EVALUATION OF ANTIOXIDANT POTENTIAL AND ANTIBACTERIAL EFFICACY OF SOME INDIAN MEDICINAL PLANTS

BY

A. SUMATHI

(04MP42)

A THESIS SUBMITTED TO THE AVINASHILINGAM INSTITUTE FOR HOME SCIENCE AND HIGHER EDUCATION FOR WOMEN - DEEMED UNIVERSITY, COIMBATORE - 641 043

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF PHILOSOPHY IN BIOCHEMISTRY

SEPTEMBER - 2005

CERTIFICATE

This is to certify that the thesis entitled **"Evaluation of Antioxidant Potential and Antibacterial Efficacy of Some Indian Medicinal Plants"** submitted to Avinashilingam Institute For Home Science and Higher Education for Women - Deemed University, Coimbatore, in partial fulfilment of the requirements for the award of the degree of Master of Philosophy in Biochemistry is a record of original research work done by **Ms. A. SUMATHI** during the period of her study in the Department of Biochemistry and Biotechnology, Avinashilingam Institute For Home Science and Higher Education for Women-Deemed University, Coimbatore, under my supervision and guidance and the thesis has not formed the basis for the award of any degree / Diploma / Associateship / Fellowship or other similar title to any candidate of any University.

28/9/05

Signature of the Guide

Parvatham 28/9/05

Signature of the
Head of the Department

Forwarded

DECLARATION

I hereby declare that the dissertation entitled **"Evaluation of Antioxidant Potential and Antibacterial Efficacy of Some Indian Medicinal Plants"** submitted to the Avinashilingam Institute For Home Science and Higher Education for Women - Deemed University, Coimbatore, in partial fulfilment of the requirements for the award of the degree of **Master of Philosophy in Biochemistry** is a record of original research work done by me under the supervision and guidance of **Dr. A. Pushpa**, M.Sc., M.Phil., (Madras) Ph.D., (Avinashilingam), Reader, Department of Biochemistry and Biotechnology, Avinashilingam Institute For Home Science and Higher Education for Women-Deemed University, Coimbatore, and it has not formed the basis for the award of any degree / Diploma / Associateship / Fellowship or similar title to any candidate of any University.

28/09/05
Signature of the Candidate

Counter signed

28/9/05
Signature of the Guide

ACKNOWLEDGEMENT

ACKNOWLEDGEMENT

The author expresses her thanks to Dr. K.Kulandaivel, M.A., (Ohio state), Ph.D., (Madras), Chancellor, Dr. Saroja Prabhakararan, M.A., and Dip. Ed., (Madras), Ph.D., (Mother Terasa), Vice Chancellor, and Dr. S.Sivakamasundari, M.Sc., (Annamalai), M.Phil., Ph.D., (Madras), Dean, Faculty of Science, Avinashilingam Institute for Home Science and Higher Education for Women, Deemed University, Coimbatore, for providing all the facilities needed for conducting the study.

The author acknowledges her sincere and reverential gratitude to Dr. R. Parvatham, M.Sc., M.Phil., (Madras), Ph.D., (Avinashilingam), Professor and Head of the Department of Biochemistry and Biotechnology, Avinashilingam Deemed University, Coimbatore for her moral support, motivation and encouragement throughout the project period.

The author records her heartfelt thanks to her guide Dr. A. Pushpa M.Sc., M.Phil., (Madras), Ph.D., (Avinashilingam), Reader, Department of Biochemistry and Biotechnology, Avinashilingam Deemed University, Coimbatore, for her immense help and efforts rendered throughout the study.

The author wishes to express her thanks to all the Staff members in the Department of Biochemistry and Biotechnology who offered their unwavering assistance and abundance.

The author also extends her thanks to Mr. C.Manoharan of Manoo Graphics for his excellent work in bringing out the thesis.

The author wholeheartedly expresses her thanks to Dr. J. Kaliamurthy, M.Sc., Ph.D., Department of Microbiology, Joseph Eye Hospital, Trichy for his constructive help and valuable suggestions.

CONTENTS

CONTENTS

S.NO.	TITLE	PAGE NO.
	LIST OF TABLES	
	LIST OF FIGURES	
	LIST OF PLATES	
	LIST OF APPENDICES	
1	INTRODUCTION	1
2	REVIEW OF LITERATURE	4
3	EXPERIMENTAL PROCEDURE	33
4	RESULTS AND DISCUSSION	43
5	SUMMARY AND CONCLUSION	76
	REFERENCES	
	APPENDICES	

LIST OF TABLES

TABLE NO	TITLE	PAGE NO
I	MAJOR EXOGENOUS CAUSE OF OXIDATIVE STRESS INVOLVED IN CARCINOGENESIS	1
II	MAJOR CLASSES OF ANTIMICROBIAL COMPOUNDS FROM PLANTS	29
III	QUALITATIVE SCREENING OF THE PLANT SAMPLES FOR THE PRESENCE OF ALKALOIDS, PHENOLICS AND FLAVONOIDS	45
IV	ENZYMATIC ANTIOXIDANTS IN THE PLANT SAMPLES	47
V	LEVELS OF ENZYMATIC ANTIOXIDANTS IN THE PLANT SAMPLES	50
VI	NON-ENZYMATIC ANTIOXIDANTS IN THE PLANT SAMPLES	52
VII	PROTEIN AND CARBOHYDRATE IN THE PLANT SAMPLES	55
VIII	EFFECT ON *IN VITRO* LIPID PEROXIDATION	55
IX	PERCENTAGE INHIBITON OF SUPEROXIDE GENERATION *IN VITRO*	59
X	PERCENTAGE INHIBITION OF NITRIC OXIDE GENERATION *IN VITRO*	60
XI	ANTIBIOTIC SENSITIVITY TEST (AST) USING DIFFERENT ANTIBIOTICS	63
XII	ANTIBACTERIAL ACTIVITY OF THE AQUEOUS EXTRACTS	66
XIII	ANTIBACTERIAL ACTIVITY OF THE ALCOHOLIC EXTRACTS	67
XIV	EFFECT OF THE AQUEOUS EXTRACTS ON BACTERIAL ISOLATES	73
XV	EFFECT OF THE ALCOHOLIC EXTRACTS ON BACTERIAL ISOLATES	74

LIST OF FIGURES

FIGURE NO	TITLE	PAGE NO
1	CONTROL OF OXIDATIVE STRESS	5
2	INVOLVEMENTOF FREE RADICALS AND ANTIOXIDANTS INTHE PATHOGENSIS OF SEVERAL DISEASES	14
3	ACTION OF BIOLOGICAL GUARDMEN (ANTIOXIDANTS) IN THE REMOVAL OF CULPRITS (FREE RADICALS)	19
4	HALLIWELL-ASADA PATHWAY	25
5	ENZYMATIC ANTIOXIDANTS IN THE PLANT SAMPLES	49
6	NON-ENZYMATIC ANTIOXIDANTS IN THE PLANT SAMPLES	53
7	PROTEIN AND CARBOHYDRATE IN THE PLANT SAMPLES	56
8	EFFECT OF PLANT SAMPLES ON PERCENTAGE INHIBITION OF *IN VITRO* LIPID PEROXIDATION	57
9	PERCENTAGE OF INHIBTION OF NITRIC OXIDE GENERATION *IN VITRO*	61
10	ANTIBIOTIC SENSITIVITY TEST (AST)	64
11	ANTIBACTERIAL ACTIVITY OF CRUDE ALCOHOLIC EXTRACTS	68

LIST OF PLATES

PLATE NO	TITLE	PAGE NO
I	FRESH AND POWDERED SAMPLES	34
II	FRESH AND POWDERED SAMPLES	35
III	ANTIBIOTIC SENSITIVITY TEST (AST) USING DIFFERENT ANTIBIOTICS	65
IV	ANTIBACTERIAL ACTIVITY OF AQUEOUS EXTRACTS AGINST *E.COLI*	69
V	ANTIBACTERIAL ACTIVITY OF ALCOHOLIC EXTRACTS AGINST *S.FLEXINERI*	70
VI	ANTIBACTERIAL ACTIVITY OF ALCOHOLIC EXTRACTS AGINST *P.AERUOGINOSAE*	71

