore as a marker for protein oxidative damage is 3-nitrotyrosine(Stadtman, 1986). This adduct is produced following the interaction between $ONOO^{-}$ and other nitrogen reactive radicals with the amino acid tyrosine. Following $OH^{\cdot}$ attack, a series of compounds can be formed, including hydroxyproline, glutamyl semialdehyde, and others. Following protein oxidation, modified proteins are susceptible to many changes in their function. These include chemical fragmentation, inactivation, and increased proteolytic degradation (Kasprzak, 2002).

2.3.2.3. DNA

DNA is a stable, well-protected molecule; ROS can interact with it and cause several types of damage: modification of DNA bases, single- and double-DNA breaks, loss of purines (apurinic sites), damage to the deoxyribose sugar, DNA-protein cross-linkage, and damage to the DNA repair system. Not all ROS can cause damage; most is attributable to

induced by ionizing irradiation, a variety of adducts are formed. The $OH^{\cdot}$ can attack guanine at its C-8 position to yield an oxidation product, 8- hydroxydeoxyguanosine (8-OHdG). Other positions could be attacked, and other possible products could be formed. Hydroxyl radicals can also attack other bases like adenine to yield 8 (or 4-, 5-)- hydroxyadenine. Other products are the result of interactions between pyrimidines and hydroxyl radicals leading to the formation of thymine peroxide, thymine glycols, 5- (hydroxymethyl) uracyl, and other such products (Dizdaroglu *et al.*, 2002,).

The direct interaction of DNA with other less reactive ROS, such as O.2 and H_2O_2, does not lead to damage at their physiological concentrations; however, these species serve as sources for other reactive intermediates that can easily attack and cause damage. For example, H_2O_2 and superoxide might lead the production of the OH. via the Haber-Weiss reaction, and NO and $O_2^{\cdot -}$ might lead to the formation of $ONOO^{-}$ that can easily cause DNA damage similar to that obtained when hydroxyl radicals are involved. Transition metals like iron that possess high binding affinity to DNA sites can catalyze the production of $OH^{\cdot}$ in close proximity to the DNA molecule, thus ensuring repeated attack upon the DNA by an efflux of hydroxyl radicals (Halliwell 1999, Kasprzak 2002, Ron Kohen *et al.*, 2002).

2.4. Free radical mediated diseases

Oxygen-derived free radicals are very important mediators of cell injury and death. Not only are these highly reactive chemical species important in the aging process, but they are either directly or indirectly involved in a wide variety of clinical disorders, such as atherosclerosis, reperfusion injury, pulmonary toxicity, macular degeneration, cataractogenesis, and cancer. In addition, they play an important role in chronic granulomatous disease and act as secondary sources of cellular injury in chronic granulomatous disease and act as secondary sources of cellular injury in chronic inflammatory processes and several disorders of the central nervous system. Furthermore, a wide variety of drugs and xenobiotics are themselves either converted to, or stimulate the formation of, free radicals.

2.4.1. Radiation injury

Radiation injury represents an important cause of ROS-mediated disease. With respect to more commonly encountered levels of radiation, depending upon the situation,

generated secondarily. This applies not only to the acutely toxic forms of radiation injury, but the long-term, mutagenic (and hence carcinogenic) effects as well.

An important clinical application of this principle is encountered regularly in the treatment of cancer by radiation therapy. Large tumors often outgrow their blood supplies and tumor cells die within the center, despite being well-oxygenated at the periphery. Between these two regions is an area of tumor that is poorly oxygenated, yet remains viable. Radiation therapy of such tumors is particularly effective at the periphery, where an abundant concentration of oxygen is available to form tumorcidal ROS.

Oxidative damage to cells is caused by the activity of free radicals, which are released during normal cell processes. This results in oxidative stress, a process that is believed to result in tissue inflammation, long suspected to be a cause of Alzheimer's disease. Brain tissue is particularly susceptible to free radical damage because, unlike many other tissues, it does not contain large amounts of protective antioxidant compounds (Knight,1995).

2.4.2. Cancer and other malignancies

All entail unconstrained cell growth and proliferation based upon changes in the cell's genetic information. In most cases, for example, one or more genes that normally constrain cell growth and replication is mutated, or otherwise inactivated. These genetic deficiencies correspond directly with deletions and sequence changes in the genetic code, resident in the cell's DNA. A frequently seen final common cause of such DNA damage is free radical injury. Of the myriad injuries sustained by our DNA on a daily basis, most are repaired by normal DNA repair mechanisms within the cell, while some result in cell death (Scott Gottlieb, 1998).

Since such injuries are sporadic and distributed somewhat randomly across the genome, most lethal DNA injuries are clinically inconsequential, resulting in the loss of a few cells among millions. However, when a single cell sustains an injury that impairs growth regulation, it can proliferate disproportionately and grow rapidly to dominate the cell population by positive natural selection. The result is a tumor, frequently a malignant one, where the constraint of growth and proliferation is particularly deficient. Therefore, free radical injury to the genetic material is a major final common pathway for carcinogenesis.

ROS can be generated within the cell not only by external sources of radiation, but also within the body as a byproduct of normal metabolic processes. An important source of endogenous free radicals is the metabolism of some drugs, pollutants, and other chemicals and toxins, collectively termed xenobiotics. While some of these are directly toxic, many others generate massive free radical fluxes via the very metabolic processes that the body uses to detoxify them.

2.4.3. Atherosclerosis

Atherosclerosis is a complex process that leads to heart attack, stroke, and limb loss by the plugging of the arteries with atherosclerotic plaque. This plaque is a form of oxidized fat. When free radicals react with lipids, the consequence is lipid peroxidation, the same process by which butter turns rancid when exposed to the oxygen in the air. While a number of factors influence the development and severity of atherosclerosis, a major factor is the ROS-mediated peroxidation of low density lipoproteins (LDLs, or "bad cholesterol"). The dietary approach to the prevention of heart disease and stroke is based partially on adding dietary antioxidants to limit LDL oxidation, as well as decreasing the intake of fat itself.

In human ageing and with many pathologies correlated to the senile functional decay of cells, membrane damage often occurs in some organ or tissue, which provokes lipid peroxidation in the membrane and accelerates the disorder in structure and function of the membrane. When lipid peroxides accumulate sufficiently, they leak from the organ or tissue into the bloodstream and increase the lipid peroxide level in blood lipoproteins. The increased lipid peroxides in the blood attack the blood vessel and promote atherogenesis.

2.4.4. Degenerative neurological diseases

Degenerative neurological diseases It affects millions of Americans. A number of these diseases, including amyotrophic lateral sclerosis (ALS, or Lou Gehrig's disease), Parkinson's disease, and Alzheimer's disease, appear to have ROS toxicity as a central component of their underlying mechanism of nerve cell destruction. Eating more dietary or even pharmocologic antioxidants will prevent or arrest the neural degeneration.

2.4.5. Ischemia/reperfusion injury

Ischemia/reperfusion injury is a particularly fascinating example of ROS-mediated disease. When an organ is deprived of its blood supply (ischemia) it is injured, not just by the temporary loss of oxygen, but also by the ROS that are generated by reaction with the oxygen that is reintroduced at reperfusion, when the blood supply is restored. In some clinical situations, this injury is prevented by giving antioxidants, sometimes even *after* the period of ischemia, but just prior to reperfusion.

For example, the preservation of kidneys, livers, and other organs in solutions that contain antioxidants, as well as other agents, is now routine prior to their transplantation. Another example is the use of drugs that block the function of free radical generating enzymes prior to stopping the heart for cardiac surgery. These drugs help prevent reperfusion injury when the heart is restarted and flow is restored. This reperfusion injury mechanism also has been found to play an important role in patients suffering from multiple organ failure after trauma, massive surgery, or shock.

2.4.6. Aging

Aging is a remarkably complex process that has managed to remain relatively opaque to scientific understanding. However, it to be a process *per se*, i.e., a series of controlled mechanisms, But if aging is a series of processes, it's logical to conclude that it is potentially controllable, or at least modifiable. One of the most important of these processes is comprised of an accumulation of the molecular injuries that are mediated by free radicals and other ROS. The therapeutic manipulation of ROS metabolism can actually extend the total life span of mice to a significant degree. This was the first time that life span has been successfully altered experimentally by treatment (Stadtman, 1986).

2.5. Counteracting free radical damage

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

System of enzymes, including glutathione peroxidases, superoxide dismutases and

essential minerals including selenium, copper, manganese and zinc are necessary for the formation or activity of these enzymes. Hence, if the nutritional supply of these minerals is inadequate, enzymatic defences against free radicals may be impaired.

Defence against free radical damage is the presence of antioxidants. An antioxidant is a molecule stable enough to donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage. Some antioxidants, including glutathione, ubiquinol and uric acid, are produced during normal metabolism in the body. Other lighter antioxidants are found in the diet. Although about 4000 antioxidants have been identified, the best known are vitamin E, vitamin C and the carotenoids. Many other non-nutrient food substances, generally phenolic or polyphenolic compounds, display antioxidant properties and, thus, may be important for health((Kiffin, 2006).

Although a wide variety of antioxidants in foods contribute to disease prevention, the bulk of research has focused on three antioxidants which are essential nutrients or precursors of nutrients. These are vitamin E, vitamin C and the carotenoids. Each of these antioxidant nutrients have specific activities and they often work synergistically to enhance the overall antioxidant capability of the body The balance between the production of free radicals and the antioxidant defences in the body has important health implications. If there are too many free radicals produced and too few antioxidants, a condition of "oxidative stress" develops which may cause chronic damage.

As mentioned above, free radicals have been implicated in several health problems. Cancer, atherosclerosis, cerebrovascular accidents, myocardial infarction, senile cataracts, acute respiratory distress syndrome and rheumatoid arthritis are just a few examples. Numerous studies have shown the protective effects of antioxidant nutrients on these health problems.

Vitamin E is the collective name for eight compounds, four tocopherols and four tocotrienols, found in nature. It is a fat-soluble substance present in all cellular membranes and is mainly stored in adipose tissue, the liver and muscle. Vitamin E is a principal antioxidant in the body and protects polyunsaturated fatty acids in cell membranes from peroxidation. It is a single oxygen quencher, neutralizing these highly reactive and unstable singlet oxygen molecules. In fact, singlet oxygen can damage DNA and be mutagenic.

Vitamin E also protects the double bonds of β-carotene from oxidation and thus exhibits a sparing effect. Due to the ability of vitamin E to work at higher oxygen pressures, free radicals are scavenged and tissue injury is minimized. Besides its anti-aging properties, vitamin E is known to afford protection against cancer, ischaemia and reperfusion injury, cataract, arthritis and certain neurological disorders (Bagchi *et al.*, 1998).

2.6. Antioxidants

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

Antioxidants are reducing agents, and limit oxidative damage to biological structures by passivating free radicals. Although oxidation reactions are crucial for life, they can also be damaging. Hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells (Miller, 1997)..

Antioxidants are also widely used as ingredients in dietary supplements in the hope of maintaining health and preventing diseases such as cancer and coronary heart disease. Although initial studies suggested that antioxidant supplements might promote health, later large clinical trials did not detect any benefit and suggested instead that excess supplementation may be harmful (Bjelakovic *et al.*, 2007.). In addition to these uses of natural antioxidants in medicine, these compounds have many industrial uses, such as preservatives in food and cosmetics and preventing the degradation of rubber and gasoline.

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

These compounds is synthesized in the body or obtained from the diet. The action of one antioxidant may therefore depend on the proper function of other members of the antioxidant system. The amount of protection provided by any one antioxidant will also depend on its concentration, its reactivity towards the particular reactive oxygen species being considered, and the status of the antioxidants with which it interacts.

The different antioxidants are present at a wide range of concentrations in body fluids and tissues, with some such as glutathione or ubiquinone mostly present within cells, while others such as uric acid are more evenly distributed. Some antioxidants are only found in a few organisms and these compounds can be important in pathogens and can be virulence factors. (Benzie and Strain,1996)

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

2.6.1. Enzyme systems

Cells are protected against oxidative stress by an interacting network of antioxidant enzymes. Here, the superoxide released by processes such as oxidative phosphorylation is first converted to hydrogen peroxide and then further reduced to give water. This detoxification pathway is the result of multiple enzymes, with superoxide dismutases catalysing the first step and then catalases and various peroxidases removing hydrogen peroxide. As with antioxidant metabolites, the contributions of these enzymes to antioxidant defenses can be hard to separate from one another, but the generation of transgenic mice lacking just one antioxidant enzyme can be informative.

2.6.1.1. Superoxide dismutase

Superoxide dismutases (SODs) are a class of closely related enzymes that catalyze the breakdown of the superoxide anion into oxygen and hydrogen peroxide.(Johnson and

Superoxide dismutase enzymes contain metal ion cofactors that, depending on the isozyme, can be copper, zinc, manganese or iron. In humans, the copper/zinc SOD is present in the cytosol, while manganese SOD is present in the mitochondrion. There also exists a third form of SOD in extracellular fluids, which contains copper and zinc in its active sites. (Bannister *et al.*, 1987)

The mitochondrial isozyme seems to be the most biologically important of these three, since mice lacking this enzyme die soon after birth. (Van Camp *et al.*, 1999)In contrast, the mice lacking copper/zinc SOD (Sod1) are viable but have numerous pathologies and a reduced lifespan, while mice without the extracellular SOD have minimal defects (sensitive to hyperoxia). In plants, SOD isozymes are present in the cytosol and mitochondria, with an iron SOD found in chloroplasts that is absent from vertebrates and yeast. (Bisseling *et al.*, 1997)

2.6.1.2. Catalases

Catalases are enzymes that catalyse the conversion of hydrogen peroxide to water and oxygen, using either an iron or manganese cofactor (Chelikani *et al.*, 2004). This protein is localized to peroxisomes in most eukaryotic cells (del Río, *et al.*,1992). Catalase is an unusual enzyme since, although hydrogen peroxide is its only substrate, it follows a ping-pong mechanism. Here, its cofactor is oxidised by one molecule of hydrogen peroxide and then regenerated by transferring the bound oxygen to a second molecule of substrate. Despite its apparent importance in hydrogen peroxide removal, humans with genetic deficiency of catalase "acatalasemia" .

2.6.1.3. Peroxiredoxins

Peroxiredoxins are peroxidases that catalyze the reduction of hydrogen peroxide, organic hydroperoxides, as well as peroxynitrite. They are divided into three classes: typical 2-cysteine peroxiredoxins; atypical 2-cysteine peroxiredoxins; and 1-cysteine peroxiredoxins. These enzymes share the same basic catalytic mechanism, in which a redox-active cysteine (the peroxidatic cysteine) in the active site is oxidized to a sulfenic acid by the peroxide substrate. Over-oxidation of this cysteine residue in peroxiredoxins inactivates these enzymes, but this can be reversed by the action of sulfiredoxin. Peroxiredoxins seem to be

lifespan and suffer from hemolytic anaemia, while plants use peroxiredoxins to remove hydrogen peroxide generated in chloroplasts (Balinsky and Bernstein,1963)

2.6.1.4. Glutathione system

The glutathione system includes glutathione, glutathione reductase, glutathione peroxidases and glutathione *S*-transferases(Nicoletta Pellegrini *et al.*, 2003). This system is found in animals, plants and microorganisms. Glutathione peroxidase is an enzyme containing four selenium-cofactors that catalyzes the breakdown of hydrogen peroxide and organic hydroperoxides (Lenzi, 2002). There are at least four different glutathione peroxidase isozymes in animals. Glutathione peroxidase 1 is the most abundant and is a very efficient scavenger of hydrogen peroxide, while glutathione peroxidase 4 is most active with lipid hydroperoxides. Surprisingly, glutathione peroxidase 1 is dispensable, as mice lacking this enzyme have normal lifespans, but they are hypersensitive to induced oxidative stress. In addition, the glutathione *S*-transferases show high activity with lipid peroxides. These enzymes are at particularly high levels in the liver and also serve in detoxification metabolism. (Beutler, 1984)

2.6.1.5. Importance of enzymatic antioxidants

The activity of SOD, catalase and GSH-Px ensure an efficient scavenging action against reactive oxygen intermediates, thus preventing them from rapidly diffusing into oocyte membranes during the period of resumed meiotic activity. It is well known that germ cell membranes are particularly vulnerable to attack by ROS, being very rich in polyunsaturated fatty acids(Zhu, 2001). The high level of GST activity in this compartment can further contribute to an efficient detoxification from ROS byproducts (Zelko 2002).

The presence of the Alpha and Pi GST isoenzymes in human follicular fluid has already been described (Bisseling *et al.*, 1997). Significant amounts of Se-independent GSH-Px activity is showed, therefore emphasizing the antioxidant function of GST in follicular fluid. Finally, the significant levels of GSSG-Rx in this compartment may efficiently supply GSH, which, besides being a cofactor essential for both GSH-Px and GST activity, is also one of the most efficient non-enzymatic antioxidants. As a final result, the coordinate action of antioxidant enzymes, possibly working in concert with non-enzymatic antioxidants, may

provide maximum protection against ROS and their byproducts(WUYTS Nathalie *et al.*,1987).

Nonalcoholic steatohepatitis (NASH), an advanced form of nonalcoholic fatty liver disease (NAFLD), is a cause of hepatitis that may progress to cirrhosis. The pathogenesis of NASH involves two major stages; accumulation of triglyceride within hepatocytes as the first step, and oxidative stress that leads to inflammation, cellular injury, and progressive fibrosis as the second step. Cellular damage induced by mitochondrial oxidative stress is a widely accepted explanation for steatosis to the progression of steatosis to necroinflammation and fibrosis(Zámocký *et al.*,1999). Increased intrahepatic levels of fatty acids provide a source of oxidative stress. It has been proposed that the vulnerable fatty liver is injured by reactive oxygen species (ROS) generated from microsomal, mitochondrial, and/or other hepatocellular pro-oxidant pathways when the antioxidant defenses are critically lowered. ROS-mediated liver injury may be triggered by 3 main mechanisms: lipid peroxidation, cytokine induction, and Fas ligand induction.

Antioxidant enzymes reduce the levels of lipid peroxides as well as hydrogen peroxide and are important in preventing lipid peroxidation and maintaining the structure and function of biologic membranes. SOD catalyses the dismutation of peroxide to hydrogen peroxide and GSH-Px catalyses the oxidation of glutathione. It has been reported that certain antioxidants (eg, vitamin E, betaine, and acetylcysteine) are effective in decreasing serum transaminases and improving hepatic histopathology in patients with NAFLD (Abdelmalek *et al.*,2000, Gulbahar *et al.*, 2000)

The state of oxidative stress exists when there is imbalance between pro-oxidant and antioxidant chemical species Decreased serum SOD activity, increased serum GSH, and slight but statistically insignificant increases of serum GSH-Px and GR levels in NASH patients observed. This indicates that the hepatic antioxidant enzymatic defense system in NASH is impaired. The balance between oxidative stress and antioxidant defense mechanisms may be impaired by depletion of enzymatic antioxidants and increased serum levels of MDA and NO in patients with NASH. The enzyme glutathione reductase plays a pivotal role in replenishing and maintaining optimum concentrations of reduced glutathione in biological systems(Zeron *et al.*, 2001).

2.6.2. Non enzymatic antioxidants:

2.6.2.1. Ascorbic acid (or) vitamin C

Ascorbic acid or vitamin C is a monosaccharide antioxidant found in both animals and plants. As one of the enzymes needed to make ascorbic acid has been lost by mutation during human evolution, it must be obtained from the diet and is a vitamin. Most other animals are able to produce this compound in their bodies and do not require it in their diets. In cells, it is maintained in its reduced form by reaction with glutathione, which can be catalysed by protein disulfide isomerase and glutaredoxins. Ascorbic acid is a reducing agent and can reduce, and thereby neutralize, reactive oxygen species such as hydrogen peroxide. In addition to its direct antioxidant effects, ascorbic acid is also a substrate for the antioxidant enzyme ascorbate peroxidase, a function that is particularly important in stress resistance in plants. Ascorbic acid is present at high levels in all parts of plants and can reach concentrations of 20 millimolar in chloroplasts.

The skin is exposed to various exogenous sources of oxidative stress, including ultraviolet radiation. These spectral components are generally viewed as responsible for the extrinsic type of skin aging, sometimes termed photo-aging. It has been shown not only that increased levels of protective low molecular weight antioxidants through a diet rich in phytochemicals, but also by direct topical dermal application of low molecular weight antioxidants, notably vitamins C and E, as well as lipoic acid, may confer protective effects against oxidative stress

2.6.2.2. Vitamin E

Vitamin E is a family of α-, β-, γ-, and δ-tocopherols and corresponding four tocotrienols. Vitamin E is a fat-soluble antioxidant that stops the production of reactive oxygen species formed when fat undergoes oxidation. It protects cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction. This would remove the free radical intermediates and prevent the oxidation reaction from continuing. The oxidised α-tocopheroxyl radicals produced in this process may be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, retinol or ubiquinol. Other forms of vitamin E have their own unique properties. For example,

γ-tocopherol (also written as gamma-tocopherol) is a nucleophile that can react with electrophilic mutagens (Brigelius-Flohé *et al.*, 1999).

2.6.2.3. Importance of non-enzymatic antioxidants

Chronic renal failure (CRF) in children is associated with decreased concentrations of plasma antioxidant vitamins. This reduction is most expressed in children on maintenance hemodialysis (HD) and particularly concerns plasma vitamin C and erythrocyte vitamin E concentrations. The low levels of plasma vitamin A, E and C might result in reduced activity of the non-enzymatic antioxidant defense system and might be responsible for increased oxidative stress occurring in children with CRF(Lavine, 2000).

The generation of free radicals is a normal physiological process and free radicals act on lipids to cause lipid peroxidation. The cells have evolved a number of counteracting antioxidant defences. These antioxidant defence mechanisms can be categorized under the heads of free radical scavenging and chain breaking antioxidants (vitamin C and vitamin A). Reduced glutathione, alpha tocopherol, ascorbic acid and retinol are nonenzymatic chain breaking antioxidants which limit the cellular concentration of free radicals and prevent excessive oxidative damage (Corrocher *et al.*, 1986).

Vitamin C and vitamin A protect polyunsaturated fatty acids from peroxidative damage by donating hydrogen to the lipid peroxyl radical. Because of the lipophilic property of the tocopherol molecule vitamin E is the major free radical chain terminator in the lipophilic environment. Vitamin C as a reducing agent, directly reacts with superoxides, hydroxyl radicals and various lipid hydroperoxides. In addition it can also restore the antioxidant properties of oxidised vitamin E (Patil, 2006).

A significant increase in the lipid peroxidation as pregnancy advances is showed, which is associated with decreased antioxidant levels. Supplementation of natural antioxidants like alpha tocopherol, ascorbic acid and beta carotene may be beneficial in preventing complications like pregnancy induced hypertension.

Levels of the enzymes glutathione peroxidase and superoxide dismutase and of the nonenzymatic components of the antioxidant system including reduced glutathione, ascorbic acid, α-tocopherol, lycopene, and β-carotene were significantly lower in children with asthma compared with healthy controls. Reduced glutathione is an effective reductant and plays an

glutathione synthesis, glycine and glutamine were significantly lower in children with asthma. The majority of the amino acid susceptible to oxidative stress displayed lower levels in children with asthma. Childhood asthma is associated with significant decreases in various components of both enzymatic and nonenzymatic antioxidant defenses (Cansin Sackesen *et al.*, 2008).

2.6.3. Phytochemicals

Phytochemicals are chemical compounds such as beta-carotene that occur naturally in plants. The term is generally used to refer to those chemicals that may affect health, but are not yet established as essential nutrients. While there is abundant scientific and government support for recommending diets rich in fruits and vegetables, there is only limited evidence that health benefits are due to specific phytochemicals. Phytochemicals in fruits and vegetables may reduce the risk of cancer, possibly due to dietary fibers, polyphenol antioxidants and anti-inflammatory effects.

Some phytochemicals with physiological properties are elements rather than complex organic molecules. Abundant in many fruits and vegetables, selenium, for example, is involved with major metabolic pathways, including thyroid hormone metabolism and immune function. Particularly, it is an essential nutrient and cofactor for the enzymatic synthesis of glutathione, an endogenous antioxidant. There are many phytochemicals and each works differently. These are some possible actions:

- **Antioxidant** - Most phytochemicals have antioxidant activity and protect our cells against oxidative damage and reduce the risk of developing certain types of cancer. Phytochemicals with antioxidant activity: allyl sulfides (onions, leeks, garlic), carotenoids (fruits, carrots), flavonoids (fruits, vegetables), polyphenols (tea, grapes).

- **Hormonal action** - Isoflavones, found in soy, imitate human estrogens and help to reduce menopausal symptoms and osteoporosis.

- **Stimulation of enzymes** - Indoles, which are found in cabbages, stimulate enzymes that make the estrogen less effective and could reduce the risk for breast cancer. Other phytochemicals, which interfere with enzymes, are protease inhibitors (soy and beans), terpenes (citrus fruits and cherries).

- **Interference with DNA replication** - Saponins found in beans interfere with the replication of cell DNA, thereby preventing the multiplication of cancer cells. Capsaicin, found in hot peppers, protects DNA from carcinogens.

- **Anti-bacterial effect** - The phytochemical allicin from garlic has anti-bacterial properties.

- **Physical action** - Some phytochemicals bind physically to cell walls thereby preventing the adhesion of pathogens to human cell walls. Proanthocyanidins are responsible for the anti-adhesion properties of cranberry. Consumption of cranberries will reduce the risk of urinary tract infections and will improve dental health.

2.6.3.1. Carotenoids

Carotenoids are natural pigments synthesized by plants and microorganisms, but not by animals. Carotenoids are classified as follows: 1) Carotenoid hydrocarbons are known as carotenes and contain specific end groups. Lycopenes have two acyclic end groups. ß-Carotene has two cyclohexene type end groups. 2) Oxygenated carotenoids are known as xanthophyls. Examples of these compounds are a zeaxanthin and lutein (hydroxy), b) spirilloxanthin (methoxy), c) echinenone (oxo), and d) antheraxanthin (epoxy) .Of the over 600 carotenoids found in nature, about 40 are present in a typical human diet. Of these carotenoids, only 14 and some of their metabolites have been identified in blood and tissues. Many epidemiologic studies have associated high carotenoid intake with a decrease in the incidence of chronic disease. carotenoids can be converted to retinoids (i.e. have provitamin A activity), can modulate the enzymatic activities of lipoxygenases (proinflammatory and immunomodulatory molecules), can have antioxidants properties of vitamin A.

The antioxidant actions of carotenoids are based on their singlet oxygen quenching properties and their ability to trap peroxyl radicals. The best documented antioxidant action of carotenoids is their ability to quench singlet oxygen. This results in an excited carotenoid, which has the ability to dissipate newly acquired energy through a series of rotational and vibrational interactions with the solvent, thus regenerating the original unexcited carotenoid, which can be reused for further cycles of singlet oxygen quenching.

The quenching activity of a carotenoid mainly depends on the number of conjugated

(cyclic or acyclic) or the nature of substituents in carotenoids containing cyclic end groups. Lycopene (eleven conjugated and two nonconjugated double bonds) is among the most efficient singlet oxygen quenchers of the natural carotenoids. The prevention of lipid peroxidation by carotenoids has been suggested to be mainly via singlet oxygen quenching.

ß-Carotene is also scavenger of peroxyl radicals, especially at low oxygen tension. This activity may be also exhibited by others carotenoids. The interaction of carotenoids with peroxyl radicals may proceed via an unstable ß-carotene radical adduct. Carotenoid adduct radicals have been shown to be highly resonance stabilized and are predicted to be relatively unreactive. They may further undergo decay to generate non-radical products and may terminate radical reactions by binding to the attacking free radicals. Carotenoids act as antioxidants by reacting more rapidly with peroxyl radicals unsatured acyl chains. In this process, carotenoids are destroyed.

2.6.3.2. Polyphenols

A polyphenol antioxidant is a type of antioxidant containing a polyphenolic substructure. Numbering over 4,000 distinct species, these compounds have antioxidant activity in vitro but are unlikely to have antioxidant roles *in vivo*. a polyphenol antioxidant's ability to scavenge free radicals and up-regulate certain metal chelation reactions. That is to say various reactive oxygen species must be continually removed from cells to maintain healthy metabolic function. Some specific free radicals affected are the reactive oxygen species singlet oxygen, peroxynitrite and hydrogen peroxide. Diminishing the concentrations of reactive oxygen species can have several benefits. Since reactive oxygen species are linked to mobilization of ion transport systems, they are known to have roles in redox signaling.

2.6.3.3. Flavonoids

Flavonoids (both flavonols and flavanols) are most commonly known for their antioxidant activity in vitro. Research conducted at the Linus Pauling Institute and evaluated by the European Food Safety Authority indicates that, following dietary intake, flavonoids themselves are of little or no direct antioxidant value. Flavonoids and other polyphenols are poorly absorbed (less than 5%), with most of what is absorbed being quickly metabolized and

flavonoid-rich foods is not caused directly by flavonoids themselves, but most likely is due to increased uric acid levels that result from metabolism of flavonoids.

Most ingested flavonoids are extensively degraded to various phenolic acids, some of which still possess a radical-scavenging ability. Both the absorbed flavonoids and their metabolites may display an in vivo antioxidant activity, which is evidenced experimentally by the increase of the plasma antioxidant status, the sparing effect on vitamin E of erythrocyte membranes and low-density lipoproteins, and the preservation of erythrocyte membrane polyunsaturated fatty acids.

2.6.3.4. Alkaloids

The antioxidant action of a series of benzylisoquinoline alkaloids has been investigated. Laudanosoline, protopapaverine, anonaine, apomorphine, glaucine, boldine, bulbocapnine, tetrahydroberberine and stepholidine produced a dose-dependent inhibition of microsomal lipid peroxidation induced by Fe^{2+}/ascorbate, CCl_4/NADPH or by Fe^{3+}ADP/NADPH. The presence of a free hydroxyl group or preferably of a catechol group is a feature relevant for inhibition of lipid peroxidation and NBT reduction, nevertheless the antioxidant activity of benzylisoquinoline alkaloids cannot be only ascribed to the formation of phenoxy radicals and other free radical species may be formed during aporphine and tetrahydroprotoberberine oxidation. The influence of this series of compounds on the time course of lipid peroxidation suggests that some of them, like apomorphine and boldine act as chain-breaking antioxidants.

2.6.4. Plant Antioxidants

There are various forms of phytochemicals and antioxidants present in the foods, e.g. carotenoids and polyphenol compounds including flavonoids and anthocyanins. Seventy Fiji grown fruits and vegetables, and some other commonly consumed products, were analysed for their total antioxidant capacity (TAC), total polyphenol content (TPP), total anthocyanin content (TAT) as well as the major flavonol and carotenoid profiles. These data will be used to estimate the phytochemical and antioxidant intake of the Fijian population and will be a useful tool in future clinical trial. Green leafy vegetables had the highest antioxidant capacity, followed by the fruits and root crops (Tan DX, *et al.*,2000).

Main antioxidants in higher plants include glutathione, ascorbate, tocopherol, proline,

signaling components that interact with biomembrane-related compartments. As an evolutionary consequence of aerobic life for higher plants, reactive oxygen species (ROS) are formed by partial reduction of molecular oxygen. The above enzymatic and non-enzymatic antioxidants in higher plants can protect their cells from oxidative damage by scavenging ROS. In addition to crucial roles in defense system and as enzyme cofactors, antioxidants influence higher plant growth and development by modifying processes from mitosis and cell elongation to senescence and death (Sanchez-Moreno, 2002).

Most importantly, they provide essential information on cellular redox state, and regulate gene expression associated with biotic and abiotic stress responses to optimize defense and survival. An overview of the literature is presented in terms of main antioxidants and redox signaling in plant cells. Special attention is given to ROS and ROS-antioxidant interaction as a metabolic interface for different types of signals derived from metabolism and from the changing environment, which regulates the appropriate induction of acclimation processes or, execution of cell death programs, which are the two essential directions for higher plants(Vertuani *et al.*, 2004).

Plant antioxidants include a variety of structural types with a wide range of antioxidant activity. Phenolic acids are common antioxidants, ubiquitous in fruits, vegetables, legumes and grains. They exist primarily as substituted benzoic and cinnamic acid compounds. Flavonoids are more concentrated in fruits and vegetables, but are also found in grains. They number in the thousands and have a basic three-ring structure in common, but activity varies greatly dependent primarily on number and location of hydroxyl groups. Enzymatic hydroxylation, O-methylation, O-glycosylation or esterification can modify the basic structures of phenolic acids and flavonoids. The compounds may be absorbed directly during digestion or after hydrolysis. After absorption, sulfate, phosphate or glucuronide derivatives may be formed. Such transformations complicate direct analysis of biovailability(Shao HB, 2008).

Cereals have remarkably high antioxidant content, and even the most refined products from rice and corn are higher than essentially all vegetables and a majority of fruits. Whole grain products are generally recognized as healthful foods and important to a balanced diet. Whole grains provide a wide range of nutrients and phytochemicals that may work synergistically to optimize human health. Fruits and vegetables provide protection against age related diseases. It is believed their high content of antioxidant compounds is key to such protection (Subhasree *et al.*, 2009).

Antioxidants can be naturally obtained from fruits, vegetables, nuts and legumes and also synthesized in human body. Antioxidants are also available in specially formulated supplements. The following fruits are among the foods high in antioxidant activity

Table.2.1. List of food sources containing high levels of antioxidant activity

Antioxidant Food Sources	**Antioxidant Content/Activity**
Blackberry	51.53
Redcurrant	44.86
Raspberry	43.03
Olive (black)	39.99
Strawberry (wild)	28.00
Olive (green)	24.59
Strawberry (cultivated)	22.74
Orange	20.50
Blueberry	18.61
Pineapple	15.73
Plum (red)	12.79
Grape (black)	11.09
Grapefruit (yellow)	10.20
Tangerine	9.60
Clementine	8.88
Cherry	8.10
Kiwi fruit	7.41
Prickly pear	6.97
Peach (yellow)	6.57
Fig	5.82
Melon (cantaloupe)	5.73
Pear	5.00
Apricot	4.02

Grape (white)	3.25
Apple (yellow Golden)	3.23
Loquat	2.70
Banana	2.28
Melon (honeydew)	2.27
Watermelon	1.13

Acai is a fruit that looks similar to a grape and is native to Central and South America. Acai is rich in polyunsaturated fats, dietary fiber, calcium, as well as antioxidants like Vitamin E, and certain phytochemicals know as anthocyanins. After harvest, acai berries deteriorate rapidly. Acai is generally seen as a powder in foods like granola, or sold as a supplement in capsule or juice form.

Goji berry also known as wolfberry, is primarily grown in China and has long been a part of traditional Chinese medicine. Nutritionally, it has high concentrations of many vitamins and minerals, as well as phytochemicals such as beta-carotene, zeaxanthin, and lycopene. Goji berries are red-orange in color and look similar to raisins. They are generally sold as dried berries, and can be found in granola and trail mix, as well as in liquid form as a juice.

Manosteen grows on tropical evergreen trees in Pacific tropics. While the fruit itself is unpigmented and therefore has low nutrient value, the rind is a deep, reddish purple color, which contains phytochemicals known as xanthones. Mangosteen is generally seen as a puree of the whole fruit in either juice or capsule form.

2.6.6. Antioxidants in banana

During banana ripening, the starch converted into reducing sugars and sucrose increasing with ripeness. Banana a tropical plant may protect itself from the oxidative stress caused by strong sunshine and high temperature by producing large amounts of antioxidant. Banana should be considered to be a good source of natural antioxidant for foods and functional food source against cancer and heart disease. Therefore, attention in recent times has been focused on the isolation, characterization and utilization of natural antioxidants, especially growing interest in polyphenols as potential disease preventing agents. As these compounds are predominantly found in most of fruit tissues, it would be worthwhile

investigating the nature of polyphenols that are present in banana peel (Vijayakumar *et al.*, 2008).

Fruits and vegetables however, contain many different antioxidant and antimicrobial components. The majority of the antioxidant capacity of a fruit or vegetable may be from compounds such as other vitamin C, vitamin E or [beta]-carotene. 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. The antioxidant and antibacterial power of banana fruit peel and to identify the responsible compounds for those activities was evaluated (Kanazawa, 2000)

The fresh green and yellow banana peel (Musa, cv. Cavendish) fruits were treated with 70% acetone, which were partitioned with chloroform and ethyl acetate (EtOAc), sequentially. The antioxidant activities of the extracts were evaluated by using the thiocyanate method, [beta]-carotene bleaching method and 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical elimination. While, antimicrobial activities of the extracts and isolated components were evaluated using paper disc methods and Minimum Inhibition Concentration (MIC). The EtOAc and water soluble fractions of green peel displayed high antimicrobial and antioxidant activity, respectively. Antioxidant activity of water extracts was comparable to those of synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene. Among all isolated components B-sitosterol, malic acid, succinic acid, palmatic acid, 12-hydroxystrearic acid, glycoside, the d-malic and 12-hydroxystrearic acid were the most active against all the Gram-negative and positive bacterial species tested. The MIC of d-malic and succinic acid was varying between 140-750 ppm, respectively

The antioxidant compounds from commercial bananas, *Musa Cavendish*, were studied. One of the antioxidants, gallocatechin, was identified in the banana. The gallocatechin was isolated (using HPLC) from the banana peel extract, which showed strong antioxidant activity. Gallocatechin was more abundant in peel (158 mg/100 g dry wt.) than in pulp (29.6 mg/100 g dry wt.). The antioxidant activity of the banana peel extract, against lipid autoxidation, was stronger than that of the banana pulp extract. This result was consistent with the gallocatechin analysis. The higher gallocatechin content may account for the better

gallocatechin content. Bananas should be considered as a good source of natural antioxidants for foods.

A strong water-soluble antioxidant was identified in the popular commercial banana *Musa cavendishii*. It is dopamine, one of the catecholamines. For suppressing the oxygen uptake of linoleic acid in an emulsion and scavenging a diphenylpicrylhydrazyl radical, dopamine had greater antioxidative potency than glutathione, food additives such as butylated hydroxyanisole and hydroxytoluene, flavone luteolin, flavonol quercetin, and catechin, and similar potency to the strongest antioxidants gallocatechin gallate and ascorbic acid. Banana contained dopamine at high levels in both the peel and pulp. Dopamine levels ranged from 80–560 mg per 100 g in peel and 2.5–10 mg in pulp, even in ripened bananas ready to eat. Banana is thus one of the antioxidative foods

The antioxidant activity of flavonoids from banana (*Musa paradisiaca*) was studied in rats fed normal as well as high fat diets. Concentrations of peroxidation products namely malondialdehyde, hydroperoxides and conjugated diens were significantly decreased whereas the activities of catalase and superoxide dismutase were enhanced significantly. Concentrations of glutathione were also elevated in the treated animals (Smirnoff, 2000)

2.6.7. Taxonomy and classification of banana

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

The edible banana cultivars are mostly derived from two wild species of genus *Musa,* namely, *Musa acuminata* and *Musa balbisiana. Musa acuminata* is a diverse species and consists of at least nine subspecies while *Musa balbisiana* is less diverse and no subspecies has been suggested so far. All the edible cultivars originated from these two species belong to various genome groups. They are differed from each other depending on whether the clones are pure *acuminata* and *balbisiana*, diploid or triploid derivative and whether they are diploid, triploid or tetraploid hybrids of two wild species (Siddhurajua, 2002)Hence, a classification system was developed by Simmonds and Shepherd (1955) to classify all the edible banana cultivars systematically. On the basis of 15 vegetative and reproductive morphological characters, the differences between *Musa acuminate* and *Musa balbisiana* could clearly be discerned (Table 2.2). It is also necessary to determine the ploidy of a clone before it can be satisfactorily classified. Ploidy and relative contribution of the two species to

Musa acuminata and *Musa balbisiana* are designated as A and B, respectively. Basically, all edible banana cultivars can be classified into six groups which are AA, BB, AAA, AAB, ABB, and ABBB. They are respectively diploid, triploid and tetraploid. However, most of them are triploid.

Table 2.2. Characters used in distinguishing banana cultivars

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

Male flower Colour	Creamy white	Variably flushed with pink
Stigma colour	Orange or rich yellow	Cream, pale yellow or pale pink

2.6.8. Antioxidants and Phytochemicals in parts of Musa species

Musa sapientum commonly known as banana is widely used in Indian folk medicine for the treatment of diabetes mellitus. Oral administration of 0.15, 0.20 and 0.25 g/kg body weight of the chloroform extract of the flowers for 30 days resulted in a significant reduction in blood glucose and glycosylated haemoglobin and an increase in total haemoglobin. The extract prevented a decrease in body weight, and also resulted in a decrease in free radical formation in the tissues. Thus the study shows that banana flower extract (BFEt) has an antihyperglycaemic action. The decrease in thiobarbituric acid reactive substances (TBARS) and the increase in reduced glutathione (GSH), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) clearly shows the antioxidant property of BFEt

To understand the mechanisms of sucrose-induced acclimation in relation to plant cryopreservation, sugars, sterols, fatty acids of different lipid fractions (neutral lipids, glycolipids and sphingolipids and phospholipids), as well as free fatty acids were analyzed in proliferating meristem cultures of different banana varieties.

The banana flower is a good source of Vitamin C and Vitamin A. It is a fair source of calcium and iron, which help make strong bones and teeth (calcium) and help keep the blood healthy (iron).