LIST OF APPENDICES

APPENDIX NO	TITLE
I	QUALITATIVE PHYTOCHEMICAL ANALYSIS
II	ESTIMATION OF CATALASE ACTIVITY
III	ESTIMATION OF PEROXIDASE ACTIVITY
IV	ESTIMATION OF SUPEROXIDE DISMUTASE ACTIVITY
V	ESTIMATION OF POLYPHENOL OXIDASE ACTIVITY
VI	ESTIMATION OF GLUTATHIONE-S-TRANSFERASE ACTIVITY
VII	ASSAY OF GLUTATHIONE PEROXIDASE ACTIVITY
VIII	ESTIMATION OF GLUTATHIONE REDUCTASE ACTIVITY
IX	ESTIMATION OF ASCORBIC ACID
X	ESTIMATION OF α-TOCOPHEROL
XI	ESTIMATION OF REDUCED GLUTATHIONE
XII	ESTIMATION OF POLYPHENOL
XIII	ESTIMATION OF TOAL CAROTEINOIDS AND LYCOPENE
XIV	ESTIMATION OF PROTEIN
XV	DETERMINATION OF TOTAL CARBOHYDRATE BY ANTHRONE METHOD
XVI	EXTENT OF INHIBITION OF *IN VITRO* LIPID PEROXIDATION
XVII	DETRMINATION OF SUPEROXIDE PRODUCTION *IN VITRO*
XVIII	DETERMINATION OF NITRIC OXIDE GENERATION *IN VITRO*

INTRODUCTION

1.0 INTRODUCTION

"May food be your medicine and medicine - your food"

Traditional cultures worldwide have an extended repertoire of knowledge about plants with healing properties. Medicinal herbs or plant parts are thought to be both effective and safe as they are gleaned directly from loving lap of nature (Datta, 2004). A large number of indigenous plant species are used as sources of herbal therapies (Ljubuncic *et al.*, 2005). Traditional herbal remedies are used by a large proportion of people worldwide (Snyman *et al.*, 2005). Over the last few decades, a global resurgence in the use of herbal remedies has fuelled the growing multibillon dollar international trade of botanical products (Clement *et al.*, 2005).

Non-allopathic Indian medicine, referred to elsewhere in the world as complementary and alternative medicine have gathered increasing recognition in recent years with regard to both treatment options and health hazards. Ayurvedha, Sidha, Unani and Homeopathy are practised in India as non-allopathic systems (Gogtay *et al.*, 2002). 'Ayurvedha' the Indian traditional system of medicine which dates back to many centuries, uses many herbal extracts to cure a variety of diseases (Padmavathi *et al.*, 2005).

Scientific evidence validates the pharmacological actions of medicinal plants (Abel and Busia, 2005). The demand for medicinal and aromatic plants will continue to increase in developing and industrialized countries. About 25% of drugs in modern pharmacopoeia are derived from plants (Phytomedicines) and many others are synthetic analogues built on prototype compounds isolated from plants (Rao[b] *et al.*, 2004). Medicinal plants constitute an effective source of both traditional and modern medicine. Herbal medicine has been shown to have genuine utility and about 80% of rural population depends on it for their primary healthcare

(Akinyemi *et al.*, 2005). Globally about 85% of the traditional medicines used for primary healthcare are derived from plants (Kala *et al.*, 2004).

Plants have been used as a source of medicinal and pharmaceutical agents in the form of isolates, extracts or as lead compounds for synthetic optimization (Venu *et al.*, 2003). Herbal medicine remains one of the common forms of therapy available to much of the world's population (Rao[a] *et al.*, 2004). Herbal medicine is the form of complementary and alternative medicine often used by individuals seeing traditional medical practitioners (Roy-Byrne *et al.*, 2005). It is one of the most popular choices of complementary therapies for women (Huntley, 2004). Medicinal plants produced marked therapeutic effects in the body. They accumulate individual elements or groups of elements at concentration much higher than their average contents in plants of same origin (Lovkova *et al.*, 2001).

Medicinal plants contain a variety of phytochemicals as well as minerals, vitamins and trace elements. Some of the phytochemicals are pharmacologically active and can exert a therapeutic action on the body (http://www.friedii.com/herbs/intro.html). Structural diversity of medicinal herbs makes them valuable source of novel lead compounds against therapeutic targets that are newly discovered by genomics, proteomics and high-throughput screening (Suk, 2005). The major hinderance in the amalgamation of herbal medicine into modern medical practices is the lack of scientific and clinical data, better understanding of efficacy and safety of the herbal products (Seth and Sharma, 2004).

Plant extracts have the potential for scavenging Reactive Oxygen Species. A great number of aromatic, spicy, medicinal, and other plants contain chemical compounds exhibiting antioxidant properties. However scientific information on antioxidant properties of various plants, particularly those that are

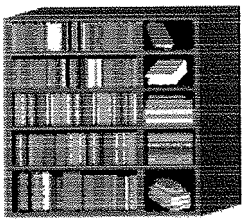
widely used in culinary and medicine, is still rather scarce. Therefore the assessment of such properties remains an interesting and useful task particularly for finding new sources for natural antioxidants (Milliauskas *et al.*, 2004).

The growing interest in the substitution of synthetic food antioxidants by natural antioxidants as neutraceuticals has fostered research on vegetable sources and screening of raw materials for identifying antioxidants (Klewala and Ananthanarayan, 2004). Investigation of antioxidant activity of traditional folk plants and their constituents is one of the successful strategies that may help in preventing many oxidative stress related disorder (Gamel-Eldeen *et al.*, 2004).

Several plants have been identified for their possible antimicrobial properties (Radha *et al.*, 2003). The main characteristic of antimicrobials (synthetic or natural) is their selective toxicity. This feature is based on the presence of target only or mainly on the infectious agents, which allows their systemic administration without deleterious effects to the host cells (Lima *et al.*, 2005).

Based on the above information, the plants namely *Acalypha indica*, Linn, *Eclipta prostrata*, Linn, *Mollugo latoides*, Linn and *Leucas aspera*, Spreng and the flowers of *Nelumbo nucifera*, Gatern were selected for the present investigation with the following objectives:

- To identify the presence of phytochemicals qualitatively in the extracts.
- To screen the antioxidant status (both enzymatic and non-enzymatic antioxidants) of the medicinal plants.
- To determine the extent of lipid peroxidation *in vitro*.
- To analyze the radical scavenging effects of the samples against oxidative injury.
- To evaluate the antimicrobial activity of the crude extracts against bacterial isolates.



REVIEW OF LITERATURE

2.0 REVIEW OF LITERATURE

Herbal medicine is still the mainstay of about 75-80% of the world population mainly in the developing countries for primary health care (Kamboj, 2000). According to the World Health Organisation (WHO) the use of herbal remedies throughout the world exceeds that of the conventional drugs by two to three times (Chatterjee *et al.*, 2004). Rising demand for medicinal plants has led to increased pressure on wild plant populations (Botha *et al.*, 2004).

The review of literature pertaining to the present study is discussed under the following headings :