Polyphenol oxidase activity (PPO, EC 1.14.18.1, monophenol monooxygenase, and EC 1.10.3.2, o-diphenoloxidase) has been extensively studied in banana fruit for its role in enzymatic browning. Rapid discolouration of leaf, stem and root tissue after injury and strong pigmentation of tissue extracts indicate that PPO and phenolic compounds are ubiquitous in vegetative tissue of banana as well. They hamper biochemical and molecular studies in banana, as cumbersome adaptations of extraction protocols are required. PPO and phenolic compounds could be an important part of the plant's defence system against pests and diseases, including root parasitic nematodes.

To facilitate future studies in this area, extraction and assay conditions for PPO from

activities were obtained in a 0.2 M phosphate buffer at pH 7.0 with 5% insoluble polyvinylpyrrolidone and 0.25% Triton X-100. The lowest K_m values were obtained for dopamine and D-catechin. Banana flowers and unexpanded leaf roll had high PPO activities with lower activities observed in mature leaves, roots and stem (Gooding *et al.*, 2001).

Musa paradisiaca is a plant of family Musaceae. The different parts of this plant (leaf, stem, flower) has been used as a folk medicine for treatment of peptic ulcers,analgesic, anti-asthmatic.The antioxidant activity of banana (*Giant Cavendishii*, AAA) leaf is determined with ferric thiocyanate method, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assay,reducing power determination, chelating effect on Fe(II) and liposomal model system (Kuo *et al.*,1999). The antioxidant activity of banana flower was determined by Hafidth *et al.*,(2008).

Chemical constituents of the Acitan a natural product obtained from *Musa paradisiaca* stem have been investigated, and some of them identified (tannins and phenols). Acitan scavenged both superoxide anions and hydroxyl radicals, and inhibited the degradation of deoxyribose mediated by hydroxyl radicals (Perez Capote *et al.*, 2007).

2.6.9. Evaluation of Antioxidant potential in parts of banana varieties

Among the various natural food sources that act as antioxidants, parts of banana contribute majorly. Many research findings quote that the bioavailability of antioxidants is greater in leaf part of banana. Parts of banana varieties have a great nutritive value and have been used in traditional medicine for the treatment or prevention of free radical induced diseases like arthritis, diabetes, cancer, etc. Banana resembled some properties of tea leaf including high content of phenolic compounds, polyphenol oxidase activity and lipoxygenase activity. This project therefore aims to bring limelight the *in vitro* and *in vivo* antioxidant and phytochemical activities of the parts (leaf, flower, stem) extracts from *Musa spp,* viz.,

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

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

MATERIALS AND METHODS

3. 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. Plant materials

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

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

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

The following parts of *Musa spp.* (*Rasthali, Karpooravalli*) were used:

- Leaf
- Stem
- Flower

3.3. Methods

Different methods were used to estimate the free radical scavenging activity of the parts of the *Musa spp* extracts by *in vitro* antioxidant capacity assays.

3.3.1. Preparation of the parts of *Musa spp.* 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.

3.3.2. *Invitro* Free radical Scavenging Assays

3.3.2.1. 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 reagent solution(0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate).The tubes were capped with silver foil and incubated at 95°C for 90 minutes. The tubes were then cooled to room temperature and the absorbance was measured at 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).

3.3.2.2 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

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 parts of the *Musa spp* 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 taken as blank. The percentage inhibition of the radicals due to the antioxidant property of the extract was calculated using the formula:

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

3.3.2.3. Determination of ABTS cation radical scavenging activity (Re *et al.*, 1999)

Principle

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

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

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 according to the formula :

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

3.3.2.4. 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.

3.3.2.5. Determination of Hydroxyl radical scavenging activity

(Umamaheshwari and Chatterjeel , 2008).

Principle

Hydroxyl radical scavenging activity was measured by the ability of the different fractions of banana peel samples to scavenge the hydroxyl radicals generated by the Fe^{3+} - ascorbate – EDTA – H_2O_2 system (Fenton reaction). These damage the substrate deoxyribose, which undergoes degradation to form malondialdehyde. This produces a pink chromogen with TBA. The antioxidants inhibit this reaction and hence there is a decrease in the colour intensity of the chromogen as the antioxidant potential increases

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.

3.3.2.6. Determination of superoxide radical scavenging activity

Reagents

1) Phosphate buffer

2) Riboflavin

3) EDTA

4) NBT

5) Sodium cyanide

Procedure

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

3.3.2.7. 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 of ferrous to ferric or by chelating the iron itself.

Reagents

1) Phosphate buffer saline(pH 7.4)

2) 0.07M Ferrous sulphate

4) 0.8%TBA in 1.1% SDS

5) 20% TCA

6) 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.

3.3.2.8. 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.

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.

3.3.2.9. 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 (1-10mg/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

3.3.3. Estimation of Enzymatic and Non- enzymatic antioxidants

3.3.3.1. Estimation of Enzymatic antioxidants

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

Principle:

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

Reagents:

1) 50mM phosphate buffer, pH 7.4

2) 20mM L- methionine.

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

4) 10mM Hydroxylamine hydrochloride.

5) 50 µM EDTA.

6) 50 µM Riboflavin.

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

Procedure:

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

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

3.3.3.1.2. Estimation of Catalase (Sinha, 1972)

Principle:

Catalase causes rapid decomposition of hydrogen peroxide to water.

$$H_2O_2 \longrightarrow H_2O + O_2$$

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

Reagents:

1) 0.01 M phosphate buffer, pH 7.0

2) 0.2 M Hydrogen peroxide.

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

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

Procedure:

The assay mixture contained 1.0 ml of buffer, 0.5 ml of hydrogen peroxide and 0.4 ml of water. 0.2 ml of sample was added to initiate the reaction. 2.0 ml of the dichromate/acetic

was added after the addition of the acid reagent. The tubes were then kept in boiling water bath for 10 min and the colour developed was read at 610nm. The activity of catalase was expressed as mole of hydrogen peroxide decomposed/min/mg protein.

3.3.3.1.3. Estimation of Glutathione Peroxidase (Ellman, 1959)

Reagents:

1) 0.4 M sodium phosphate buffer, pH 7.0

2) 10mM sodium azide.

3) 2.5mM Hydrogen peroxide.

4) 4mM reduced glutathione.

5) 10% TCA.

6) 0.3M phosphate solution.

7) 0.04% DTNB in 1% sodium citrate.

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

Procedure:

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

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

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

Principle:

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

CDNB + GSH ⟶ CDNB-S-glutathione.

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

Reagents:

1) 0.5 M phosphate buffer, pH 6.5

2) 30mM CDNB in 95% ethanol.

3) 30mM reduced glutathione.

Procedure:

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

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

3.3.3.1.5. Estimation of Glutathione Reductase (Beutler, 1984)

Principle:

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

$NADPH\ (NADH) + H^+ + GSSG \dashrightarrow NADP^+\ (NAD)^+ + 2GSH$

Reagents:

1) 0.3 M Phosphate buffer, pH 6.8

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

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

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

Procedure:

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

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

3.3.3.1.6. Estimation of Glucose 6 Phosphate Dehydrogenase

(Balinsky and Bernstein, 1963)

Principle:

Glucose 6 phosphate dehydrogenase is assayed by measuring the increase in absorbance which occurs at 340 nm when NADP reduces to NADPH. This reaction takes place when electrons are transferred from glucose 6 phosphate to NADP in the reaction catalysed by Glucose 6 phosphate dehydrogenase.

Reagents:

1) 0.1 M Tris HCl buffer, pH 8.2

2) 0.2 mM NADP

3) 0.1 M Magnesium chloride

4) 6 mM Glucose 6 phosphate

Procedure:

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

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

3.3.3.1.7. Estimation of Polyphenol Oxidase

(Rocha and Morais, 2001,Yemenicioglu, 2002)

Reagents:

1) 0.1M phosphate buffer, pH 6.0.

2) 0.001M Catechol dissolved in phosphate buffer.

Procedure:

1g of the sample was taken and ground in a mortar and pestle using 10ml of 0.1M phosphate buffer, pH 6.0. This was centrifuged at 6000 rpm for 10 minutes. 2ml of the supernatant was taken in a clean and dry test tube. To this 3.0ml of phosphate buffer and 1ml of catechol was added and mixed well. The absorbance was read at 495nm at an interval of 30 seconds for 3 minutes.

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

3.3.3.2. Estimation of Non- Enzymatic antioxidants

3.3.3.2.1. Estimation of Total Reduced Glutathione

Principle:

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

Reagents:

1) 0.2M phosphate buffer, pH 8.0

2) 0.4M Disodium hydrogen phosphate.

3) 0.04% DTNB in 1% sodium citrate.

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

Procedure:

1.0 ml of 10% homogenate was mixed with 1.0 ml of TCA. The solution was centrifuged at 1000 rpm for 10 min. To 1.0 ml of supernatant, 1.0 ml of disodium hydrogen phosphate and 2.0 ml of DTNB reagent was added. The absorbance was read within 2 min at 412nm against a reagent blank. A set of standards was also treated in the above manner. The amount of glutathione was expressed as mg g^{-1} fresh tissue.

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

Principle:

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

Reagents:

1) 4% oxalic acid.

2) 0.5N sulphuric acid.

3) 2% DNPH in 0.5N sulphuric acid.

4) 10% thiourea.

5) 80% sulphuric acid.

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

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

Procedure:

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

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

3.3.3.2.3. Estimation of Vitamin E

Principle:

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

Reagents:

1) Absolute ethanol

2) Xylene

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

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

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

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

Procedure:

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

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

3.3.3.3. Estimation of Phytochemicals

3.3.3.3.1. Estimation of Carotenoids and Lycopene

Principle:

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

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

Reagents:

1) 12% KOH.

2) Ethanol.

3) Petroleum ether.

4) Sodium sulphite.

Procedure:

5-10g of sample was saponified using 12% KOH in ethanol and kept for 30 minutes in a shaker. The sample was transferred to a separating funnel containing 10-15ml of petroleum ether and mixed gently. This was allowed to stand till the layers were separated completely. The pigments were collected in the petroleum layer. This was then transferred to the separating funnel and extracted using petroleum ether homogenous phase. The extraction of the aqueous phase was repeated with petroleum ether until it became colourless. The aqueous layer was discarded. To the extract sodium sulphite was added to remove turbidity. The final volume of extract was noted to a known volume. The absorbance was read at 450nm and 503nm.

3.3.3.3.2. Estimation of Flavonoids

Reagents:

1) Methanol.

2) Sodium nitrite.

3) Aluminium chloride.

4) 1M NaOH.

Procedure:

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

3.3.3.3.3. Estimation of Total Phenols

Principle:

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

Reagents:

1) Ethanol.

2) Folin- Ciocalteau reagent.

3) Sodium carbonate.

Procedure:

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

3.3.3.3.4. Estimation of Alkaloids

Reagents:

1) 0.1M acetic acid.

2) 0.01M Sodium meta periodate

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

Procedure:

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

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

Principle:

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

Reagents:

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

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

(Reagent B).

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

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

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

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

Procedure:

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

3.3.4. 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. RESULTS AND DISCUSSION

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

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

Oxidative stress has been implicated in the pathology of many diseases, inflammatory conditions, cancer and aging. And the best way to eliminate free radicals is with the help of antioxidants like Vitamins A, E and C. Antioxidants may offer resistance against oxidative stress by scavenging the free radicals, inhibiting lipid peroxidation, etc. and thus prevent the onset of deadly diseases.

Literature suggests that antioxidant activity is high on herbal plants and vegetables. Fruits and vegetables are rich in many nutrients. Green leafy vegetables are highly rich in nutrients like Vitamin A and Vitamin C, which are potent non-enzymatic antioxidants. Studies carried out by researchers have however shown that low consumption of vegetables is associated with an increased risk of cancer. This clearly indicates that apart from the antioxidants synthesized naturally, the body requires a supplement of dietary antioxidants. This can be obtained only by the consumption of an antioxidant rich diet.

Antioxidants are those substances that when present in low concentrations compared to those of an oxidizable substrate significantly delays or prevents oxidation of that substance. Antioxidants may inhibit these deleterious effects of ROS on the organism and therefore, a number of studies have evaluated the effect of various antioxidants *in vitro* and *in vivo* studies.

A great number of *in vitro* methods have been developed to measure the efficiency of

divided into two major groups: 1) Hydrogen atom transfer reactions like Oxygen Radical Absorbance Capacity (ORAC), Total radical trapping antioxidant potential (TRAP) and β carotene bleaching; 2) Electron transfer reactions like trolox equivalent antioxidant capacity (TEAC), ferric reducing antioxidant power (FRAP), 1,1-diphenyl 2-picryl hydrazyl radical scavenging assay (DPPH), superoxide anion radical scavenging assay, hydroxyl radical scavenging assay, nitric oxide radical scavenging assay and total phenol assay. However, it is essential to use more than one method to evaluate antioxidant capacity of plant materials because of the complex nature of phytochemicals. Generally, any part of the plant can be used for antioxidant studies but most commonly used part is leaf followed by fruit(Dizdaroglu *et al.*, 2002).

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. 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.

4.1. Free radical scavenging assays

4.1.1. Total antioxidant capacity assay

The total antioxidant activities of various parts of *Musa spp* extracts are depicted in Table 4.1.1.1. The total antioxidant assay gives an estimate of the overall antioxidant potential of parts of the *Musa spp*. 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 parts of the *Musa spp* extracts was expressed as number of equivalents of ascorbic acid.

Table 4.1.1.1 Total antioxidant activity of ethanolic extracts of parts of the *Musa spp* expressed as ascorbic acid equivalents.

Extract	Ascorbicacid equivalent(AAE) mM g^{-1}
Rasthali.Leaf	$5.69^{c} \pm 0.33$
Rasthali.Flower	$4.70^{b} \pm 0.02$
Rasthali.Stem	$3.60^{a} \pm 0.06$
Karpooravali.Leaf	$5.39^{c} \pm 0.06$
Karpooravali.Flower	$3.06^{a} \pm 0.03$
Karpooravali.Stem	$4.26^{b} \pm 0.11$

Values represent mean ± SD of 3 replicates.

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

Different parts extracts exhibited various degrees of antioxidant capacity .The ethanolic extract of *Rasthali leaf* showed higher activity in the range of 5.69 mM g^{-1} in comparison to other parts of *Musa spp*, whereas the ethanolic extract of *Karpooravalli stem* showed least activity.

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).

Sample concentration(mg/ml)										EC_{50} (mg/ml)
1	2	3	4	5	6	7	8	9	10	
$87^{g}\pm1.34$	$65.15^{f}\pm1.88$	$68.20^{f}\pm1.49$	$73.28^{e}\pm1.54$	$76.20^{de}\pm1.71$	$78.59^{cd}\pm1.74$	$80.64^{c}\pm1.87$	$86.17^{b}\pm1.75$	$87.61^{b}\pm2.28$	**$92.33^{a}\pm2.00$**	**0.15**
$01^{h}\pm1.79$	$57.49^{g}\pm1.75$	$63.65^{f}\pm1.60$	$68.47^{e}\pm1.58$	$71.00^{de}\pm1.51$	$74.17^{cd}\pm1.99$	$76.40^{bc}\pm1.81$	$77.04^{bc}\pm1.85$	$78.63^{b}\pm2.00$	$84.63^{a}\pm1.73$	0.50
$56^{g}\pm1.14$	$51.07^{g}\pm1.76$	$63.76^{f}\pm1.45$	$67.96^{e}\pm1.75$	$68.53^{e}\pm1.67$	$70.20^{de}\pm1.73$	$73.65^{cd}\pm1.88$	$76.39^{bc}\pm1.94$	$78.41^{b}\pm1.81$	$82.46^{a}\pm1.70$	1.60
$73^{i}\pm1.13$	$41.60^{h}\pm1.45$	$43.20^{h}\pm1.18$	$48.81^{g}\pm1.60$	$51.96^{f}\pm1.33$	$57.56^{e}\pm1.71$	$60.76^{d}\pm1.39$	$66.70^{c}\pm1.71$	$69.89^{b}\pm1.63$	$78.54^{a}\pm1.86$	4.2
$44^{g}\pm1.35$	$34.30^{g}\pm1.59$	$37.74^{f}\pm1.65$	$44.60^{e}\pm1.06$	$51.43^{d}\pm1.60$	$53.74^{d}\pm1.21$	$58.17^{c}\pm1.99$	$59.13^{c}\pm1.53$	$70.47^{b}\pm1.86$	$74.53^{a}\pm1.69$	4.95
$54^{g}\pm1.33$	$32.89^{g}\pm0.93$	$36.36^{f}\pm1.62$	$42.51^{e}\pm1.45$	$48.81^{d}\pm1.60$	$51.06^{d}\pm1.16$	$56.47^{c}\pm1.73$	$58.52^{c}\pm1.85$	$66.47^{Sb}\pm1.46$	$70.54^{a}\pm1.74$	5.40
10	20	30	40	50	60	70	80	90	100	6.07
48.43	55.58	64.67	73.55	79.05	84.56	87.04	90.56	93.45	96.45	6.64

ean ± SD of 3 replicates.

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

Figure 4.1.2.1. Scavenging activity (%) on DPPH radical by ethanolic extracts of parts of *Rasthali* variety.

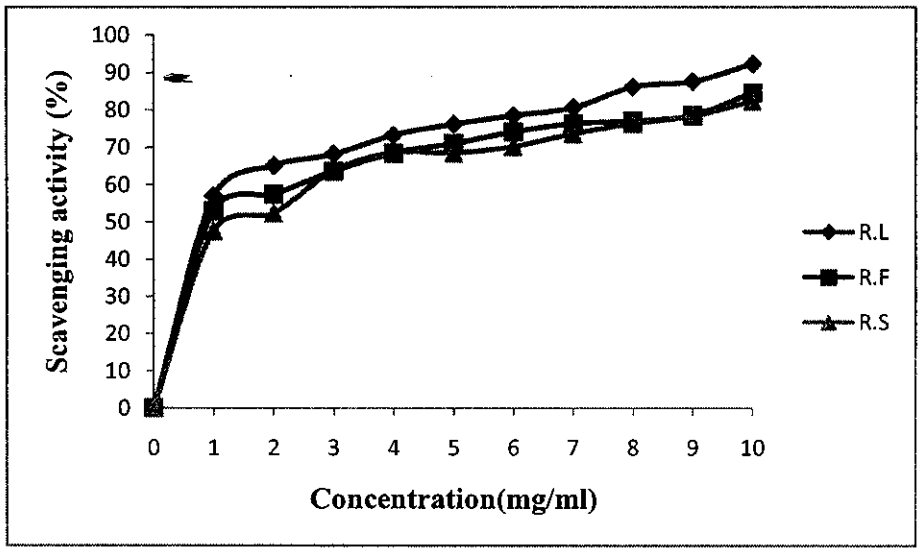


Figure 4.1.2.2. Scavenging activity (%) on DPPH radical by ethanolic extracts of parts of *Karpooravalli* variety.

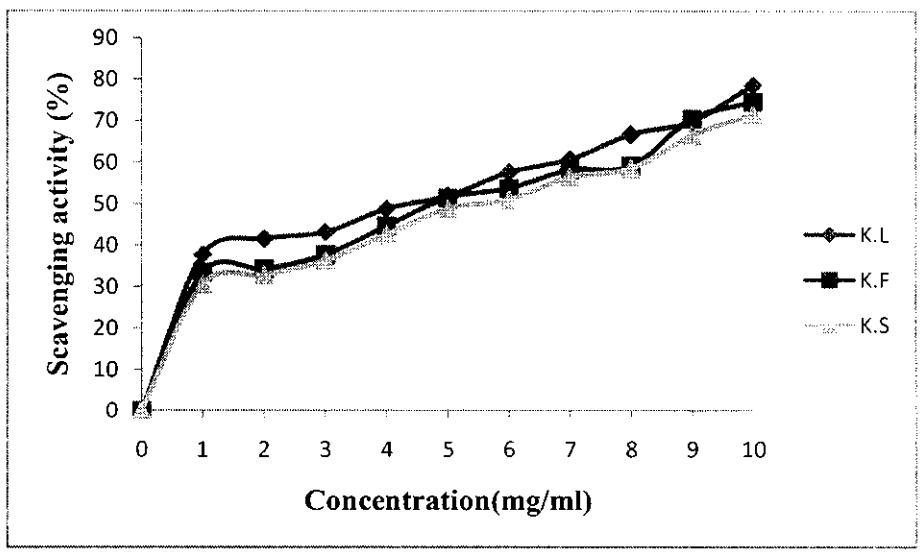
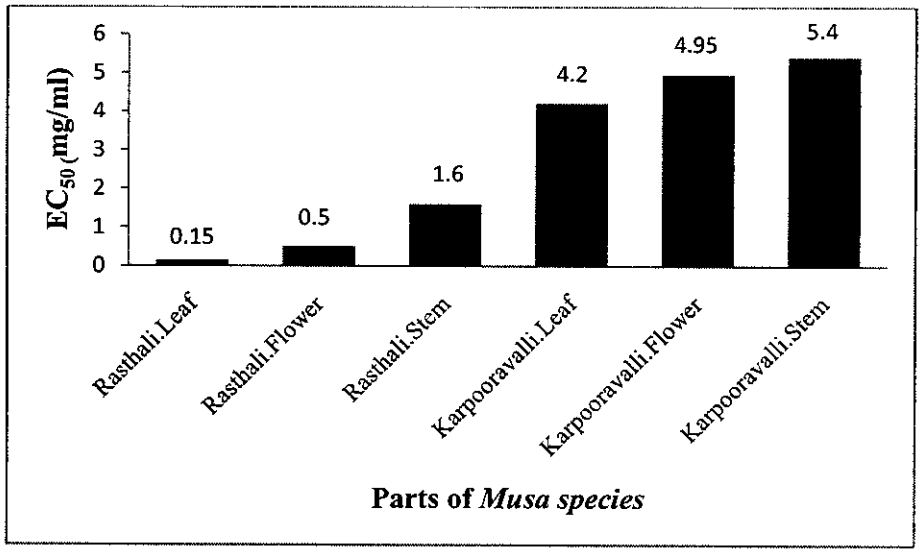


Figure 4.1.2.3.Comparison of EC_{50} values of DPPH inhibition activity



Free radical scavenging potential of the ethanolic extracts of parts of *Musa spp* 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 *Rasthali* leaf, flower, stem and *karpooravalli* leaf, flower, stem showed the scavenging activity of 56.87-92.33%, 53.01-84.63%, 47.56-82.46%, 37.73-78.54%, 33.44-74.53%, 30.54-71.54%, respectively. However, at 10mg ml^{-1}, Rasthali leaf extract exhibited highest DPPH scavenging activity. With respect to the EC_{50} value, *Rasthali* leaf showed least value, which represent highest antioxidant activity. Statistically, the scavenging activity of antioxidants was effective in the order of Rasthali leaf > Rasthali flower > Rasthali stem > Karpooravalli leaf > karpooravalli flower > karpooravalli stem.

Studies by Kuo *et al* (1999) have also showed the scavenging activity of *Musa* spp leaf extract on DPPH radical.

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).

3.1 ABTS cation radical scavenging activity (%) of ethanolic extract of parts of *Musa spp.*

Sample concentration(mg/ml)										EC_{50} (mg/ml)
1	2	3	4	5	6	7	8	9	10	
$4.72^{h}\pm1.22$	$62.02^{g}\pm1.35$	$66.76^{f}\pm1.48$	$71.55^{e}\pm1.80$	$79.43^{d}\pm1.65$	$82.65^{cd}\pm1.80$	$84.40^{c}\pm1.97$	$87.76^{b}\pm1.85$	$90.54^{ab}\pm1.90$	**$93.18^{a}\pm2.07$**	**0.30**
$2.04^{h}\pm1.19$	$54.43^{h}\pm1.15$	$59.27^{g}\pm1.61$	$63.17^{f}\pm1.64$	$67.43^{e}\pm1.86$	$73.19^{d}\pm1.80$	$77.66^{c}\pm1.66$	$82.16^{b}\pm1.66$	$85.57^{a}\pm1.83$	$88.36^{a}\pm1.82$	0.70
$9.76^{g}\pm1.07$	$55.51^{f}\pm1.50$	$57.87^{f}\pm1.24$	$62.94^{e}\pm1.38$	$63.99^{e}\pm1.38$	$72.03^{d}\pm1.55$	$76.63^{c}\pm1.72$	$80.42^{b}\pm1.93$	$83.73^{a}\pm1.70$	$84.60^{a}\pm1.72$	1.10
$8.83^{h}\pm1.01$	$53.71^{g}\pm1.21$	$60.47^{f}\pm1.28$	$63.81^{e}\pm1.51$	$68.57^{d}\pm1.39$	$73.52^{c}\pm1.80$	$77.79^{b}\pm1.93$	$81.56^{a}\pm1.82$	$83.63^{a}\pm2.07$	$84.76^{a}\pm1.72$	1.30
$5.95^{i}\pm1.04$	$49.42^{h}\pm1.20$	$53.33^{g}\pm1.19$	$57.93^{f}\pm1.20$	$63.99^{e}\pm1.38$	$69.35^{d}\pm1.49$	$72.87^{c}\pm1.53$	$77.88^{b}\pm1.67$	$83.32^{a}\pm1.69$	$83.64^{a}\pm1.69$	2.20
$2.13^{i}\pm1.04$	$45.80^{h}\pm1.42$	$49.55^{g}\pm1.09$	$51.75^{g}\pm1.38$	$56.15^{f}\pm1.37$	$59.29^{e}\pm1.42$	$63.79^{d}\pm1.43$	$68.49^{c}\pm1.38$	$74.55^{b}\pm1.84$	$80.42^{a}\pm1.93$	3.40
10	20	30	40	50	60	70	60	90	100	5.86
18.56	23.67	34.54	41.56	46.67	52.69	57.68	67.90	77.67	87.67	5.90

esent mean ± SD of 3 replicates.

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

Figure 4.1.3.1. Scavenging activity (%) on ABTS radical by ethanolic extracts of parts of *Rasthali* variety.

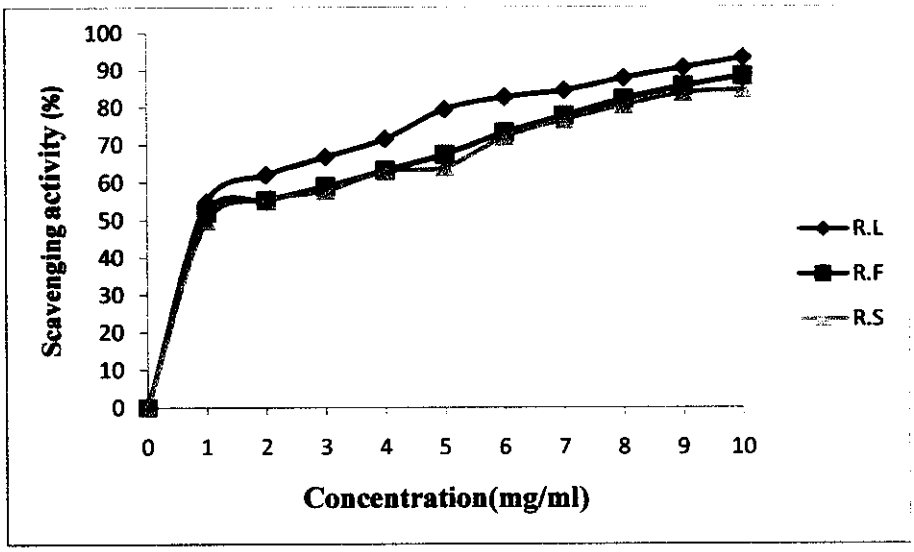


Figure 4.1.3.2. Scavenging activity (%) on ABTS radical by ethanolic extracts of parts of *Karpooravalli* variety.

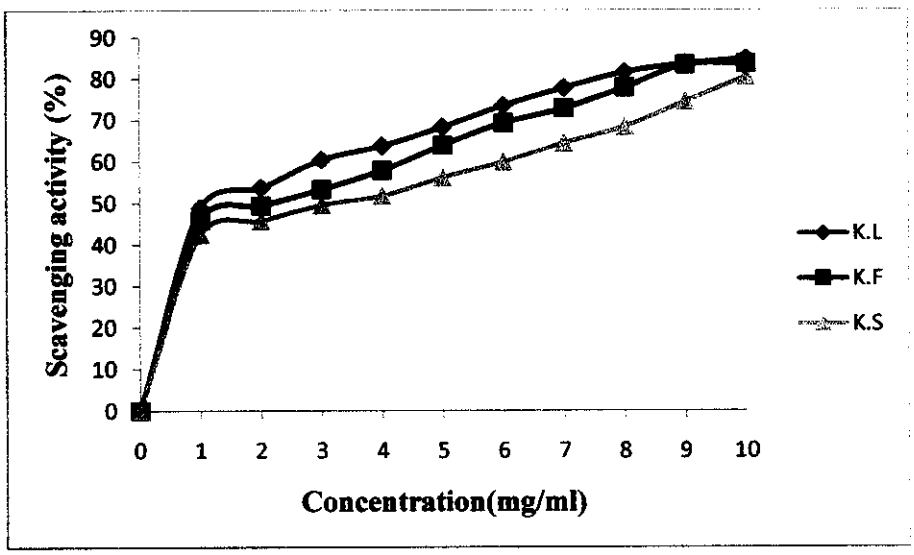
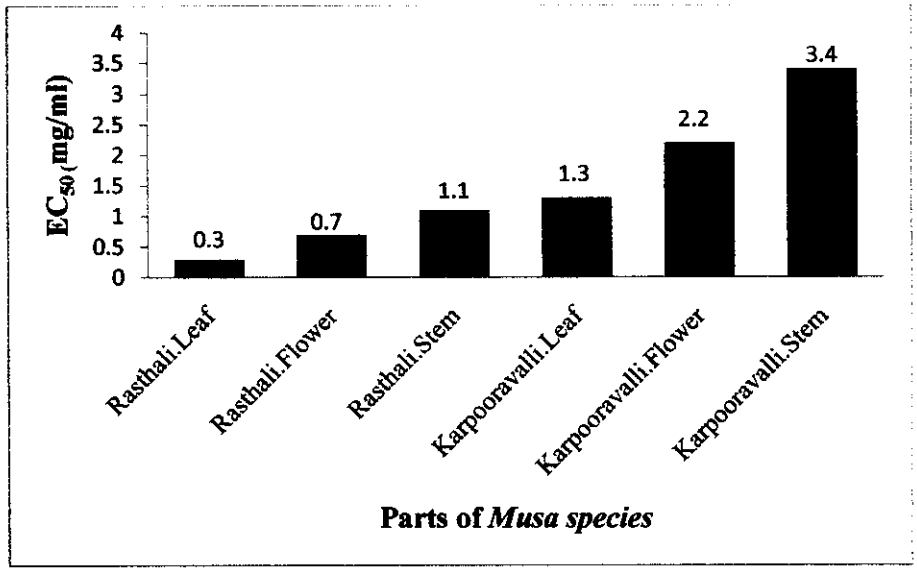


Figure 4.1.3.3 Comparison of EC_{50} values of ABTS radical scavenging activity



Free radical scavenging potential of the ethanolic extracts of parts of *Musa spp* 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 *Rasthali* leaf, flower, stem and *karpooravalli* leaf, flower, stem shows the percentage inhibition of 54.72%-93.18%, 52.04%-88.36%, 49.76%-84.60%, 48.83%-84.76%, 45.95%-83.94%, 42.46%-80.42% respectively. However, at 10mg ml^{-1}, *Rasthali* leaf extract exhibited highest ABTS scavenging activity. With respect to the EC_{50} value of *Rasthali* leaf showed least value, which represent highest antioxidant activity. Statistically, the scavenging activity of antioxidants was effective in the order of Rasthali leaf > Rasthali flower > Rasthali stem > Karpooravalli leaf > karpooravalli flower > karpooravalli stem.

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).

.4.1. Nitric oxide radical scavenging activity (%) on ethanolic extract of parts of *Musa spp.*

Sample concentration(mg/ml)										EC_{50} (mg/ml)
1	2	3	4	5	6	7	8	9	10	
4.44^{h}±0.78	41.17^{g}±0.92	44.17^{f}±1.09	47.74^{e}±0.99	50.62^{d}±1.26	50.88^{d}±1.16	52.91^{d}±1.35	56.93^{c}±1.30	66.32^{b}±1.55	**73.58^{a}±1.62**	**4.80**
5.41^{i}±0.59	29.53^{h}±0.77	31.37^{h}±0.87	34.34^{g}±0.88	37.54^{f}±0.91	40.84^{e}±1.05	47.55^{d}±1.16	52.91^{c}±1.24	62.63^{b}±1.43	70.37^{e}±1.65	7.45
4.49^{h}±0.57	27.33^{g}±0.67	28.79^{g}±0.69	32.00^{f}±0.78	33.68^{f}±0.89	36.37^{e}±0.99	41.42^{d}±0.97	48.12^{c}±1.06	54.81^{b}±1.37	64.66^{a}±1.68	8.30
2.45^{i}±0.74	35.16^{h}±0.77	35.60^{h}±0.96	39.71^{g}±1.05	43.79^{f}±0.95	46.24^{e}±0.99	50.71^{d}±1.15	59.52^{c}±1.49	66.67^{b}±1.46	70.66^{a}±1.79	6.90
3.52^{i}±0.65	27.64^{h}±0.64	30.20^{g}±0.87	34.04^{f}±1.03	35.39^{f}±0.93	38.49^{e}±0.95	43.03^{d}±1.18	45.29^{c}±1.17	55.03^{b}±1.70	64.77^{a}±1.59	8.50
1.32^{h}±055	25.46^{g}±0.76	27.76^{f}±0.69	29.93^{ef}±0.68	30.09^{e}±0.78	34.51^{d}±0.97	36.62^{d}±1.29	43.03^{c}±1.03	50.94^{b}±1.76	60.45^{a}±1.69	8.90
10	20	30	40	50	60	70	60	90	100	5.80
43.34	48.67	52.78	57.45	62.45	66.78	70.78	75.67	81.99	87.56	5.88

resent mean ± SD of 3 replicates.

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

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 parts of *Rasthali* variety.

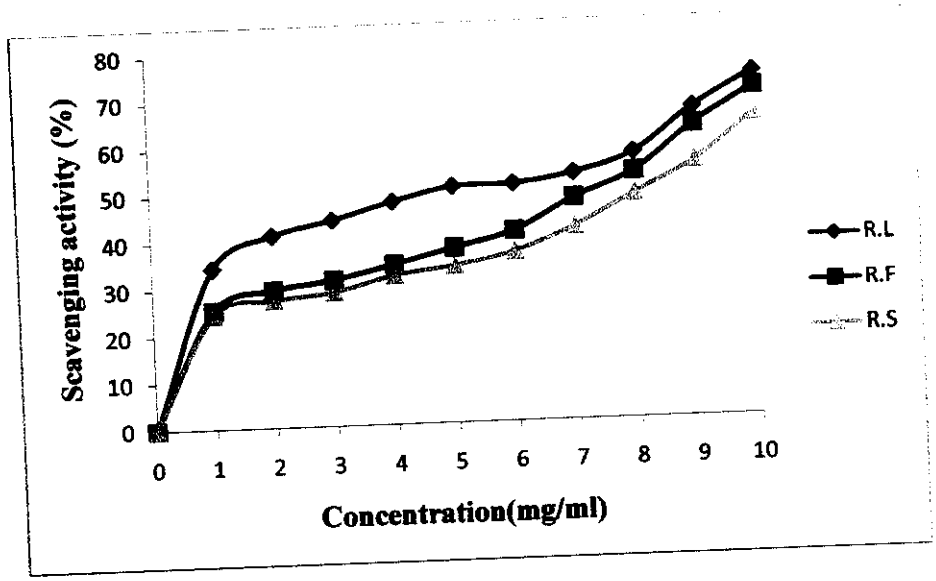


Figure 4.1.4.2. Scavenging activity (%) on nitric oxide radical by ethanolic extracts of parts of *Karpooravalli* variety.

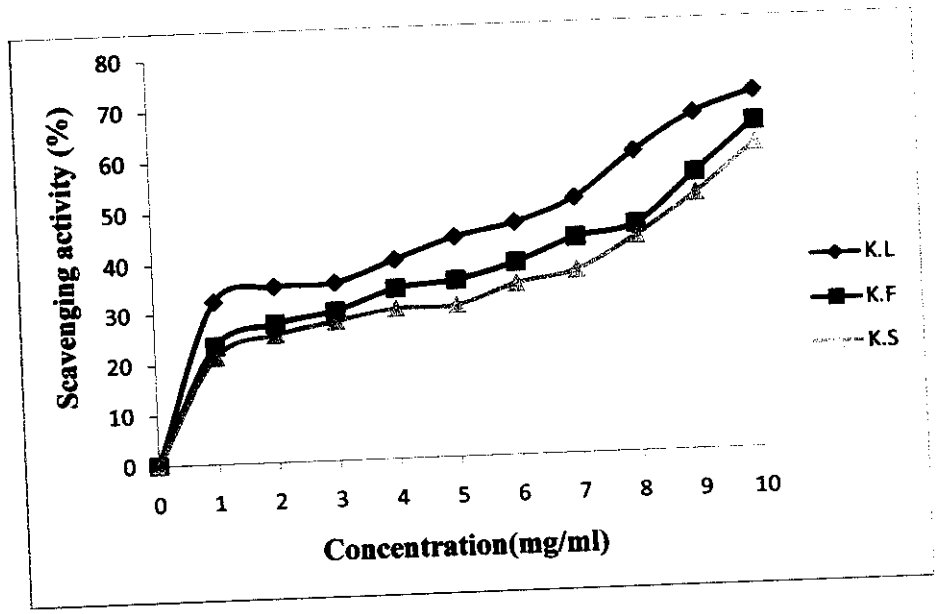
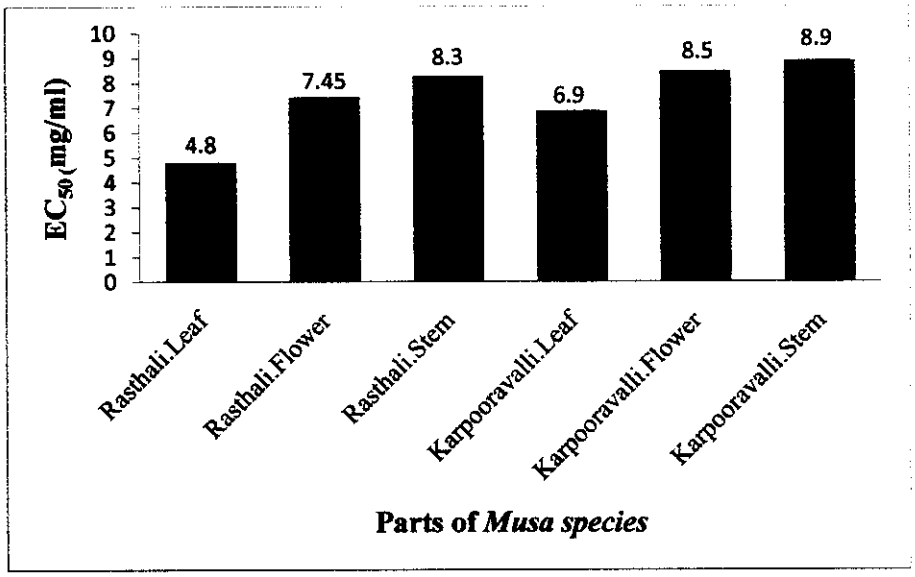


Figure 4.1.4.3. Comparison of EC_{50} values of nitric oxide radical inhibition activity



Free radical scavenging potential of the ethanolic extracts of parts of *Musa spp* is shown in Table 4.1.4.1 which increases with the increase in concentration

At 1 -10 mg ml^{-1}, the ethanolic extract of *Rasthali* leaf, flower, stem and *karpooravalli* leaf, flower, stem shows the percentage inhibition of 34.42%-73.58%, 25.41%-70.37%, 24.49%-64.66%, 32.45%-70.66%, 23.52%-64.77%, 21.32%-60.45% respectively. However, at 10mg ml^{-1}, *Rasthali* leaf extract exhibited highest nitric oxide scavenging activity. With respect to the EC_{50} value, Rasthali leaf showed least value, which represent highest antioxidant activity. Statistically, the scavenging activity of antioxidants was effective in the order of Rasthali leaf > Karpooravalli leaf >Rasthali flower > karpooravalli flower > Rasthali stem > karpooravalli stem.

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.

.5.1. Hydroxy radical scavenging activity (%) on ethanolic extract of parts of *Musa spp.*

Sample concentration(mg/ml)										EC_{50} (mg/ml)
1	2	3	4	5	6	7	8	9	10	
^{i}±0.72	25.45^{h}±0.54	35.57^{g}±0.75	40.26^{f}±1.49	49.83^{e}±1.04	53.62^{d}±1.24	61.35^{c}±1.56	67.44^{b}±1.80	73.44^{a}±1.58	**74.79^{a}±1.58**	**5.10**
^{j}±0.60	21.25^{i}±0.55	33.78^{h}±0.61	38.59^{g}±0.88	44.11^{f}±0.1.08	47.52^{e}±1.32	52.22^{d}±1.56	55.87^{c}±1.32	61.02^{b}±1.61	66.51^{a}±1.57	6.40
^{j}±0.54	18.54^{i}±0.89	23.59^{h}±0.88	33.66^{g}±0.92	37.80^{f}±1.18	45.69^{e}±1.56	49.89^{d}±1.18	53.35^{c}±1.61	59.68^{b}±1.58	64.54^{a}±1.61	7.10
^{j}±0.64	26.65^{i}±0.73	29.79^{h}±0.92	36.75^{g}±1.06	42.49^{f}±1.08	47.70^{e}±1.09	53.84^{d}±1.50	56.41^{c}±1.56	59.57^{b}±1.47	62.20^{a}±1.61	4.40
^{j}±0.76	24.34^{i}±0.54	28.30^{h}±0.72	33.99^{g}±1.21	38.01^{f}±0.89	42.23^{e}±1.40	45.80^{d}±1.48	50.04^{c}±1.34	54.90^{b}±1.40	58.7^{ba}±1.43	7.90
^{h}±0.89	22.47^{g}±0.97	26.17^{f}±1.06	33.51^{c}±1.04	34.48^{e}±1.49	38.15^{d}±1.51	40.38^{d}±1.84	45.16^{c}±1.72	48.27^{b}±1.46	51.82^{a}±1.74	9.50
10	20	30	40	50	60	70	60	90	100	6.85
9.20	14.45	21.78	28.67	33.27	38.63	44.56	50.67	58.67	65.78	6.95

sent mean ± SD of 3 replicates.