2.1 Oxidative Stress

2.2 Free Radicals – The Foes of Cells

2.3 Biological Markers of Oxidative Damage

2.4 Oxidative Stress Induced Dysfunctions and Diseases

2.5 Antioxidants – The Body's Premier Resources of Protection

2.5.1 Enzymatic Antioxidants

2.5.2 Non-enzymatic Antioxidants

2.6 Medicine from Nature–A Search for Antimicrobial Compounds of Plant Origin

2.7 Medicinal Plants - Traditional Remedies

2.7.1 Antioxidant Activity of the Plants

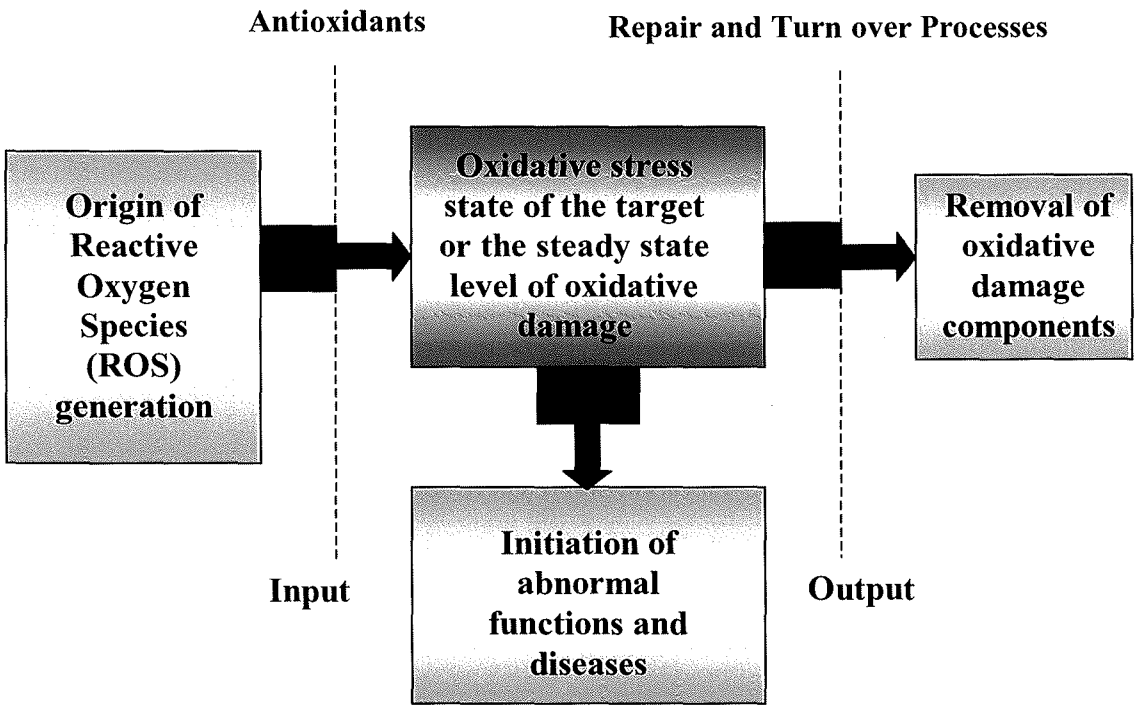
2.7.2 Antibacterial Activity of the Plants

2.1 OXIDATIVE STRESS

Oxidative stress results from an imbalance between the levels of free radicals generated (during aerobic metabolism, inflammation and infection) and the safe breakdown of these species by endogenous and exogenous scavengers

(Percy *et al.*, 2005). Oxidative stress is imposed on cells as a result of one of three factors: 1) an increase in oxidant generation, 2) a decrease in antioxidant protection or 3) failure to repair oxidative damage (http://www.sigmaaldrich.com/Area_of_Interest/Life_Science/Cell_Signalling/Scientific_Resources/Pathway_Slides_Charts/Oxidative_Stress.html).

FIGURE 1
CONTROL OF OXIDATIVE STRESS
(http://genox.com.why.htm)



An acute bout of exercise is known to increase the activities of antioxidant enzymes including superoxide dismutase and catalase in the tissues of rat (SriRam and Lakshmi, 2001). Endurance exercise can increase oxygen utilization from 10 to 20 times over the resting state. This greatly increases the generation of free radicals, prompting concern about enhanced damage to muscles and other tissues.

Regular physical exercise enhances the antioxidant defense system and protects against exercise induced free radical damage. Intense exercise in untrained individuals overwhelms defenses resulting in increased free radical damage. Thus the "Weekend warrior" who is predominately sedentary during the week but engages in vigorous bouts of exercise during the weekend may be doing more harm than good (http:// www.rice.edu/~jenkly / sports/antiox.html).

Free radical theory of aging implies that aging changes are caused by free radical reactions leading to oxidative damage of multiple cellular components (Klapcinska *et al.,* 2000). An imbalance caused by increased generation of free radicals and decreased functional efficiency of antioxidant defense system has been suggested to be one of the primary factors that contribute to the aging process (Lawler *et al.*, 1997). Aging is reported to stimulate superoxide formation (Nohl *et al.*, 2004). Aging is associated with an increase in liver peroxidation (Bukan *et al.*, 2003). Free radicals ($O_2^{\bullet -}$, H_2O_2 and $^{\bullet}OH$) are considered as pathogenic agents. The main target of oxidative injury is mitochondria, an organelle known to accumulate damages in post mitotic tissues during aging (Everekiloglu *et al.*, 2003).

2.2 FREE RADICALS – THE FOES OF CELLS

Aerobic metabolism produces as its by-products, various highly reactive molecules collectively termed as "Oxidants". These oxidants include a variety of electron – stealing molecules known as free radicals, as well as highly reactive singlet form of oxygen (Rana *et al.,* 2004). These species may be either oxygen derived (ROS - Reactive Oxygen Species) or nitrogen derived (RNS - Reactive Nitrogen Species). The oxygen derived species include $O^{\bullet}{}_2^-$ (superoxide), $^{\bullet}OH$ (hydroxyl), HO_2 (hydroperoxyl), $ROO^{\bullet}$(peroxyl),

RO (alkoxyl) as free radicals and H_2O_2 (hydrogen peroxide), HOCl (hypochlorus acid), O_3 (ozone) and $^1O_2$ (singlet oxygen) as non-radicals. Similarly, nitrogen derived oxidant species are mainly NO $^{\bullet}$(nitric oxide), $ONOO^-$ (peroxynitrite), NO_2 (nitrogen oxide) and N_2O_3 (dinitrogen trioxide) (Irshad and Chaudhuri, 2002).

a) Superoxide ($O_2^{\bullet -}$)

Superxoide is a reactive species of oxygen that is produced by free electrons interacting with dioxygen.

$$O_2 + e^- \longrightarrow O_2^{\bullet -}$$

(Rose, 2001)

Xanthine oxidases (XOD) catalyze the oxidation of hypoxanthine / xanthine to uric acid and generate superoxide radicals (Singh and Pushpa, 2005). Superoxide anion either directly or indirectly, may alter vascular cell behaviour, gene expression and injury (Cathcart, 2004).

b) Hydrogen peroxide (H_2O_2)

H_2O_2 is formed intracellularly by mitochondria, endoplasmic reticulum and peroxisomes, which contain a number of H_2O_2 generating enzymes (superoxide dismutase and several oxidases). Superoxide rapidly dismutates to form H_2O_2. This reaction can occur spontaneously or it is catalyzed by SOD.

$$O_2^{\bullet -} + O_2^{\bullet -} + 2H^+ \longrightarrow H_2O_2 + O_2$$

(Ahmad[b], 2001)

Although hydrogen peroxide is not a free radical by definition, it is a potent oxidant (Sen, 2001).

c) Hydroxyl radical (·OH)

Hydroxyl radical is highly reactive (Ray and Husain, 2002). Hydrogen peroxide is converted into highly reactive hydroxyl radical (·OH) in the presence of transition metal ions like copper (Cu^{2+}) or iron (Fe^{2+}) through Fenton reaction or Haber-Weiss reaction (Srinivasan, 2002).

$$O_2^{\bullet -} + H_2O_2 \xrightarrow{Fe^{2+}} {}^{\bullet}OH + OH^- + O_2$$

(Halliwell and Gutteridge, 1999)

Hydroxyl radicals oxidize important cellular constituents such as structural and functional proteins, membrane lipids and deplete glutathione (Tandon *et al.*, 2004).

d) Hypochlorus acid (HOCl)

Hypochlorus acid is a powerful oxidizing agent and it damages a wide variety of biomolecules. Hypochlorus acid formation is catalyzed by myeloperoxidase (MPO) in activated neutrophils (Reiter *et al.*, 2003). Neutrophils kill engulfed pathogens by using enzyme myeloperoxidase which catalyzes the reaction of hydrogen peroxide with chloride ions to produce strongly antiseptic hypochlorite ion.

$$H_2O_2 + Cl^- \longrightarrow HOCl + OH^-$$

(http://users. rcn.com/jkimball.ma.ultranet/BiologyPages/R/ROS.html)

e) Singlet oxygen ($^1O_2$)

The reversal of electron spin of oxygen induced by energy transfer from triplet state of chlorophyll, is considered as a generator of singlet oxygen, a non-radical ROS (Edreva, 2005). Singlet oxygen can be generated in biological systems by two different routes : (i) 'light reactions' due to photo-excitation and

(ii)'dark reactions' due to chemi-excitation. In mammalian tissue, one of the main candidates for the production is the activated polymorphonuclear leukocytes (Devasagayam and Kamat, 2002). Recent studies showed that $^1O_2$ is involved in cell signalling. Three pathways have been shown to be induced by $^1O_2$ - AP-1, NF-kB and AP-2 pathway (Klotz *et al.*, 2001).

f) Nitric oxide ($NO^{\bullet}$)

Nitric oxide is one of the most abundant free radicals in the body (Evereklioglu *et al.*, 2003). Nitric oxide is known to react rapidly with $O_2^{\bullet -}$ to produce peroxynitrite ($ONOO^-$) which is capable of initiating peroxidative damage through reactive species like peroxynitrite, hydroxyl radicals and $NO_2^{\bullet}$ formed through its interaction with $O_2^{\bullet -}$ (Agarwal *et al.*, 2001).