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

Figure 4.1.5.1. Scavenging activity (%) on hydroxyl radical by ethanolic extracts of parts of *Rasthali* variety.

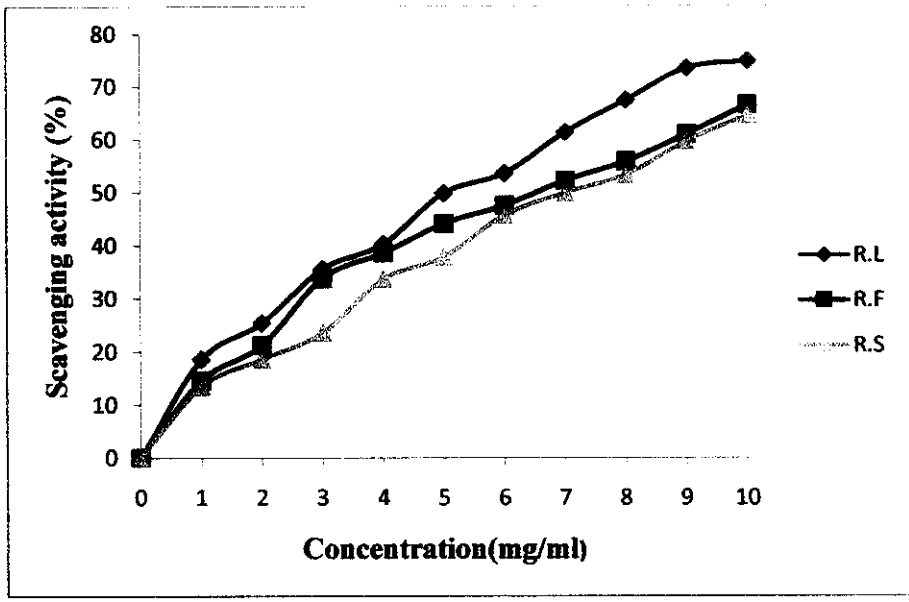


Figure 4.1.5.2. Scavenging activity (%) on hydroxyl radical by ethanolic extracts of parts of *Karpooravalli* variety.

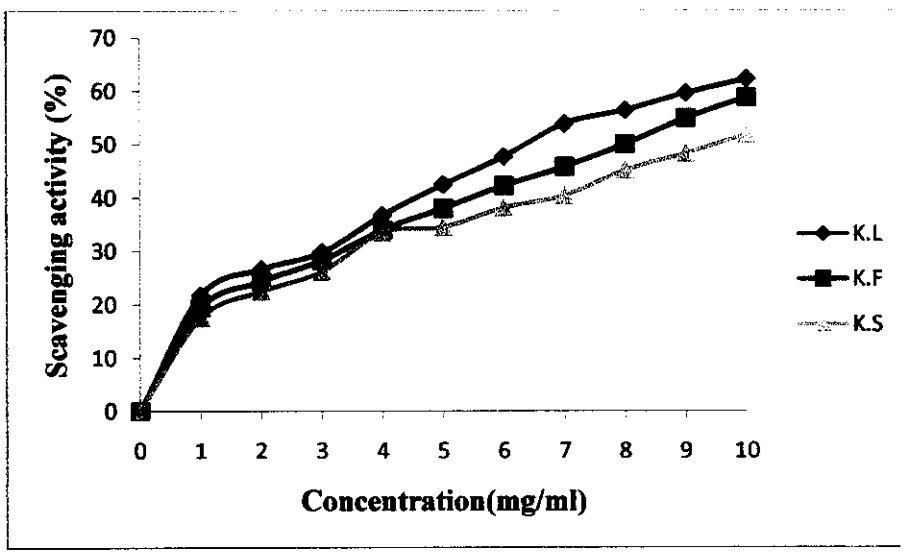
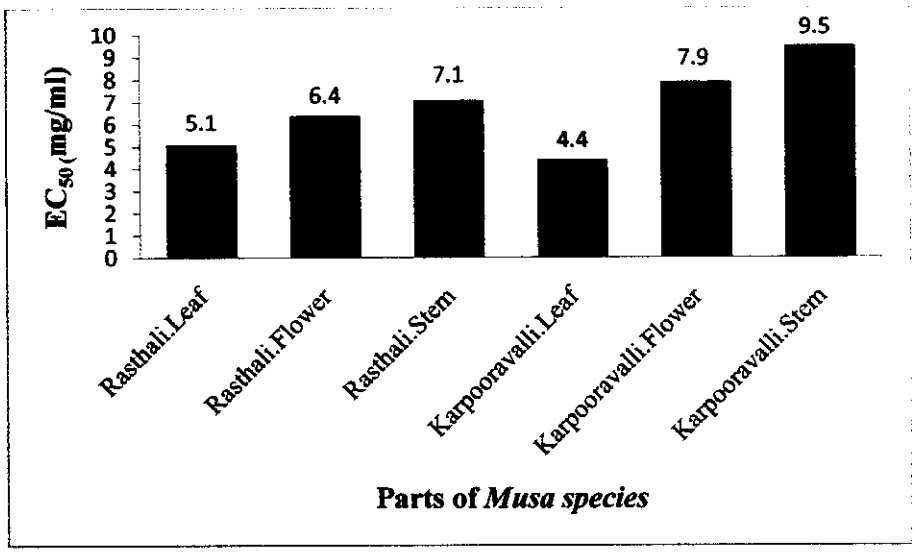


Figure 4.1.5.3 Comparison of EC_{50} values of hydroxyl radicals inhibition activity



Free radical scavenging potential of the ethanolic extracts of parts of *Musa spp* 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 *Rasthali* leaf, flower, stem and *karpooravalli* leaf, flower, stem shows the percentage inhibition of 18.54%-74.79%, 14.59%-66.51%, 13.43%-64.54%, 21.73%-62.20%, 19.44%-58.73%, 17.55%-51.82%, respectively. However, at 10mg ml^{-1}, Rasthali leaf extract exhibited highest hydroxyl radical scavenging activity. With respect to the EC_{50} value, *Rasthali* leaf showed least value, which represent highest antioxidant activity. Statistically, the scavenging activity of antioxidants was effective in the order of Rasthali leaf > Rasthali flower > Rasthali stem > Karpooravalli leaf > karpooravalli flower > karpooravalli stem.

Similar studies by Perez Capote *et al.* (2007) determined the scavenging effect of stem of *Musa spp.* on lipid peroxidation.

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).

Sample concentration(mg/ml)										
1	2	3	4	5	6	7	8	9	10	EC_{50} (mg/ml)
.39^{i}±0.63	28.53^{h}±0.65	35.45^{c}±0.81	49.67^{f}±1.09	52.84^{c}±1.27	58.54^{d}±1.35	62.94^{c}±1.47	71.80^{b}±1.62	72.91^{b}±1.57	**80.46^{a}±1.89**	**4.20**
.61^{j}±0.55	24.48^{i}±0.69	33.52^{h}±0.80	45.22^{g}±0.99	49.74^{f}±1.94	55.66^{e}±1.24	58.91^{d}±1.37	62.34^{c}±1.45	69.92^{b}±1.57	78.53^{a}±1.82	5.10
.41^{i}±0.55	22.59^{h}±0.63	29.35^{g}±0.82	38.65^{f}±1.08	45.23^{e}±1.58	50.98^{d}±1.16	57.84^{c}±1.58	61.52^{b}±1.97	68.13^{a}±1.70	70.21^{a}±1.79	5.95
.38^{i}±0.66	24.48^{i}±0.71	27.40^{h}±0.84	33.52^{g}±1.07	41.86^{f}±1.54	49.73^{e}±1.26	54.60^{d}±1.38	63.64^{c}±1.59	69.68^{b}±1.69	74.43^{a}±1.71	6.10
.59^{j}±0.69	20.65^{i}±0.65	24.58^{h}±0.73	29.57^{g}±0.87	34.64^{f}±1.16	42.43^{e}±1.12	52.11^{d}±1.21	59.05^{c}±1.33	66.95^{b}±1.72	69.88^{a}±1.75	6.85
.51^{j}±0.52	17.41^{i}±0.43	22.25^{h}±0.67	26.37^{g}±0.73	30.87^{f}±1.31	37.31^{e}±0.93	45.17^{d}±1.05	52.84^{c}±1.27	60.65^{b}±1.63	66.87^{a}±1.61	7.65
10	20	30	40	50	60	70	60	90	100	6.77
34.34	42.89	51.65	56.65	62.56	68.23	75.45	82.78	86.34	98.45	6.92

sent mean ± SD of 3 replicates.

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

Figure 4.1.6.1. Scavenging activity (%) on superoxide radical by ethanolic extracts of parts of *Rasthali* variety

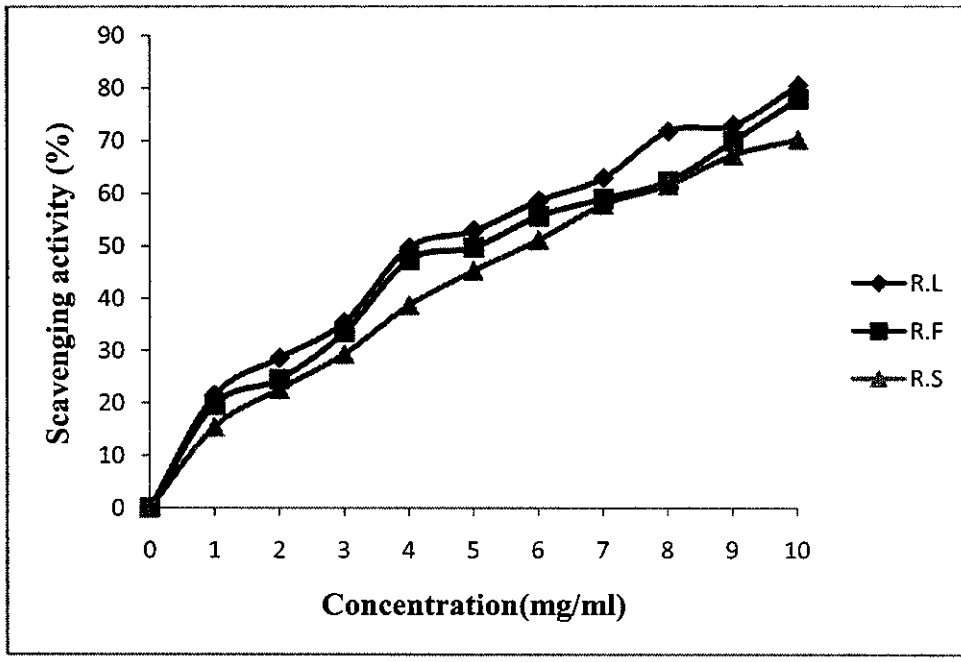


Figure 4.1.6.2. Scavenging activity (%) on superoxide radical by ethanolic extracts of parts of *Karpooravalli* variety

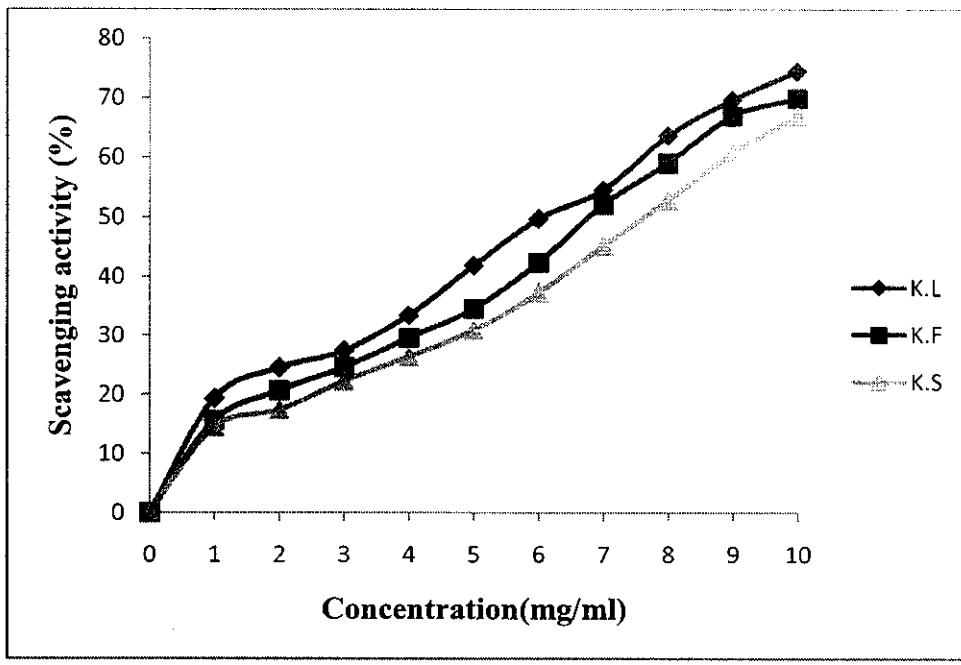
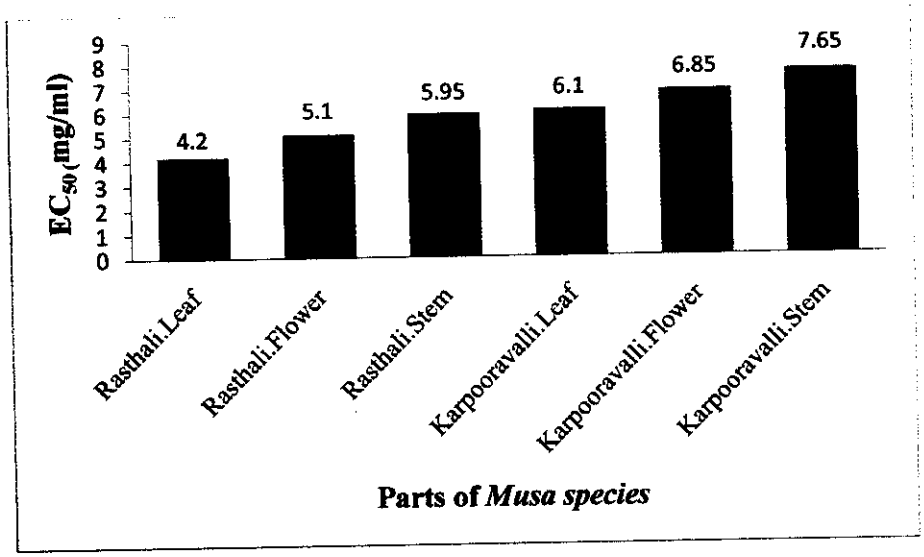


Figure 4.1.6.3 Comparison of EC_{50} values of superoxide radicals inhibition activity



Free radical scavenging potential of the ethanolic extracts of parts of *Musa spp* 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 *Rasthali* leaf, flower, stem and *karpooravalli* leaf, flower, stem shows the percentage inhibition of 21.39%-80.46%, 19.61%-77.86%, 15.41%-70.21%, 19.30%-74.43%, 15.59%-69.88%, 14.51%-66.87%, respectively. However, at 10mg ml^{-1}, *Rasthali* leaf extract exhibited highest superoxide radical scavenging activity. With respect to the EC_{50} value, Rasthali leaf showed least value, which represent highest antioxidant activity. Statistically, the scavenging activity of antioxidants was effective in the order of Rasthali leaf > Rasthali flower > Karpooravalli leaf > Rasthali stem > karpooravalli flower > karpooravalli stem.

4.1.7. Inhibition of lipid peroxidation activity

Lipid peroxides, derived from polyunsaturated fatty acids, 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

1.7.1 Lipid peroxidation inhibition (%) on ethanolic extract of parts of *Musa spp.*

Sample concentration(mg/ml)										EC_{50} (mg/ml)
1	2	3	4	5	6	7	8	9	10	
22.63^{i}±054	26.34^{i}±0.89	32.15^{h}±1.08	34.47^{g}±0.96	40.12^{f}±0.88	46.68^{e}±0.98	48.98^{d}±0.98	56.51^{c}±1.26	64.26^{b}±1.58	**75.37^{a}±1.76**	7.20
16.11^{i}±0.49	22.39^{i}±0.78	26.70^{h}±0.85	33.07^{g}±0.91	35.57^{f}±1.05	40.22^{e}±1.09	45.56^{d}±1.79	50.37^{c}±1.26	56.26^{b}±1.55	65.66^{a}±1.56	8.00
14.13^{i}±1.89	20.38^{h}±0.67	23.56^{g}±0.77	28.70^{f}±0.90	30.46^{f}±091	34.77^{e}±0.98	41.73^{d}±1.26	45.68^{c}±1.24	53.38^{b}±1.36	62.43^{a}±1.59	8.60
20.47^{i}±0.75	24.46^{h}±0.66	27.89^{g}±0.75	33.33^{f}±0.87	37.47^{e}±0.89	40.66^{d}±1.11	45.56^{c}±1.13	46.50^{c}±0.96	51.77^{b}±1.37	61.78^{a}±1.67	8.65
16.67^{i}±0.56	22.69^{h}±0.81	26.40^{g}±0.75	28.70^{f}±0.90	33.15^{e}±0.95	38.10^{d}±0.96	42.38^{c}±0.99	42.92^{c}±1.06	48.98^{b}±0.99	**57.63^{a}±1.65**	9.15
16.29^{h}±0.58	18.24^{h}±0.69	22.72^{g}±0.67	26.60^{f}±0.89	27.98^{f}±0.95	32.08^{e}±0.89	34.97^{d}±0.85	39.38^{c}±1.07	45.56^{b}±0.98	55.95^{a}±1.43	9.40
10	20	30	40	50	60	70	60	90	100	6.10
5.56	11.34	17.45	22.45	29.19	37.67	43.90	52.45	61.95	70.5	6.20

present mean ± SD of 3 replicates.

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

indicates the role of extracts as antioxidants. TBARS assay was used to determine the anti-lipid peroxidation properties of the parts of *Musa spp* extracts.

Figure 4.1.7.1. Scavenging activity (%) on lipid peroxidation by ethanolic extracts of parts of *Rasthali* variety.

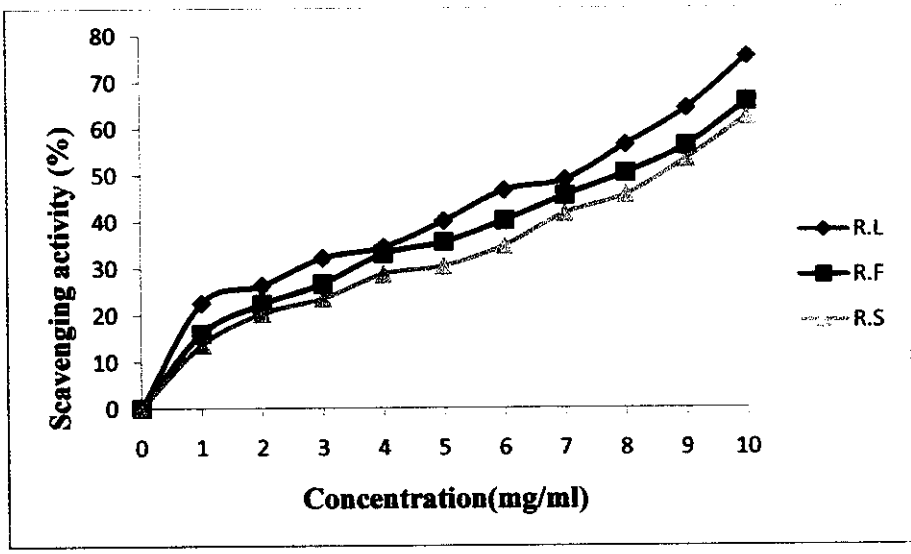


Figure 4.1.7.2. Scavenging activity (%) on lipid peroxidation by ethanolic extracts of parts of *Karpooravalli* variety.

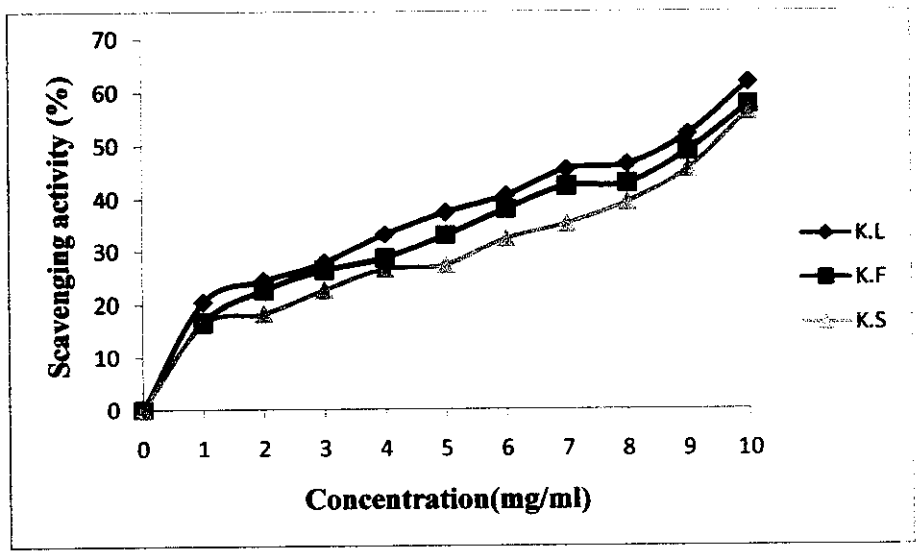
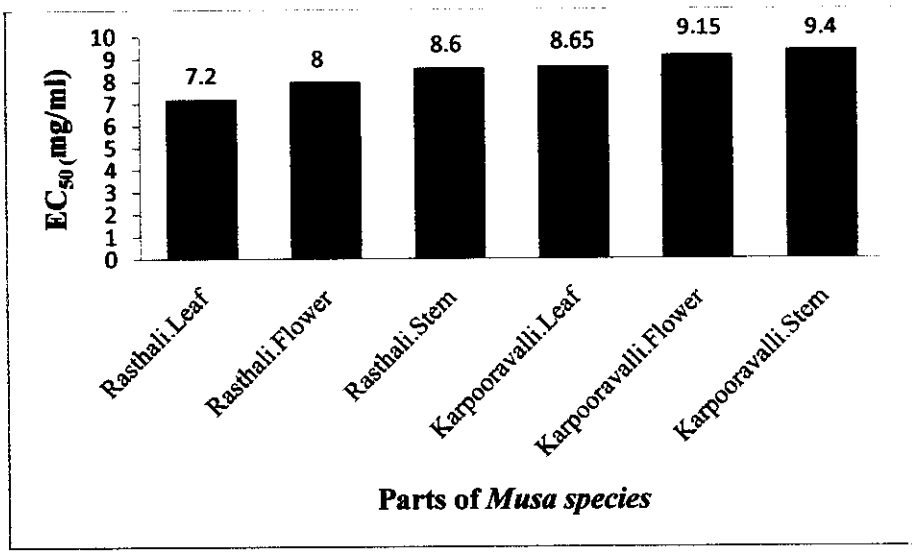


Figure 4.1.7.3 Comparison of EC_{50} values of lipid peroxidation inhibition activity



Free radical scavenging potential of the ethanolic extracts of parts of *Musa spp* 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 R*asthali* leaf, flower, stem and *karpooravalli* leaf, flower, stem shows the percentage inhibition of 22.63%-75.37%, 16.11%-65.66%, 13.69%-62.43%, 20.47%-61.78%, 16.67%-57.63%, 16.29%-56.34%, respectively. However, at 10mg ml^{-1}, Rasthali leaf extract exhibit highest lipid peroxidation inhibition activity. With respect to the EC_{50} value, Rasthali leaf showed least value, which represent highest antioxidant activity. Statistically, the scavenging activity of antioxidants was effective in the order of Rasthali leaf > Rasthali flower > Rasthali stem > Karpooravalli leaf > karpooravalli flower > karpooravalli stem.

Similar studies by Perez Capote *et al.* (2007) determined the scavenging effect of stem of *Musa spp.* on lipid peroxidation.

4.1.8. Ferric Reducing Antioxidant Potential activity

In this method, antioxidant compounds form a coloured 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 al.*, 2008)

.1.8.1 Ferric Reducing Antioxidant Potential activity on ethanolic extract of parts of *Musa spp.*

Sample concentration(mg/ml)									
1	2	3	4	5	6	7	8	9	10
$0.073^{j}\pm0.002$	$0.136^{i}\pm0.003$	$0.151^{h}\pm0.004$	$0.162^{g}\pm0.004$	$0.183^{f}\pm0.004$	$0.200^{e}\pm0.004$	$0.255^{d}\pm0.005$	$0.275^{c}\pm0.006$	$0.300^{b}\pm0.006$	**$0.345^{a}\pm0.007$**
$0.051^{j}\pm0.002$	$0.068^{i}\pm0.002$	$0.088^{h}\pm0.002$	$0.103^{g}\pm0.003$	$0.135^{f}\pm0.003$	$0.161^{e}\pm0.004$	$0.179^{d}\pm0.004$	$0.193^{c}\pm0.004$	$0.226^{b}\pm0.005$	$0.250^{a}\pm0.005$
$0.040^{j}\pm0.002$	$0.055^{i}\pm0.002$	$0.076^{h}\pm0.002$	$0.097^{g}\pm0.002$	$0.112^{f}\pm0.003$	$0.127^{e}\pm0.004$	$0.142^{d}\pm0.004$	$0.159^{c}\pm0.004$	$0.178^{b}\pm0.004$	$0.191^{a}\pm0.004$
$0.057^{j}\pm0.002$	$0.085^{i}\pm0.003$	$0.098^{h}\pm0.004$	$0.133^{g}\pm0.003$	$0.149^{f}\pm0.003$	$0.173^{e}\pm0.004$	$0.202^{d}\pm0.005$	$0.233^{c}\pm0.005$	$0.254^{b}\pm0.005$	$0.278^{a}\pm0.006$
$0.040^{j}\pm0.001$	$0.060^{i}\pm0.002$	$0.072^{h}\pm0.002$	$0.089^{g}\pm0.002$	$0.110^{f}\pm0.003$	$0.145^{e}\pm0.003$	$0.163^{d}\pm0.004$	$0.171^{c}\pm0.004$	$0.185^{b}\pm0.004$	$0.213^{a}\pm0.005$
$0.029^{j}\pm0.001$	$0.044^{i}\pm0.001$	$0.058^{h}\pm0.002$	$0.085^{g}\pm0.002$	$0.103^{f}\pm0.003$	$0.122^{e}\pm0.003$	$0.132^{d}\pm0.003$	$0.143^{c}\pm0.003$	$0.154^{b}\pm0.004$	$0.168^{a}\pm0.004$
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

esent mean ± SD of 3 replicates.

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

Figure 4.1.8.1 Reducing power of ethanolic extracts of parts of *Rasthali* variety

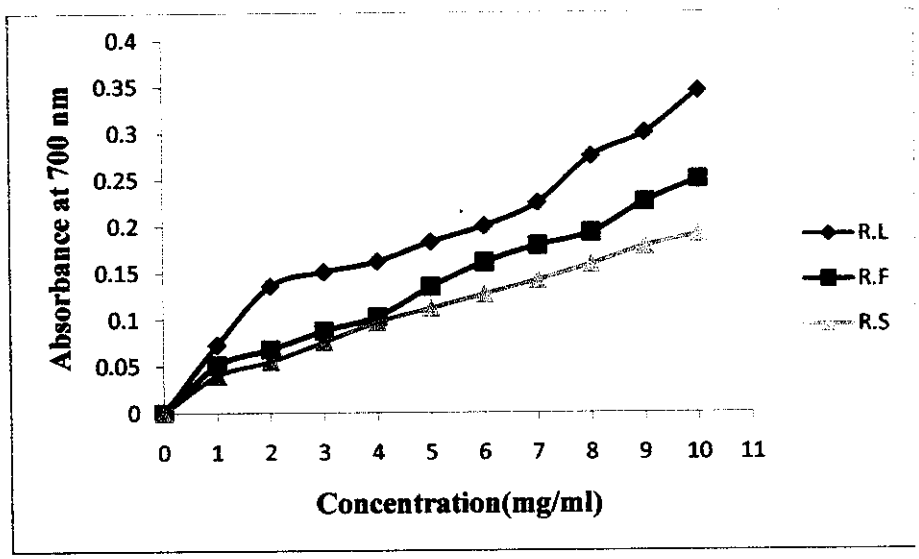
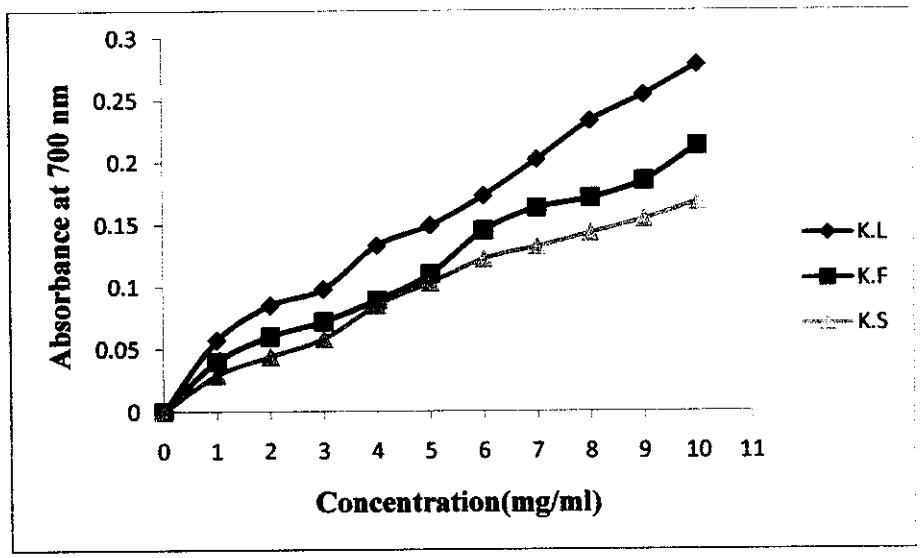


Figure 4.1.8.1 Reducing power of ethanolic extracts of parts of *Karpooravalli* variety



The reducing power of ethanolic extracts of parts of *Musa* spp. increases with the increase in concentration as depicted in the graph. The ethanolic extract of *Rasthali* leaf, flower, stem and *karpooravalli* leaf, flower, stem show the Mean absorbance of 0.073-0.345, 0.051-0.250, 0.040-0.191, 0.057-0.278, 0.040-0.213, 0.029-0.168 respectively. However, at 10mg ml^{-1}, *Rasthali* leaf extract exhibited highest reducing power.

Similar observation was made by (Kuo *et al.*, 1999) which showed significant reducing power of parts of Musa spp.

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 compounds in emulsions, accompanied with the coupled oxidation of β-carotene and linoleic acid.

Figure 4.1.9.1. Scavenging activity (%) on β-carotene bleaching by ethanolic extracts of parts of *Rasthali* variety.

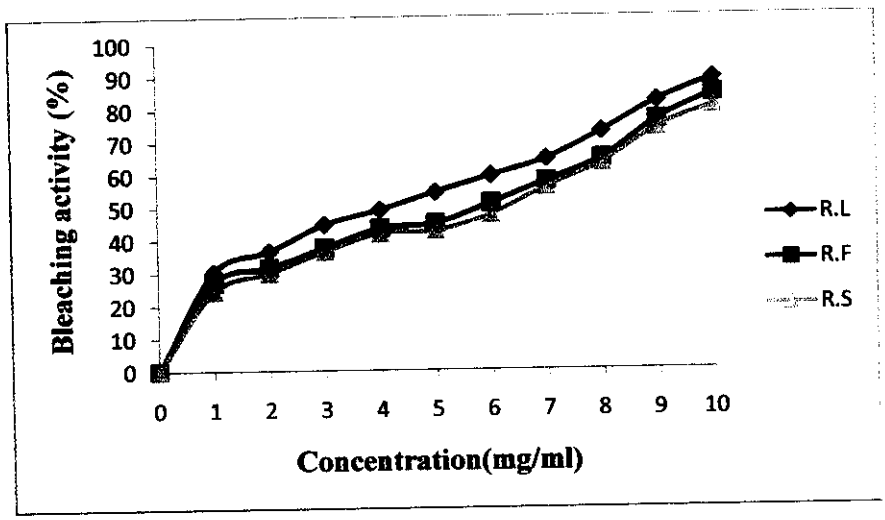
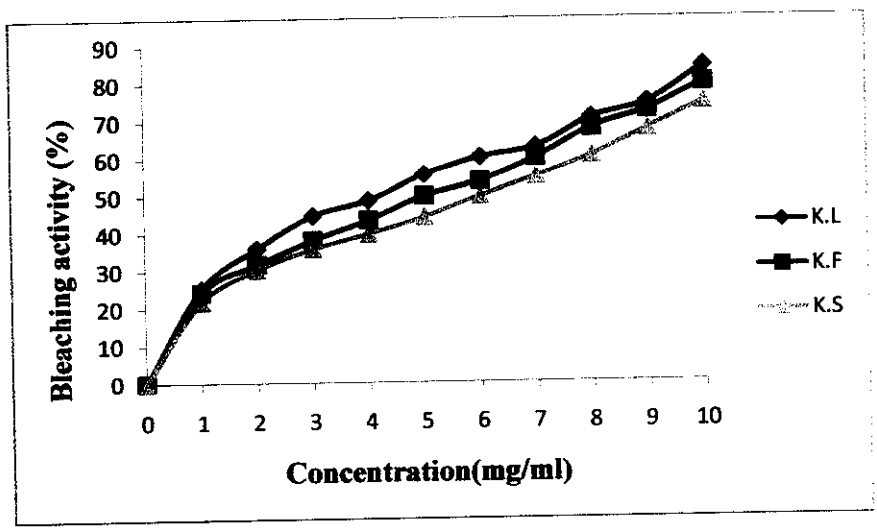


Figure 4.1.9.2. Scavenging activity (%) on β-carotene bleaching by ethanolic extracts of parts of *Karpooravalli* variety.



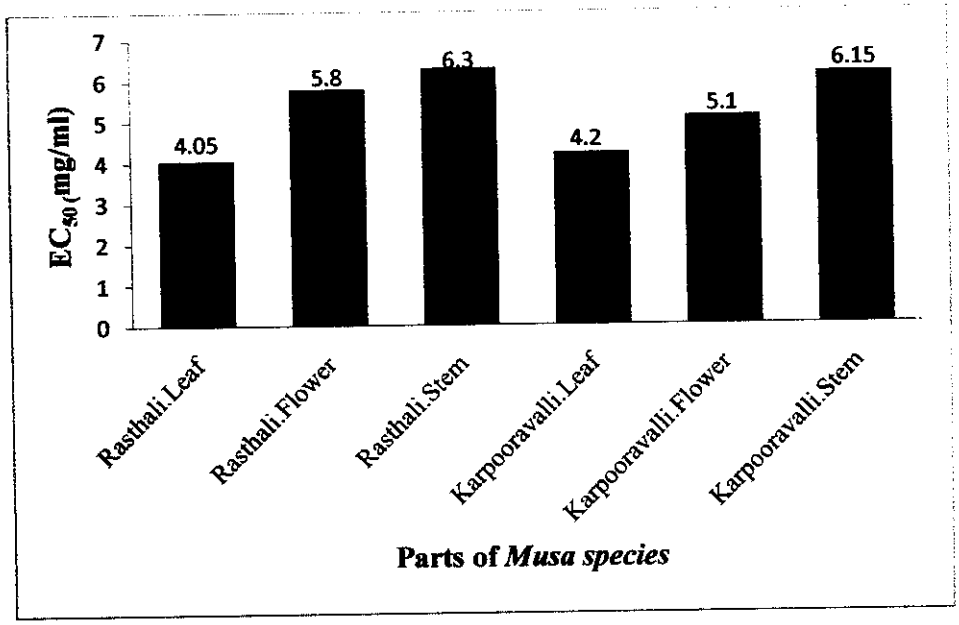
1.9.1. β-carotene bleaching (BCB) activity (%) on ethanolic extract of parts of *Musa spp.*

Sample concentration(mg/ml)										EC_{50} (mg/ml)
1	2	3	4	5	6	7	8	9	10	
$30.39^{i}\pm0.75$	$36.60^{h}\pm0.99$	$44.80^{g}\pm1.78$	$49.15^{f}\pm1.96$	$54.12^{e}\pm1.78$	$59.56^{d}\pm1.78$	$57.79^{d}\pm1.98$	$72.74^{c}\pm1.96$	$82.07^{b}\pm1.98$	**$88.85^{a}\pm1.99$**	**4.05**
$27.21^{i}\pm0.69$	$31.65^{h}\pm1.47$	$37.62^{g}\pm1.45$	$43.50^{f}\pm1.11$	$43.97^{f}\pm1.65$	$51.04^{e}\pm1.49$	$57.81^{d}\pm1.71$	$64.61^{c}\pm1.96$	$76.26^{b}\pm1.95$	$84.31^{a}\pm1.86$	5.80
$24.47^{i}\pm1.09$	$30.04^{h}\pm1.07$	$36.51^{g}\pm1.07$	$41.90^{f}\pm1.30$	$42.73^{f}\pm1.12$	$47.51^{e}\pm1.58$	$55.92^{d}\pm1.56$	$63.11^{c}\pm1.74$	$73.82^{b}\pm1.96$	$80.51^{a}\pm2.06$	6.30
$25.30^{i}\pm0.95$	$36.04^{h}\pm1.07$	$44.64^{g}\pm1.45$	$48.67^{f}\pm1.67$	$55.50^{e}\pm1.79$	$60.22^{d}\pm1.59$	$62.98^{d}\pm1.73$	$70.56^{c}\pm1.89$	$74.67^{b}\pm1.97$	$84.07^{a}\pm1.97$	4.20
$24.27^{i}\pm0.86$	$31.72^{i}\pm1.01$	$38.18^{h}\pm1.05$	$43.50^{g}\pm1.20$	$49.82^{f}\pm1.15$	$53.80^{e}\pm1.49$	$60.05^{d}\pm1.59$	$67.96^{c}\pm1.50$	$72.65^{b}\pm1.75$	$79.84^{a}\pm1.65$	5.10
$21.77^{i}\pm0.78$	$30.31^{h}\pm1.08$	$35.88^{g}\pm1.07$	$38.37^{g}\pm1.19$	$44.18^{f}\pm1.05$	$49.77^{e}\pm1.49$	$54.89^{d}\pm1.56$	$60.55^{c}\pm1.86$	$67.68^{b}\pm1.87$	$74.65^{a}\pm1.73$	6.15
10	20	30	40	50	60	70	60	90	100	6.20
5.56	11.34	17.45	22.45	29.19	37.67	43.90	52.45	61.95	70.5	6.31

resent mean ± SD of 3 replicates.

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

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



Free radical scavenging potential of the ethanolic extracts of the parts of *Musa spp* is shown in Table 4.1.9.1 which increases with the increase in concentration

At 1-10 mg ml^{-1}, the ethanolic extract of *Rasthali* leaf, flower, stem and *karpooravalli* leaf, flower, stem show the percentage inhibition of 30.39%-88.85%, 27.21%-84.31%, 24.47%-80.51%, 25.30%-84.07%, 24.27%-79.84%, 21.77%-74.65% respectively. However, at 10mg ml^{-1}, Rasthali leaf extract exhibit highest activity. With respect to the EC_{50} value of *Rasthali* leaf showed the least value, which represent highest β-carotene bleaching inhibition activity. Statistically, the scavenging activity of antioxidants was effective in the order of Rasthali leaf > Rasthali flower >Karpooravalli leaf > Rasthali stem> Karpooravalli flower > Karpooravalli stem.

4.2. Enzymatic antioxidants

Table 4.2.1 represents the activities of superoxide dismutase and catalase activities in different parts of *Musa spp*.

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

Catalase is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules. Human catalase works at an optimum temperature of 37°C.

The SOD activity in parts of *Musa spp* varied from 15.50 to 34.65 units mg^{-1} protein. The highest activity was recorded in *Rasthali* leaf and the lowest in *Karpooravalli* stem. Hence, *Rasthali* leaf has the highest potential to scavenge superoxide radicals. The catalase activity in parts of *Musa spp* varied from 12.50 to 24.23 units mg^{-1} protein. The parts extracts obtained from *Rasthali* leaf has the greatest potential to decompose hydrogen peroxide as it showed highest catalase activity.

Table 4.2.1. Activities of Hydrogen peroxide metabolizing enzymes in the different parts of *Musa spp*

Varieties	Superoxide dismutase (SOD)	Catalase (CAT)
Rasthali.Leaf	**34.65^{c}±1.146**	**24.23^{b}±0.936**
Rasthali.Flower	26.29^{b}±0.945	20.23^{a}±0.789
Rasthali.Stem	22.56^{a}±0.561	18.48^{a}±0.449
Karpooravalli.Leaf	27.41^{c}±1.352	20.70^{c}±0.968
Karpooravalli.Flower	24.71^{b}±0.603	18.50^{b}±0.489
Karpooravalli.Stem	15.50^{a}±0.253	12.50^{a}±0.457

Values represent mean ± SD of 3 replicates.