$NO^{\bullet}$ acts as a "double edged sword" in health and diseases (Irshad and Chaudhuri, 2002). Besides its beneficial effects in the regulation of blood pressure, destruction of pathogens in the immune system, and as retrograde neurotransmitter in consolidation of long-term memory, it can be highly toxic causing many neurodegenerative processes *eg.*, ischemia and reperfusion injury (Hore, 2004). Nitric oxide is a multifunctional molecule produced in a variety of mammalian cells. At physiological levels, $NO^{\bullet}$ is associated with neurotransmission and vasodilation. At higher levels $NO^{\bullet}$ has tumoricidal and bactericidal effects. In the cell mediated immune response, $NO^{\bullet}$ is produced in macrophages, neutrophils and lymphocytes (Tsumori *et al.*, 2002)

g) Peroxynitrite ($ONOO^-$)

If $NO^{\bullet}$ production is increased short-lived $NO^{\bullet}$ rapidly reacts with $O_2^{\bullet -}$ to form a potent and powerful long-lived oxidant free radical, peroxynitrite ($ONOO^-$).

$$O_2^{\bullet -} + NO^{\bullet} \longrightarrow ONOO^-$$

(Everekliogluu *et al.*, 2003)

The oxidation potential and reactivity of various ROS may be given in the following order:

$$O_2^{\bullet -} < H_2O_2 < {}^1O_2 < {}^{\bullet}OH$$

(Athar, 2002)

2.2.1 Sources of Free Radicals

Most reactive oxygen species come from the endogenous sources as by-products of normal and essential metabolic reactions, such as energy generation from mitochondria or the detoxification reactions involving the liver cytochrome P-450 enzyme system. Exogenous sources include exposure to cigarette smoke, environmental pollutants such as emission from automobiles and industries, consumption of alcohol in excess, asbestos, exposure to ionizing radiation and bacteria, fungal or viral infections (http://www.genox.com.what.htm).

2.2.2 Beneficial Role of the Free Radicals

Free radicals, such as hydroxyl ions and superoxide, occur (among others) on the activation of phagocytes. They are used by host phagocytes to kill internal pathogens, or they can be released by activated phagocytes onto a parasite surface. Although they act in a non-specific manner as effectors of resistance, their generation usually follows the activation of macrophages by cytokines from specifically stimulated lymphocytes. Free radicals are also responsible for (at least partly) for the parasite induced pathology in host (Derda *et al.*, 2004).

Growing evidence suggests that Reactive Oxygen Species (ROS) within the cell act as second messengers in intracellular signalling cascades which induce and maintain oncogenic phenotype of cancer cells. It is known that ROS can induce

cellular senescence and cell death and therefore function as anti-tumorigenic agents. Therefore, the mechanisms by which cells respond to Reactive Oxygen Species depend on the molecular background of cell and tissues, the location of ROS production and the concentration of individual ROS species (Stroz , 2005).

Free radicals produced in a regulated fashion are required participants in signalling pathways. The response to mitogenic as well as to cytokine signals can be diminished by non-enzymatic and enzymatic antioxidants, which implies a direct role for ROS as second messenger molecules in transducing receptor-initiated signalling cascades that control diverse cellular events such as proliferation, apoptosis and inflammation (Behrend *et al.*, 2003).

In the thyroid gland, relatively high levels of H_2O_2, are generated particularly in response to thyrotropin. This H_2O_2 serves as a substrate for thyroperoxidase enzyme which catalyzes the synthesis of thyroid hormones, namely thyroxine and triiodothyronine (Desphande *et al.*, 2003). A number of cell functions appear to be upregulated by the release of oxygen free radicals such as DNA expression and mitochondrial energy production (Vendemiale *et al.*, 1999). Reactive Oxygen Species could be involved in the induction of systemic acquired resistance in plants, the activation of NF-kB complex responsible for transcription of genes connected with the immune response of animal cells, in the signalling pathway dependent on platelet derived growth factor and in prevention of apoptosis (Krawiec *et al.*, 2000).

2.3 BIOLOGICAL MARKERS OF OXIDATIVE DAMAGE

Oxidative stress induced by Reactive Oxygen Species (ROS)/ Reactive Nitrogen species (RNS) leads to oxidative damage to biological targets, including DNA, proteins and lipids (Samuni *et al.*, 2002). Hydroxyl radicals are the most

Reactive Oxygen Species (ROS) and are known to have the ability to react with cellular constituents including amino acid residues and purine/pyrimidine bases in DNA; hydroxyl radicals are also able to attack cell membrane lipids causing lipid peroxidation (Reddy and Clark, 2004).

When Reactive Oxygen Species (ROS) are generated *in vitro* or by oxidative stress *in vivo*, several types of oxidative DNA lesions are formed, including small base lesions and cytotoxic adducts 8-OxodG is one of the most easily formed oxidative DNA lesions (Moller and Loft, 2002). Hydroxyl radicals produce a multiplicity of modifications in DNA. Oxidative attack by hydroxyl radical on the deoxyribose moiety will lead to the release of free bases from DNA generating strand breaks with various sugar modifications and simple abasic (AP) site. In fact, one of the major types of damages generated by ROS is AP site, a site where DNA base is lost (http://www.dojindo.com/newsletter/review_vol2.html).

Although many authors suggest that protein peroxidation may be preceded by lipid peroxidation, there is also strong evidence supporting the hypothesis that the oxidation pattern of proteins may be different from that of lipids (Antosiewicz *et al.*, 1995). Oxidative stress affecting proteins are measured by changes of carbonyl and sulfhydryl group levels (Kobiela *et al.*, 2002).

All the molecules may be attacked by free radicals but lipids are probably the most susceptible (Gopinathan *et al.*, 2004). Cell membranes enriched with PolyUnsaturated Fatty Acids (PUFAs) are more prone to lipid peroxidation (SriRam and Lakshmi, 2001). Since membranes form the basis of many cellular organelles like mitochondria, plasma membrane, endoplasmic reticulum, lysosomes, peroxisomes, *etc.*, the damage caused by LP is highly detrimental to the functioning of the cell and its survival (Lakshmi *et al.*, 2005). Lipid peroxidation, a general

mechanism of tissue damage by free radicals is known to be responsible for cell damage and may induce many pathological events (Reddy *et al.,* 2004).