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

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

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

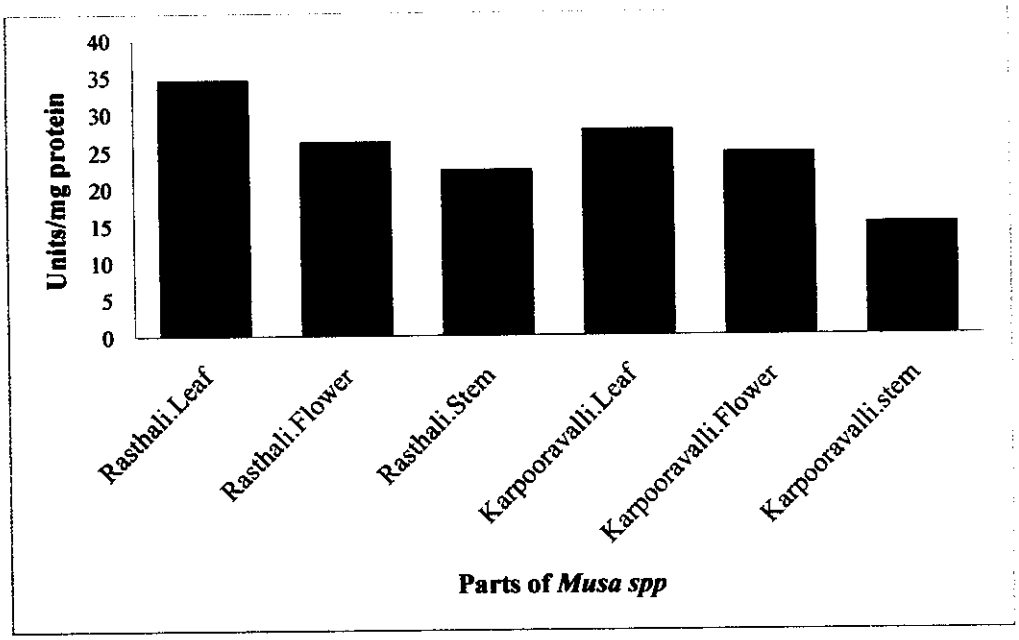


Fig. 4.2.1. Activity of superoxide dismutase in different parts of *Musa spp*.

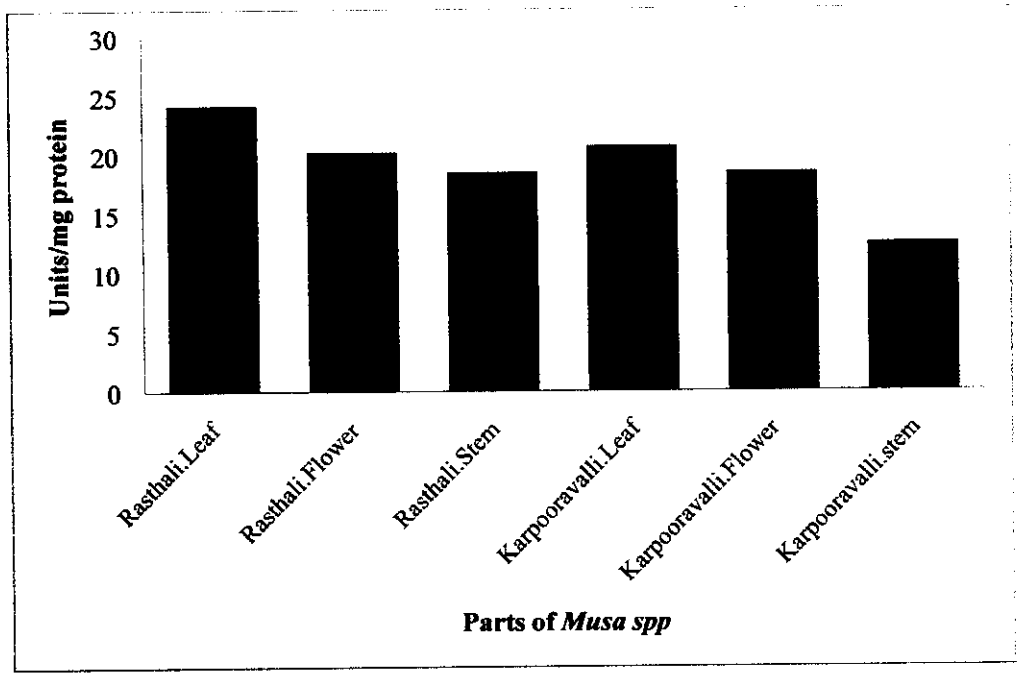


Fig. 4.2.2. Activity of catalase in different parts of *Musa spp*.

The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water. GST offers protection against lipid peroxidation by promoting the conjugation of toxic

electrophiles with GSH (Jakoby, 1988). GR maintains the cellular levels of GSH (by the reduction of GSSG), which protects the cellular membranes from peroxides.

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

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

Table 4.2.2 represents the activities of the three glutathione utilising enzymes in different parts of *Musa spp.* The values range from 0.98 units mg protein^{-1} to 1.83 mg protein^{-1} for glutathione peroxidase, the highest activity being seen in *Rasthali* leaf and least in *Karpooravalli* stem. *Rasthali* leaf again leads all other varieties in GR activity, the value being 2.99 units mg protein^{-1}. Hence, *Rasthali* leaf has the highest potential to decompose hydrogen peroxide to water as well to maintain reduced glutathione levels in the body, which in turn quenches reactive oxygen species, thereby preventing many free radical related diseases. While Karpooravalli stem showed least GR activity, the value being 2.799 units mg protein^{-1}. The values range from 7.60 units mg protein^{-1} to 5.53 mg protein^{-1} for glutathione-s-transferase, the highest activity being seen in *Rasthali* leaf and least in *Karpooravalli* stem.

Table 4.2.2. Activities of Glutathione utilizing enzymes in different parts of *Musa spp*:

Parts of the varieties	**Glutathione peroxidase**	**Glutathione-S-transferase**	**Glutathione reductase**
Rasthali.Leaf	**1.83^{b}±0.054**	**7.60^{c}±0.174**	**2.99^{c}±0.064**
Rasthali.Flower	1.34^{a}±0.065	6.70^{b}±0.195	2.05^{b}±0.091
Rasthali.Stem	1.20^{a}±0.449	6.20^{a}±0.198	1.27^{a}±0.031
Karpooravalli.Leaf	1.35^{c}±0.053	7.36^{c}±0.265	2.52^{c}±0.061
Karpooravalli.Flower	1.15^{b}±0.029	6.26^{b}±0.224	1.43^{b}±0.036

Values represent mean ± SD of 3 replicates.

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

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

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

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

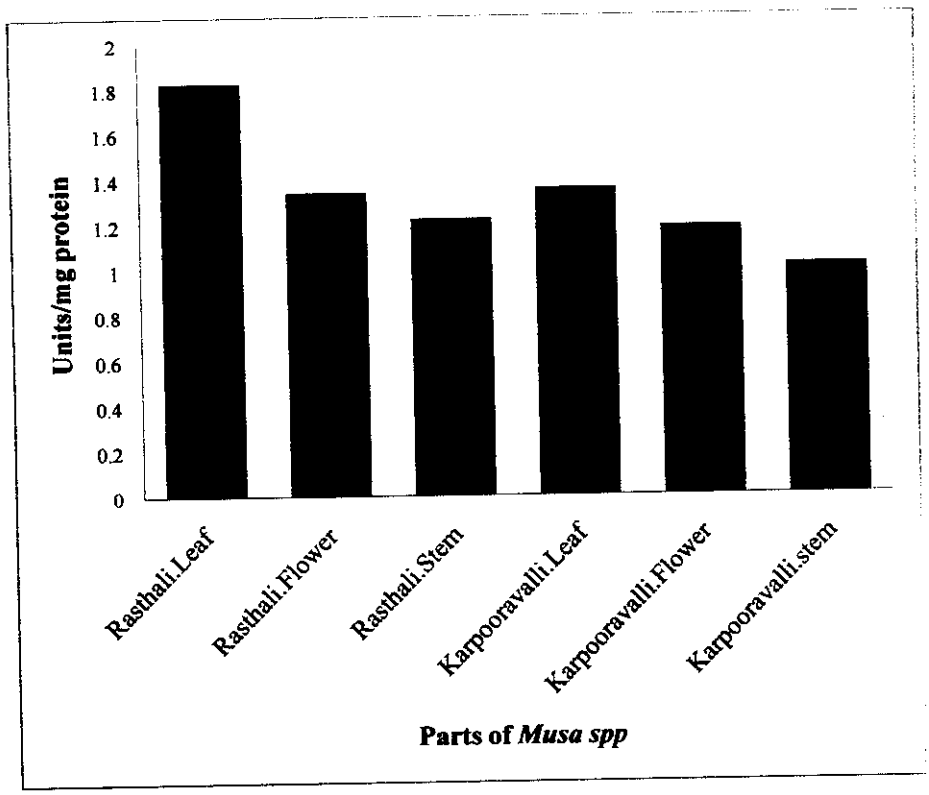


Fig. 4.2.3. Activity of glutathione peroxidase in different parts of *Musa spp.*

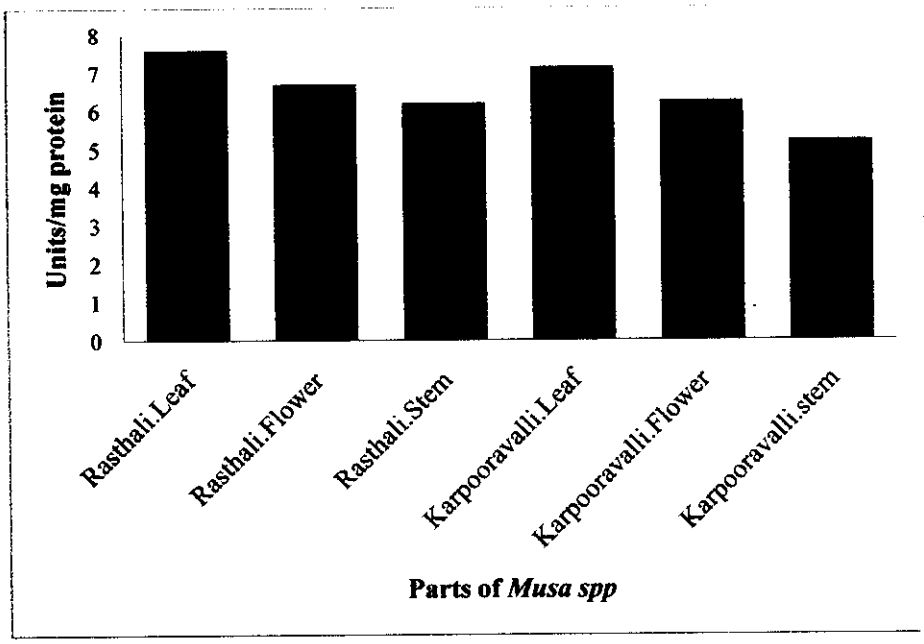


Fig. 4.2.4. Activity of glutathione-S-transferase in different parts of *Musa spp.*

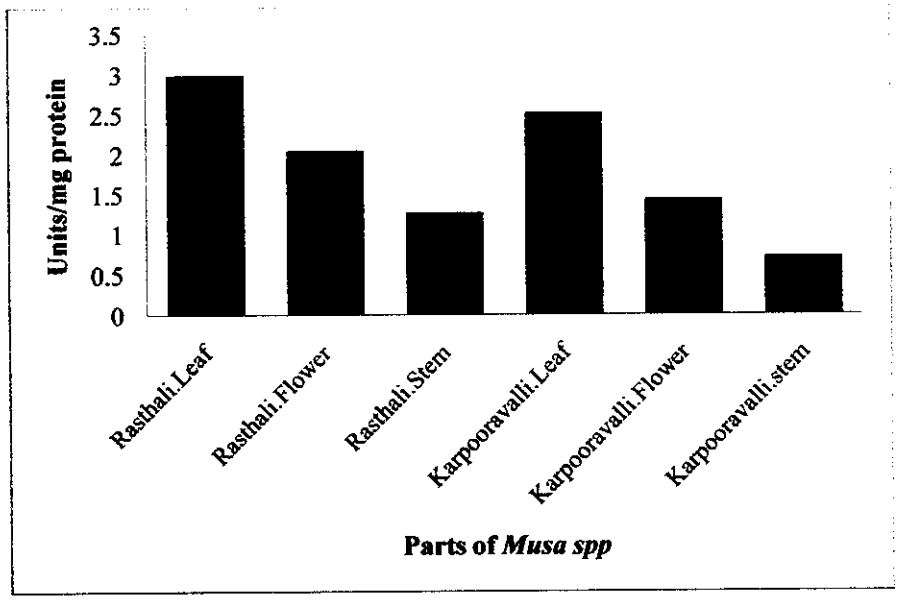


Fig. 4.2.5. Activity of glutathione reductase in different parts of *Musa spp.*

Table 4.2.2 represents the activities of glucose-6-phosphate dehydrogenase and polyphenol oxidase in different parts of *Musa spp.*

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

Table 4.2.3. Activities of Glucose-6-phosphate dehydrogenase and Polyphenol Oxidase in different parts extracts of *Musa spp*

Parts of the varieties	Glucose-6-phosphate dehydrogenase	Polyphenol oxidase
Rasthali.Leaf	**0.781^{c}±0.028**	**0.146^{c}±0.005**
Rasthali.Flower	0.662^{b}±0.026	0.120^{b}±0.004
Rasthali.Stem	0.542^{a}±0.012	0.095^{a}±0.004
Karpooravalli.Leaf	0.487^{b}±0.022	0.125^{c}±0.004
Karpooravalli.Flower	0.435^{a}±0.015	0.112^{b}±0.003
Karpooravalli.Stem	0.409^{a}±0.019	0.098^{a}±0.002

Values represent mean ± SD of 3 replicates.

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

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

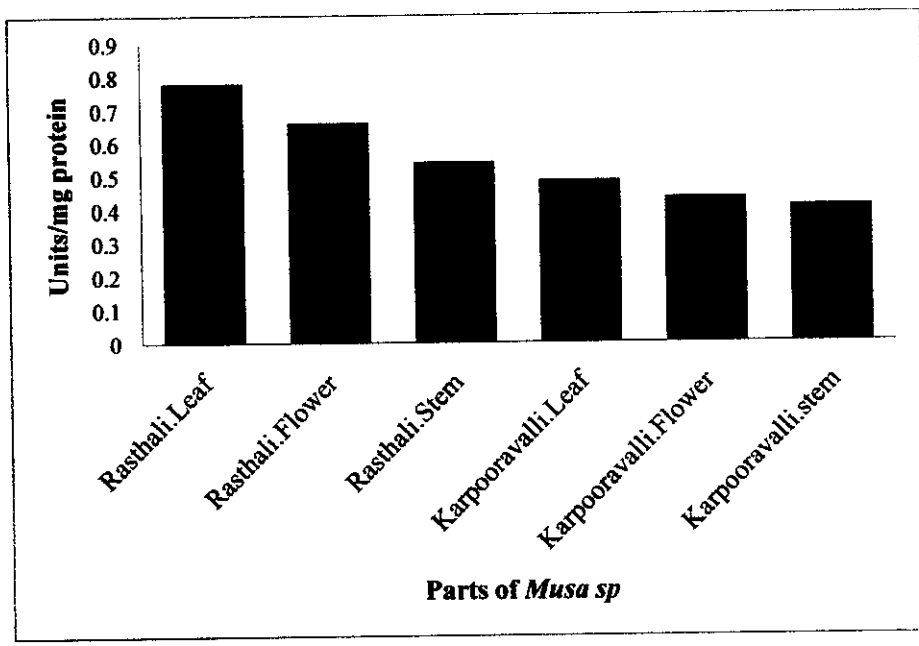


Fig. 4.2.6. Activity of glucose-6-phosphate dehydrogenase in different parts of *Musa spp.*

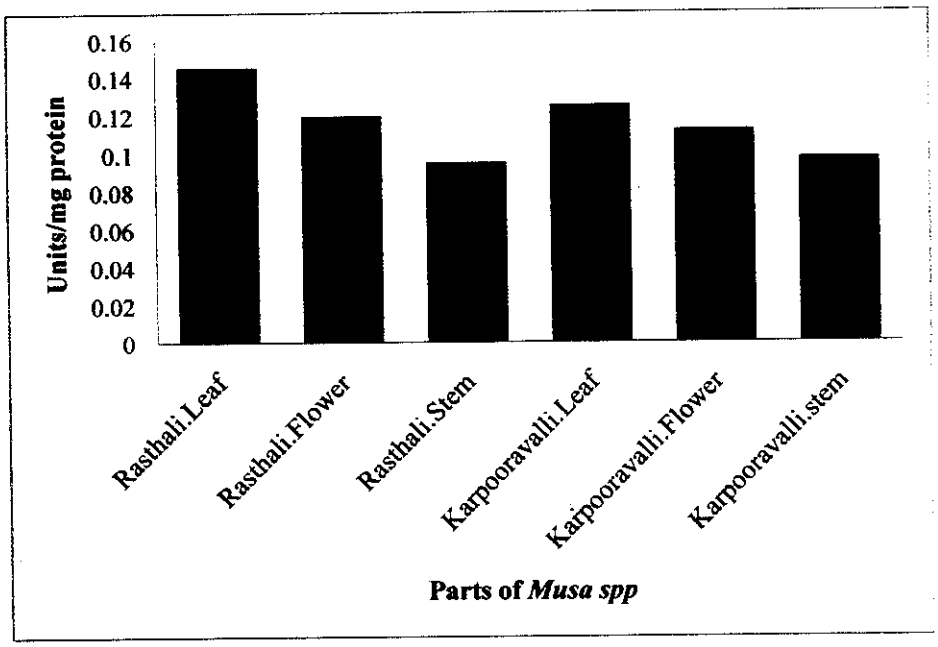


Fig.4.2.7. Activity of polyphenol oxidase in different parts of *Musa spp.*

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

G6PD activity in banana varieties ranged between 0.781 to 0.409 units mg protein^{-1}. *Rasthali* leaf again topped the list among all varieties. Hence, it helps in efficient regeneration of reduced glutathione by maintaining NADPH levels. Reduced glutathione in turn forms conjugate with free radicals thereby combating several diseases. On the contrary, least G6PD activity was observed in *Karpooravalli* stem. PPO activity was highest in Rasthali leaf extract, the least being in Rasthali.

4.3. Non- Enzymatic antioxidants

Table 4.3.1 represents total reduced glutathione, vitamin E, Ascorbic acid contents in different parts of *Musa spp.*

Ascorbic acid functions as the main water-soluble antioxidant protecting tissue from oxidative damage. It acts as a direct scavenger of free radicals and acts as a reductant in enzymatic reactions.

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

Ascorbic acid content was examined in the parts of *Musa spp* extract obtained from the two *Musa spp*. The leaf extract obtained from Rasthali showed highest ascorbic acid content. Rasthali leaf showed highest GSH content. Since, GSH converts ascorbic acid from its oxidized to its reduced state,by the enzyme dehydroascorbate reductase, Kadali banana can serve the purpose of both ascorbic acid and GSH in the body.

Table 4.3.1.Total Reduced Glutathione, vitamin E, vitamin C contents in different parts extracts of *Musa spp*

Parts of the varieties	Total reduced glutathione	Vitamin C	Vitamin E
Rasthali.Leaf	**21.26^{c}±0.571**	**2.92^{c}±0.066**	**1.47^{c}±0.035**
Rasthali.Flower	13.21^{b}±0.454	2.27^{b}±0.062	1.16^{b}±0.024
Rasthali.Stem	10.78^{a}±0.282	1.31^{a}±0.045	1.01^{a}±0.037
Karpooravalli.Leaf	20.50^{c}±0.571	2.74^{c}±0.079	1.25^{c}±0.050
Karpooravalli.Flower	12.56^{b}±0.481	1.81^{b}±0.045	1.01^{b}±0.021
Karpooravalli.Stem	9.41^{a}±0.359	0.74^{a}±0.021	0.87^{a}±0.034

Values represent mean ± SD of 3 replicates.