2.4 OXIDATIVE STRESS INDUCED DYSFUNCTIONS AND DISEASES

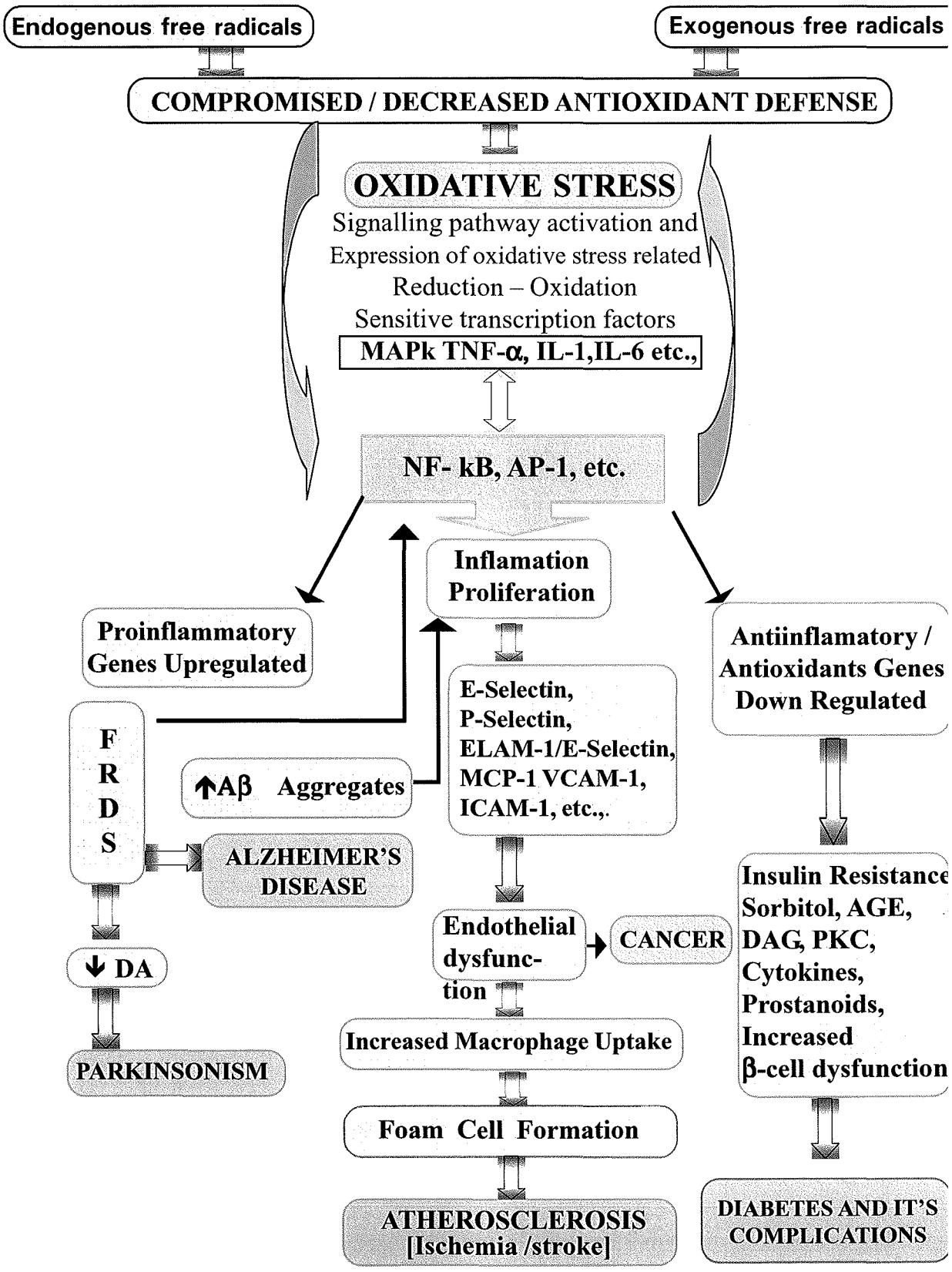
Reactive Oxygen Species (ROS) can initiate a wide range of toxic oxidative reactions such as initiation of respiratory peroxidation, direct inhibition of respiratory chain enzymes, inactivation of glyceraldehyde-3-phosphate dehydrognease, inhibition of membrane Na^+/K^+ ATPase, inactivation of membrane channels and other oxidative modifications of proteins, DNA and lipids (Sohi and Khanduja, 2003). Reactive Oxygen Species (ROS) are produced continuously in the cells as accidental by-products of metabolism which are cytotoxic (Acharya *et al.*, 2004).

Oxidative stress can be implicated in the etiopathogenesis of various diseases like Cancer, Atherosclerosis, Cataract, Diabetes mellitus, Alzhemier's disease, Parkinson's disease, etc. (Pradhan *et al.*, 2004). The excess generation of Reactive Oxygen Species (ROS) during various pathophysiological state can lead to alteration of the cellular constituents resulting in diseased conditions (Malarvannan and Devaki, 2003).

Reactive Oxygen Species (ROS) have been shown to be responsible for inducing DNA damage leading to mutagenesis, carcinogenesis and cell death if the capacity of the protective antioxidant system is impaired (Manoharan *et al.*, 2004).

FIGURE 2
INVOLVEMENTOF FREE RADICALS AND ANTIOXIDANTS INTHE PATHOGENSIS OF SEVERAL DISEASES

(Tiwari, 2004)



MAPk	=	Mitogen - Activated Protein kinase
TNF - α	=	Tumour Necrosis Factor α
IL -1	=	Interleukin - 1
IL - 6	=	Interleukin - 6
NFkB	=	Nuclear Factor Kappa B
AP	=	Activator Protein
ELAM	=	Endothelial Leukocyte Adhesion Molecule
MCP	=	Monocyte Chemoattraction Protein
VCAM	=	Vascular Cell Adhesion Molecule
ICAM	=	Intracellular Adhesion Molecule
Aβ	=	Amyloid beta-protein
DA	=	Dopamine
FRDS	=	Free Radicals
AGE	=	Advanced Glycation End products
DAG	=	Diacyl Glycerol
PKC	=	Protein Kinase C

TABLE I

MAJOR EXOGENOUS CAUSE OF OXIDATIVE STRESS INVOLVED IN CARCINOGENESIS (Athar, 2002)

Cause of oxidative stress	Oxygen free radicals	Cancer associated with exposure
Tobacco smoke	NO•, •OH	Bronchogenic carcinoma
Ultraviolet light	•OH, Organic radicals	Melanoma and other skin cancers
Fatty acids in food	Lipid peroxides	Colorectal cancer, Breast cancer
Iron and copper ions	•OH	Colorectal cancer
Ethanol	Lipid peroxides	Hepatocellular carcinoma Breast cancer

Antioxidant potential of the plant is known to be closely linked with their cancer chemopreventive properties (Singh *et al.*, 2004). The carotenoids perhaps non-nutrients like dietary fibre and other phenolic constituents influence the enzymes involved in the activation and detoxification of xenobiotics including carcinogens.

The antioxidant compounds have been well recognized to have effective role in reducing the oxidative stress and thereby reduce the risk of cancer (Anilakumar *et al.*, 2004). Studies showed a synergistic effect of chemotherapeutic agents with vitamin C, E, A or β-carotene in enhancing the effect of the agents on growth inhibition of cancer cells (Whiteside *et al.*, 2004).

The process of atherogenesis is initiated by the oxidation of lipids in Low Density Lipoproteins (LDL), termed peroxidation (Mary *et al.*, 2003). The use of vegetable oils rich in PolyUnsaturated Fatty Acids (PUFAs) has been recommended to lessen the deleterious effects of saturated fatty acids by lowering of serum cholesterol and prevention of development of atherosclerosis (Karanath *et al.*, 2004). Supplementation of diet with various compounds that have antioxidant properties before the development of vascular disease inhibited atherogenic process (Verma *et al.*, 2004).

Oxidative stress brought about by Reactive Oxygen Species (ROS) present in smoke has been implicated in the pathogenesis of cataract. (Sulochana *et al.*, 2002). Supplementation with high dose of β-carotene decreases absorption of luetin and zeaxanthin, the only carotenoids that are present in the lens, and which might protect the lens against cataract development (Mares, 2004). Padmaja and Raju (2004) stated that curcumin had a role in preventing oxidative damage in general and delaying of cataract formation in particular.

Glucose control plays an important role in the pro-oxidant / antioxidant balance (Satheesh and Pari, 2004).Oxidative stress has been shown to produce glycation of proteins inactivation of enzymes and alterations in structural functions of collagen basement membrane (Boynes, 1991). Oxidative stress may have significant effect in the glucose transport protein (GLUT) or as insulin

receptor (Jacqualine *et al.*, 1997). There is also evidence that elevation in glucose concentration may depress natural antioxidant defense such as vitamin C or glutathione. The active transport of ascorbic acid appears to be decreased by hyperglycemia and insulin deficiency. Hyperglycemia has also been shown to inhibit the uptake of dehydroascorbic acid, the oxidized species of vitamin C (Gumieniczek *et al.*, 2002). It has been noted that the oxidative eye alterations in diabetic subjects are preceded by depletion of antioxidant agents, in particular GSH, vitamin E and ascorbic acid (Vendemiale *et al.*, 1999). Diabetic brain showed decreased activity of the key antioxidant enzymes, superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase and reduced glutathione which play an important role in scavenging the toxic intermediates of incomplete oxidation (Latha and Pari, 2003).

Brain has low level of antioxidative defense system. The concentration of superoxide dismutase, glutathione peroxidase, glutathione reductase, catalase and glutathoine are very much reduced. Brain has high iron and ascorbic acid content in certain regions and it is also enriched with PolyUnsaturated Fatty Acids (PUFAs) that render them most susceptible to oxidative attack. The increased level of oxidative stress in the brain is the major contributing factor for the development of neurodegenerative diseases like Alzheimer's disease and Parkinsonisms (Srinivasan, 2002). Parkinson's and Alzheimer's diseases are associated with elevated brain iron concentrations relative to the iron storage protein, ferritin (Reddy and Clark, 2004).

The oxidation of mitochondrial DNA, and of nuclear DNA have been observed in the paritel cortex of Alzheimer's disease patients. In this disease, a protein called beta amyloid is associated with the cell damage. The effects of beta amyloid are worsened by free radicals (Christen, 2000).

Studies suggested that deficiencies of the antioxidants glutathione and peroxidase in specific areas of brain can lead to Parkinson's disease (http://www.infoaging.org/b.oxdam_9_r_age.html). Lipid peroxidation had been reported to be increased in Parkinson's disease (Lohr and Browning, 1995) and GSH levels were dramatically decreased with clinical significance (Jenner, 1994). Antioxidants seemed to have protective role in gastric ulcers and carcinomas (Tandon *et al.*, 2004).

2.5 ANTIOXIDANTS-THE BODY'S PREMIER RESOURCES OF PROTECTION

Plant and plant products are being used as a source of medicine since long. Among the most important constituents of edible plant products, low molecular weight antioxidants are the most important species (Khopde *et al.*, 2001). Antioxidants are lipid soluble or water soluble substances of either synthetic or natural origin that can prevent or delay the progress of PolyUnsaturated Fatty Acid oxidation (Velenzuela *et al.*, 2004). There is an increasing interest in the natural antioxidants contained in medicinal and dietary plants, which are candidates for the prevention of oxidative damage (Velaquez *et al.*, 2003). Based on the structural specifications, they are divided into :

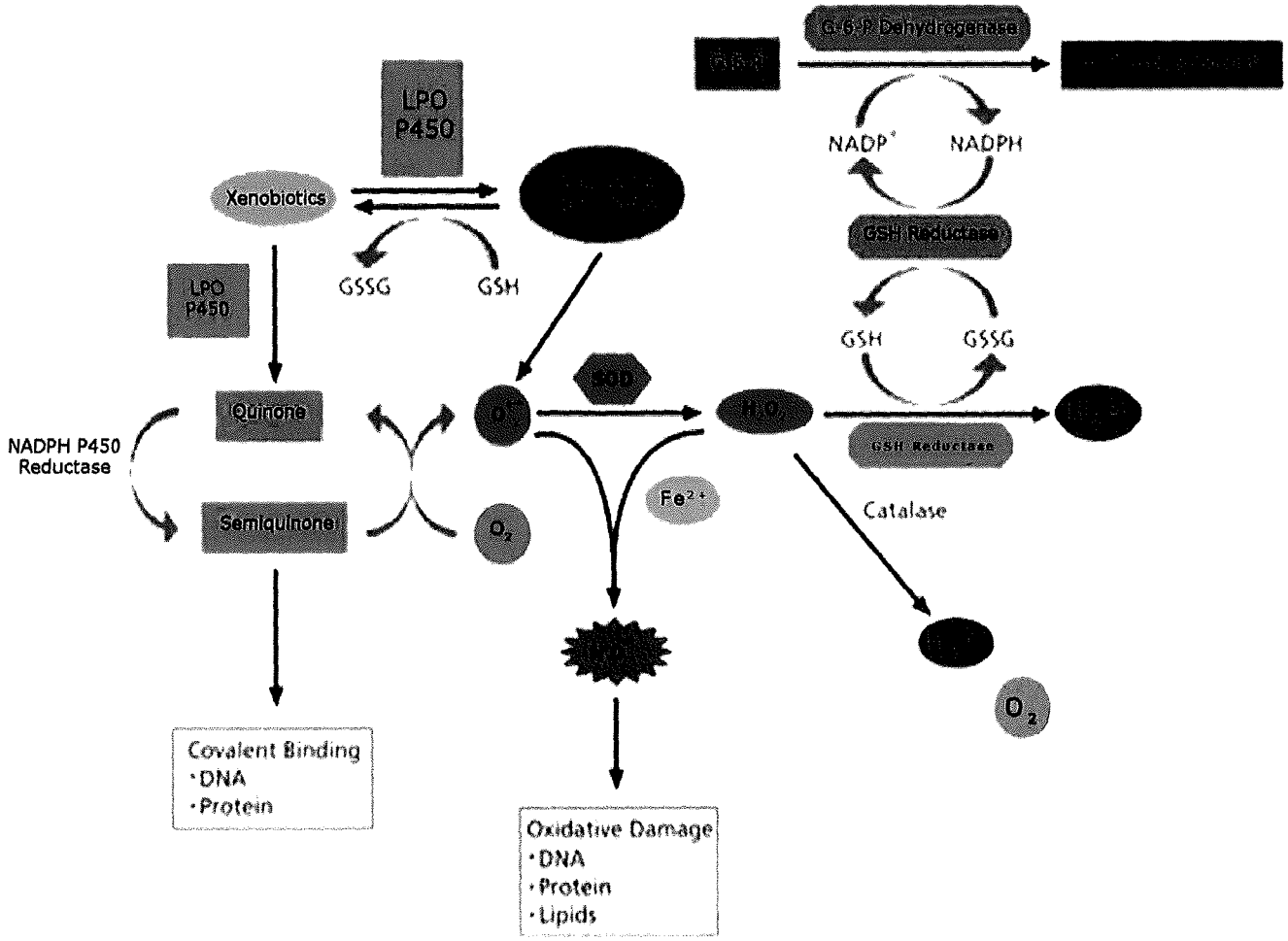
a) Enzymatic antioxidants

b) Non-Enzymatic antioxidants (Natural or Synthetic)

(Tripathi, 1998)

FIGURE 3

ACTION OF BIOLOGICAL GUARDMEN (ANTIOXIDANTS) IN THE REMOVAL OF CULPRITS (FREE RADICALS)



LPO - Lipid peroxidation

GSSG - Oxidized glutathione

GSH - Reduced glutathione

G-6-P - Glucose - 6 - phosphate

2.5.1 ENZYMATIC ANTIOXIDANTS

Antioxidant enzymes that scavenge intermediates of oxygen reduction provide the primary defense against cytotoxic oxygen radical (Selvendiran *et al.*, 2003). Most of the mammals have an effective mechanism to prevent and neutralize the free radical induced damage, which is accomplished by a set of endogenous enzymes such as superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase (Loki and Rajamohan, 2003).

a) Catalase

Catalase can function either in the catabolism of H_2O_2 or in the peroxidatic oxidation of small substrates such as ethanol, methanol, or elemental mercury. (Ahmad[a], 2001). Catalase is present in most aerobic cells. Catalase catalyzes the destruction of H_2O_2 by the following two reactions:

$$2\ H_2O_2 \xrightarrow{CAT} H_2O + O_2$$

Catalytic reaction
(at high concentration of H_2O_2)

$$H_2O_2 + SH_2 \xrightarrow{CAT} 2H_2O + S$$

Peroxidatic action
(at low concentration of H_2O_2)

where (S) is anyone of a number of hydrogen donating substrates including ethanol, methanol, formate, nitrite and quinones (Halliwell and Gutteridge, 1999).

b) Peroxidase

Peroxidases are group of enzymes that catalyze oxidation-reduction reactions. Peroxidases use H_2O_2 as electron acceptor for catalyzing different oxidative reactions. The overall reaction is as follows:

$$\text{Donor} + H_2O_2 \longleftrightarrow \text{Oxidized donor} + 2\ H_2O$$

(http:// www. chem..admu.edu.ph/vnina/rosby/main.htm)