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

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

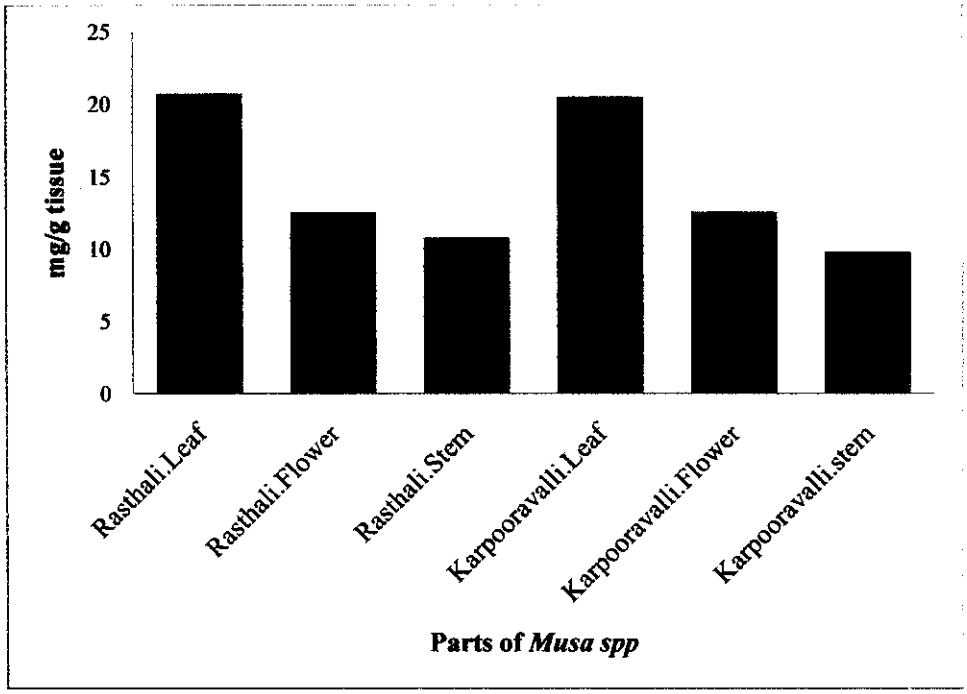


Fig. 4.3.1. Total reduced glutathione content in different parts of *Musa spp.*

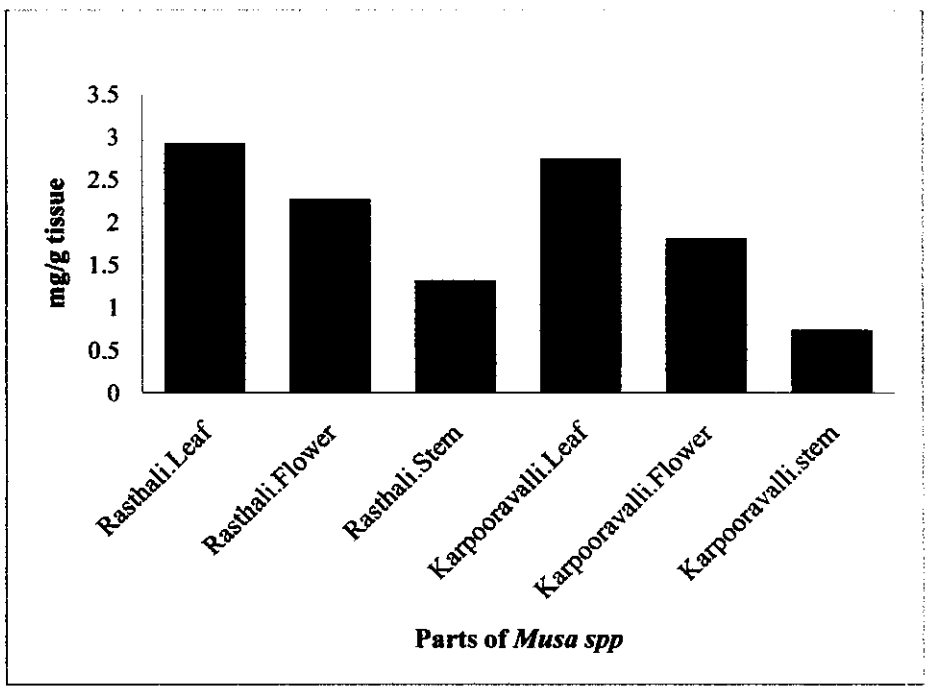


Fig. 4.3.2. Vitamin C content in different parts of *Musa spp.*

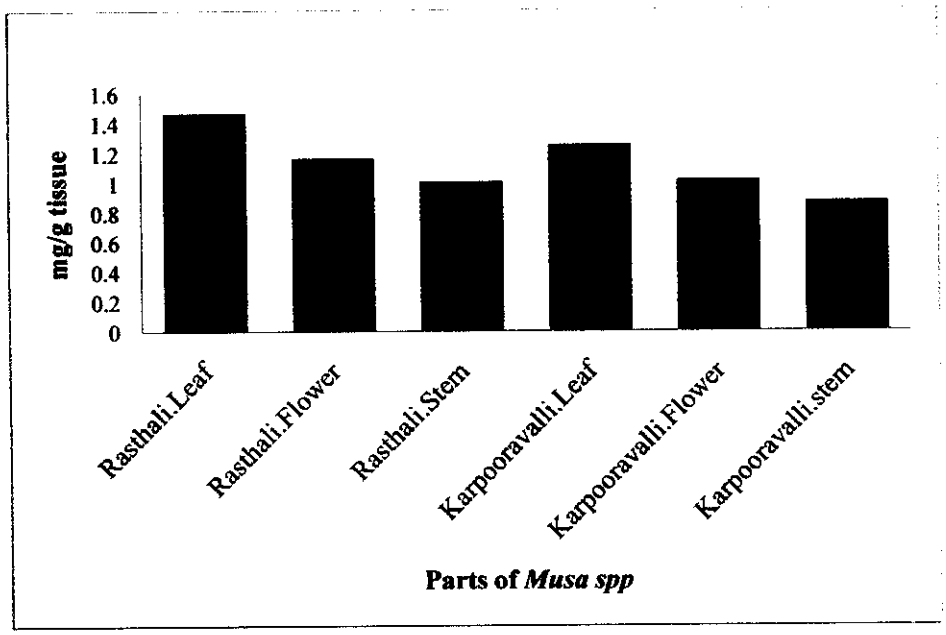


Fig. 4.3.3. Vitamin E content in different parts of *Musa spp.*

4.4. Phytochemicals

Table.4.4.1. represents the activities of Total Phenolics, Flavonoid and Alkaloid contents in different parts of *Musa spp.*

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

Flavonoids, a group of polyphenolic compounds, can widely be found in fruits and vegetables. Numerous positive health effects of flavonoids have been described. They have been reported to exhibit anti-cancer , anti-viral and anti-inflammatory effects, and to reduce the risk of cardiovascular diseases . These activities are generally associated with antioxidant or free radical scavenging properties of flavonoids. The number of flavonoid derivatives is more than 4000 and their antioxidant properties are very different. Alkaloids are a class of non- nutritive phytochemical compounds that are synthesized as secondary metablites by the plant cells. They fight against free radicals and are capable of quenching their activity.

Phenols, flavonoids and alkaloids were found in different quantities in the parts extracts from *Musa spp.* The values obtained are depicted in the Table 4.4.1. Rasthali leaf,

Karpooravalli leaf showed greatest phenol, flavonoid and alkaloid contents respectively. Thus, these parts of *Musa spp* have the capability to slow down the ageing process, fight against cancer and can reduce the risk of cardiovascular diseases. However, stem of *Musa spp* were not rich in these phytochemicals.

Table 4.4.1. Total Phenolic, Flavonoid and Alkaloid contents in different parts extracts of *Musa spp*:

Varieties	Total phenols	Flavanoids	Alkaloids
Rasthali.Leaf	**1.96^{c}±0.044**	**11.65^{c}±0.425**	**2.73^{c}±0.052**
Rasthali.Flower	0.91^{b}±0.039	1.79^{b}±0.041	1.98^{b}±0.036
Rasthali.Stem	0.61^{a}±0.022	0.94^{a}±0.031	1.42^{a}±0.033
Karpooravalli.Leaf	1.72^{c}±0.040	8.77^{c}±0.253	2.17^{c}±0.059
Karpooravalli.Flower	0.69^{b}±0.024	1.58^{b}±0.051	1.50^{b}±0.046
Karpooravalli.Stem	0.48^{a}±0.018	0.80^{a}±0.020	1.08^{a}±0.031

Values represent mean ± SD of 3 replicates.

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

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

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

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

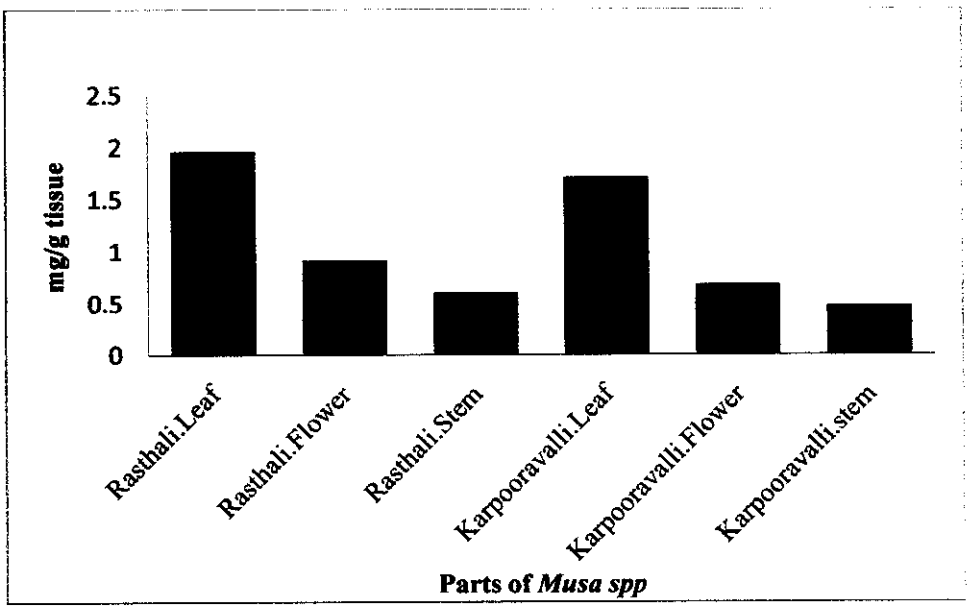


Fig. 4.4.1. Total Phenols Content in different parts of *Musa spp.*

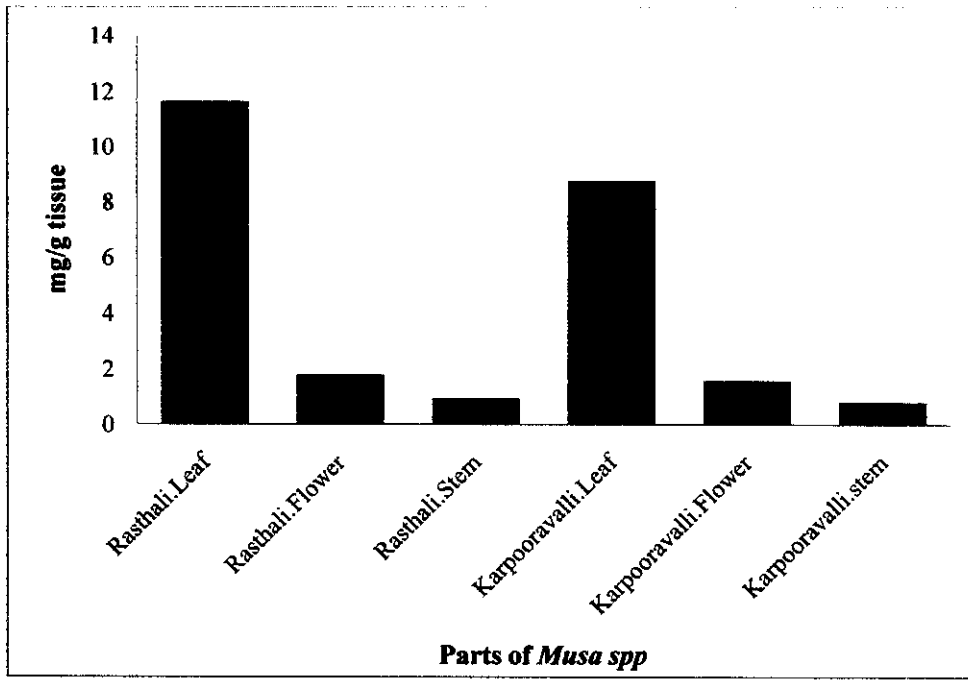


Fig. 4.4.2. Flavanoids Content in different parts of *Musa spp.*

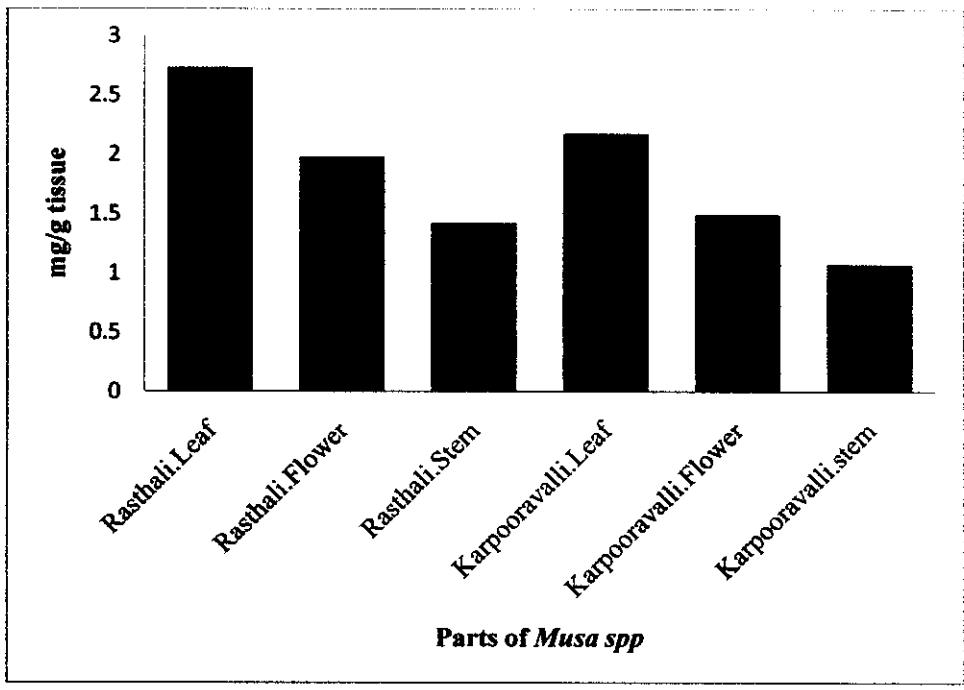


Fig. 4.4.3. Alkaloids Content in different parts of *Musa spp.*

Table.4.4.1 depicts the activities of carotenoids and lycopene contents in different parts of *Musa spp*.

Carotenoids are one of the most important classes of plant pigments and play a crucial role in defining the quality parameters of fruits and vegetables. Lycopenes are efficient quenchers of singlet oxygen. *Karpooravalli* leaf showed highest content of both carotenoids and lycopenes. Rasthali stem , on the other hand, showed lowest content of carotenoids and lycopenes respectively. The data obtained are displayed in theTable 4.15.

Table 4.4.2 Carotenoid and Lycopene contents in different parts extracts of *Musa spp*

Parts of the varieties	Carotenoids	Lycopene
Rasthali.Leaf	**1.88^{c}±0.071**	**0.097^{c}±0.007**
Rasthali.Flower	1.02^{b}±0.029	0.059^{b}±0.002
Rasthali.Stem	0.74^{a}±0.016	0.034^{a}±0.002
Karpooravalli.Leaf	2.24^{c}±0.058	0.123^{c}±0.004
Karpooravalli.Flower	1.48^{b}±0.047	0.079^{b}±0.002
Karpooravalli.Stem	0.86^{a}±0.025	0.044^{a}±0.001

Values represent mean ± SD of 3 replicates.

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

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

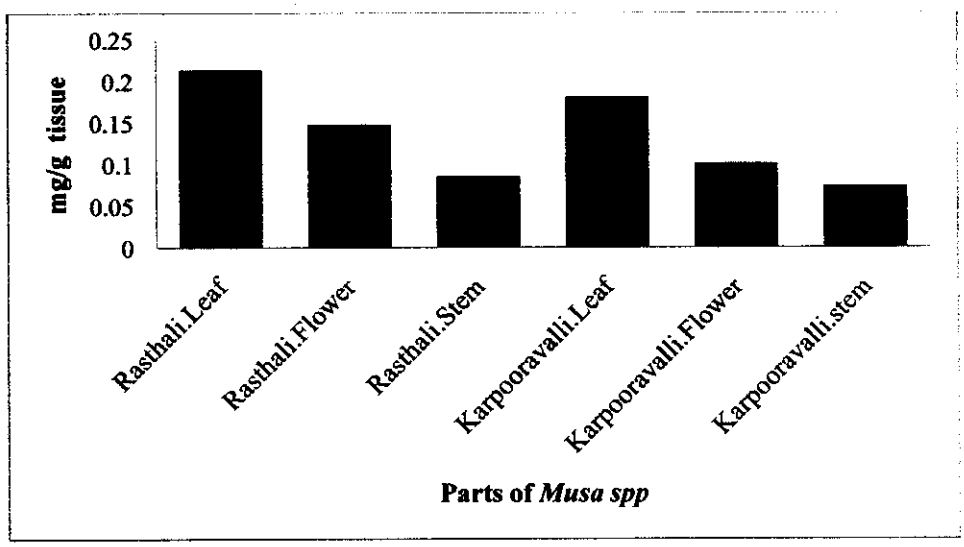


Fig. 4.4.5 Carotenoid Content in different parts of *Musa spp.*

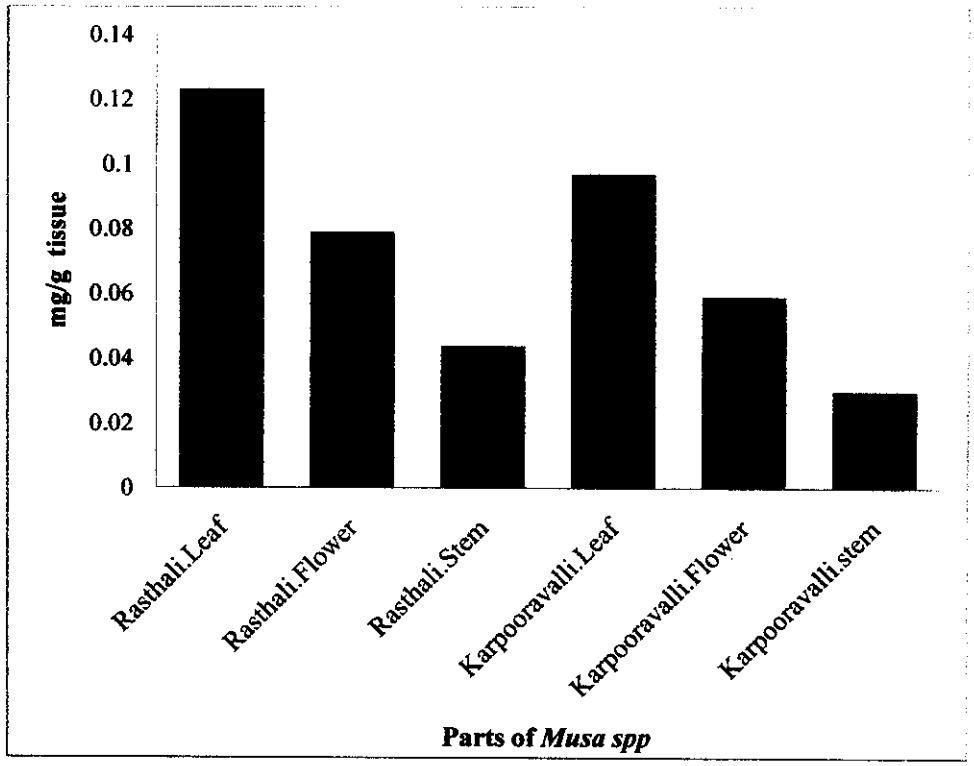


Fig. 4.4.6. Lycopene Content in different parts of *Musa spp.*

CONCLUSION

5. CONCLUSION

Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals. Free radical damage may lead to cancer. Antioxidants interact with and stabilize free radicals. Antioxidants are compounds in fruits and vegetables, which help in avoiding chronic diseases. They act as a defense system against oxidative damage in our bodies and may help in avoiding chronic diseases. The present work has been undertaken to evaluate and hence compare the antioxidant potential of three parts of banana varities, namely, *Rasthali* leaf, *Rasthali* stem, *Rasthali* flower, *Karpooravalli* leaf, *Karpooravalli* stem, *Karpooravalli* flower. The free radical scavenging ability of the parts of the *Musa spp* were tested against various free radicals generated *in vitro* and the results obtained. The parts extracts of *Musa spp* were assayed for different enzymatic, non-enzymatic and phytochemical compounds like SOD, GPx, GR, Vitamin C, Vitamin E, carotenoids, alkaloids, total phenols, flavonoids etc. The results obtained were subjected to two- way ANOVA and the parts of the varieties were ranked according to their antioxidant and phytochemical content. Even though all parts of the varieties showed significant antioxidant and phytochemical potential, *Rasthali* leaf, *Karpooravalli* leaf showed highest antioxidant and phytochemical potential. On the whole, all parts of banana varieties exhibited a good antioxidant activity with leaf part topping the list.

The main objective of this study was to evaluate and compare the antioxidants and phytochemical potentials in the parts extracts of *Musa spp.*

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