The nature of the electron donor is very dependent on the structure of the enzyme. For example, horse radish peroxidase can use a variety of organic compounds as electron donors and acceptors due to the presence of broad and accessible active site whereas cytochrome C peroxidase is very specific, because there is a very closed active site (http: //en.wikipedia.org/wiki/Peroxidase)

c) Superoxide dismutase

Superoxide dismutase, one of the important intracellular antioxidant enzymes, present in all aerobic cells has an antitoxic effect against superoxide anion. The presence of SOD in various fractions such as cytosol (Cu-Zn-SOD), mitochonidria (Mn-SOD) and plasma (EC-SOD) in body enables SOD to dismutate superoxide radicals immediately and protect the cell from oxidative damage (Singh and Pushpa, 2005). Both aerobic and unaerobic organisms possess superoxide dismutase enzyme which catalyzes the breakdown of superoxide radical. Superoxide dismutase is effective in reducing leukocyte adhesion in inflammatory conditions (Govindarjan *et al.*, 2003). In *Arogh* pretreated isoproterenol administered rats, the activities of superoxide dismutase and catalase were found to be near normal. The combined effect of the plant extracts may have protected the cells against the threat of superoxides and peroxides generated by isoproterenol (Suchalatha *et al.*, 2004).

d) Polyphenol Oxidase

Polyphenol oxidases are a group of copper proteins that are widely distributed from bacteria to mammals. They catalyze the oxidation of hydroxy phenols to their quinone derivatives, which then spontaneously polymerize. Three types of proteins related to polyphenol oxidases are catechol oxidase, laccase and crelose. They catalyze 2 reactions: the hydroxylation of

monophenols to o-diphenols (monophenolase activity) and the oxidation of o-diphenols to o-quinones diphenolase activity (Shi *et al.*, 2001).

Enzymatic browning is the main function of polyphenol oxidases in fruits and vegetables, and it often is undesirable and responsible for unpleasant sensory qualities and losses in nutrient quality (Sanchez-Amat and Solano, 1997). Polyphenol oxidase promotes peroxidase activity by generating H_2O_2 from the oxidation of phenolic compounds (Gitanjali *et al.*, 2004).

e) Glutathione-S-transferase

Glutathione-S-transferase, the important enzyme known to catalyze antioxidant processes of thiol compounds and in turn protect the cell from electrophiles, free radical induced damage and oxidative stress (Dixon *et al.*,1998). It inactivates toxic electrophillic compounds by conjugation with reduced glutathione (Baranczyk-Kuzma *et al.*, 2004). Singh *et al.*, (2004) found that there is an increase in the specific activity of hepatic glutathione-S-transferase by 2.04 and 2.19 fold compared to the control in the group of animals orally treated with low and high dose of cashew nut kernel oil respectively.

f) Glutathione peroxidase

Glutathione peroxidases are most extensively characterized selenoproteins. Selenium serves its antioxidative purpose through glutathione peroxidases. Four different glutathione peroxidases are known: cellular or cytosolic or classical (GSH-PX1), gastrointestinal (GSH-PX2), plasma or extracellular (GSH-PX3) and phospholipid hydroperoxide (GSH-PX4) (Czuczejko *et al.*, 2003). Glutathione peroxidase catalyzes the destruction of H_2O_2 and lipid hydroperoxides by reaction with reduced glutathione (GSH) to form glutathione disulfide (GSSG) and the reduction product of hydroperoxide (Loki and Rajamohan, 2003). Its reaction

with H_2O_2 is a very slow process (Sugimoto *et al.*, 1997). The activities of the antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase found to be decreased in lymphoma bearing mice compared to that of normal mice. The activities of these enzymes were found to be increased significantly in both liver and kidney after oral treatment with crude methanolic extract of *C.asiatica* on lymphoma-bearing mice (Jayashree *et al.*, 2003).

g) Glutathione reductase

Glutathione reductase is a member of pyridine nucleotide-disulfide reductase. It is a cytoplasmic flavoenzyme widely distributed in aerobic organisms. It plays a role in maintaining glutathione (GSH) in its reduced form by catalyzing the reduction of glutathione disulfide (GSSG) (http: // www.acris-online.de/sheets/ LF-P0024.pdf)

In most eukaryotic cells, glutathione reductase maintains the ratio of [GSH] / [GSSG] elevated, and participates in several vital functions such as detoxification of reactive oxygen species as well as protein and DNA biosynthesis (Picaud and Desbois, 2002). Illavarasan *et al.*, (2003) stated that liver glutathione reductase activity was significantly decreased in CCl_4-treated animals when compared to the control. A significant increase in the level of glutathione reductase was observed in aqueous extracts and methanolic extracts of bark of *Thespesia populnea* treated rats compared with CCl_4-treated animals.

2.5.2 Non-enzymatic Antioxidants

Antioxidants (glutathione, selenium, zinc, and vitamins A,E,C) and antioxidant enzymes (superoxide dismutase, catalase, glutathione reductase and peroxidase) exert synergistic actions in scavenging free radicals (Sudha *et al.*, 2004).

a) Vitamin C (Ascorbic acid)

Ascorbic acid shows antioxidant, antiinflammatory and antimutagenic properties (Khopde *et al.*, 2001). Ascorbic acid functions both as a reducing agent and as a free radical scavenger by donating either one or two electrons to more oxidized neighbouring species. It can also act as reducer by regenerating α-tocopherol from tocopheroxyl radicals that is produced via the scavenging of lipid-soluble radicals (Lee *et al.*, 2004).

Ascorbic acid, HO-Asc-OH participates in the regeneration of tocopheroxyl radicals (α- $TO^{\cdot}$) formed during interaction of a-tocopherol (α-TOH) with unsaturated phospholipid radicals in LDL ($LO^{\cdot}_2$ and $LO^{\cdot}$) according to the following reactions:

$$LO^{\circ} + \propto - TOH \longrightarrow LOOH + \propto - TO^{\circ}$$

$$(or)\ LO^{\circ} + \propto TOH \longrightarrow LOH + \propto - TO^{\circ}$$

$$\propto - TO^{\circ} + HO - Asc\text{-}OH \longrightarrow \propto - TOH + HO\text{-}Asc\text{-}O$$

$$\propto - TO^{\circ} + HO\text{-}Asc\text{-}O \longrightarrow \propto - TOH + O = Asc = O$$

(Konovalova *et al.*, 2003)

b) Vitamin E (∝–Tocopherol)

Vitamin E is considered to be the major non-enzymatic antioxidant present in the lipid structures of cells and lipoproteins. It is a reductant antioxidant, which increases LDL resistance against the oxidative modification (Safari *et al.*, 2003). ∝–Tocopherol is the principal biochemical antioxidant defense molecule against lipid peroxidation with a capacity to scavenge $O_2^{\cdot -}$, ·OH and $^1O_2$. ∝– Tocopherol has several possible mode of action:

1) as a chain breaking antioxidant by trapping fatty acyl peroxyl radical;

2) as a reductant of O_2 to produce H_2O_2 and tocopherol quinone and

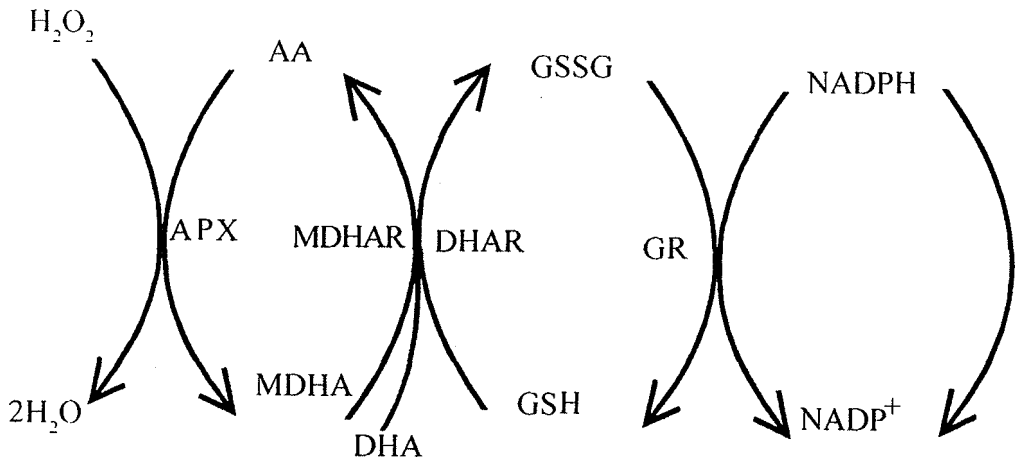
3) as a reactant with singlet oxygen.

Ascorbic acid and tocopherol can act synergistically in the reduction of free radicals (Jain *et al* ., 2004).

c) Reduced glutathione

Reduced glutathione participates non-enzymatically in protection against toxic compounds (Bukan *et al.*, 2003). Reduced glutathione (GSH) is a major low molecular weight scavenger of free radicals in the cytoplasm and an important inhibitor of free radical mediated lipid peroxidation (Bafna and Balaraman, 2004).

FIGURE 3
HALLIWELL-ASADA PATHWAY



AA – Ascorbic acid
MDHA – Monodehydro ascorbic acid
GSSG – Oxidized glutathione
GSH – Reduced glutathione
APX – Ascorbate peroxidase
MDHAR– Monodehydroascorbate reductase
DHAR – Dehydroascorbate reductase
GR – Glutathione reductase

(May *et al.*, 1998)

As a water soluble compound ascorbic acid functions most efficiently in the aqueous phase of the cell and is able to carry out the non-enzymatic regeneration of ∝–tocopherol (TOH) from the ∝–tocopheroxyl radical (TO°) in the hydrophobic surroundings (Beyer, 1994). Reduced glutathione has been shown to participate in the regeneration of the reduced form of ascorbate through non-enzymatic reduction of DHA at an alkaline pH (Noctor and Foyer, 1998).

d) Polyphenol

Phenolics are bioactive compounds and a diverse group of secondary metabolites universally present in higher plants (Yoshimoto *et al.*, 2004). Polyphenols such as flavonoids have been reported to possess strong antioxidant potential because of their lower redox potential (Panwala *et al.*, 2004). The antioxidant activity of phenolics is mainly due to their stable reaction intermediate, which allow them to act as reducing agents, free radical terminators, hydrogen donators and singlet oxygen quenchers (Gitanjali *et al.*, 2004).

e) Carotenoids and Lycopene

Carotenoids exhibit a central role against cancer, cardiovascular diseases, HIV infection and other age related disorders. Recent reports suggest that all the carotenoids except β–carotene are very efficient antioxidants (Karthikeyan and Rani, 2003). β–carotene is based on the formation of low active radicals in isoprenoid chain during interaction with active lipid radicals and/or regeneration of ∝–TO° radicals (Konovalova *et al.*, 2003). Carotenoids also have many non-antioxidant properties that affect cellular signalling pathways, modify the expression of some genes, and can act as inhibitors of regulatory enzymes which may play a part in disease prevention (Furtado *et al.*, 2004).

Lycopenes are bioflavonoids that are closely linked to β-carotene. Lycopene has been shown in experiments to have the highest oxygen quenching

capacity (strongest antioxidant). It is twice as powerful as β-carotene at neutralizing free radicals. This antioxidant property protects the cell from DNA damage. (http://holisticonline.com/cancer/cancer_lycopene.htm).

The biological activities of lycopene include its antioxidant action and control of cellular growth. Lycopene could reduce the risk of macular degeneration, the oxidation of serum lipids and cancers of the lungs, the bladder, the cervix and the skin (Rajeswari and Subbulakshmi, 2004).

2.6 MEDICINE FROM NATURE–SEARCH FOR ANTIMICROBIAL COMPOUNDS OF PLANT ORIGIN

Antibiotic resistance is as ancient as antibiotics, protecting antibiotic-producing organisms from their own products, and other originally susceptible organisms from their competitive attack in nature (Phillips *et al.*, 2004). The antibiotics in the modern therapeutic system have tremendous effect in controlling the infectious diseases. However, the advent escape mechanism (**cf.**drug resistance) adapted by most of the pathogens certainly need a suitable replacement of the presently available antibiotics. However, many antibacterial and antifungal agents are known to exhibit serious untoward effect on host tissues leading to systemic toxicity (Afaq *et al.*, 2004).

Resistance to antibiotics emerges in bacteria due to genetic mutations and consecutive selection of resistant mutants through selective pressure of antibiotics present in large amounts in soil, plants, animals and humans. Developing new antimicrobial agents does not seem to be enough to keep up pace with ever changing bacteria. Using antibiotics prudently and developing new approaches to the treatment of infections is vital for future (Andrasevic, 2004).

Inhibition of cell wall synthesis is the most common mechanism of antibiotic action. All cells including bacteria, also have a cell membrane which allows in and out of substances in the cell in a controlled manner. Synthetic compounds may affect the integrity of the cell membrane leading to cell death. Such molecules may also mimic the complex structures having *in vivo* antibacterial activity (Akgul and Kaya, 2004).

A special feature of higher plants is their capacity to produce a large amount of organic chemicals of high structural diversity, the so-called secondary metabolites. Some of these secondary metabolites are produced for self- defense. Such metabolites are divided into three categories based on their mechanisms of function i.e., chemotherapeutic, bacteriostatic, bactericidal and antimicrobial (Castello *et al.*, 2002). Many hundreds of plants worldwide are used in traditional medicine as treatment for bacterial infections. Some of these have been subjected to *in vitro* screening (Martin and Ernst, 2003). The use of herbal or natural medicines for the treatment of various disorders has a long and extensive history. Many of these herbal medicines are finding their way onto the world market as alternatives, to prescribed drugs currently available to treat various disorders/ailmetns (Huang *et al.*, 2005).

2.6.1 Major groups of antimicrobial compounds from plants

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives. In many cases, the secondary metabolites serve as plant defense mechanism against predation by microorganisms, insects, and herbivores. Useful antimicrobial phytochemicals can be divided into several categories and summarized in the following table:

TABLE II

MAJOR CLASSES OF ANTIMICROBIAL COMPOUNDS FROM PLANTS

(Cowan, 1999)

Class	Subclass	Example(s)	Mechanism
Phenolics	Simple Phenols	Catechol Epicatechin	Substrate deprivation, Membrane disruption
	Phenolic acids	Cinnamic acid	-
	Quinones	Hypericin	Bind to adhesions, Complex with cell wall, Inactivates enzymes
	Flavonoids	Chyrsin	Bind to adhesions, Complex with cell wall
	Flavones	Abyssinone	Inactivate enzyme, Inhibit HIV reverse transcriptase
	Flavonols	Totarol	Unknown
	Tannins	Ellagitanin	Bind to proteins, Bind to adhesions, Enzyme inhibition, Substrate deprivation, Complex with cell wall, Membrane disruption, Metal ion complexation.
	Coumarins	Warfarin	Interaction with eukaryotic DNA (Antiviral activity)
Terpenoids	-	Capsaicin	Membrane disruption
Alkaloids	-	Berberine Piperine	Intercalate into cell wall and / or DNA
Lectins and Polypepetides	-	Mannose specific agglutinin	Block viral fusion or absorption
	-	Fabatin	Form disulfide bridges

2.7 MEDICINAL PLANTS - TRADITIONAL REMEDIES

Indigenous medicine in India have several preparations which are implicated in the preventive medicine (Rekha *et al.*, 2001). Plants have been used worldwide for treatment of various human ailments since antiquity (Maurya *et al.*, 2004). The antioxidants may be either the natural ones or the synthetic ones. Commonly used synthetic antioxidants are butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA), propyl gallate and tertiary butyl hydroquinone. While the naturally occurring antioxidants like vitamins are a balanced mixture of redox with reduced and oxidized form, the synthetic antioxidants are unbalanced in this respect and they themselves produces harmful free radicals (Rani *et al.*, 2004). As many synthetic antioxidants have one or other side effects, there has been as upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing free radical induced tissue injury.

Numerous plant products have been shown to have antioxidant activity. The antioxidant vitamins, flavonoids and polyphenolic compounds of plant origin have been extensively reported as scavengers of free radicals and inhibitors of lipid peroxidation (Geetha *et al.*, 2003). A number of natural antioxidants are found in plant materials such as oil seeds, cereal crops, vegetables, fruits, leaves, roots, spices and herbs. Some of them exhibit significant antioxidant activity and are commonly utilized for pharmaceutical purposes and in health foods (Lakshmi *et al.*, 2005).

The antioxidants are not the remedy for metabolic diseases e.g., heart disease, diabetes, arthritis, brain disorders, *etc.*, but they definitely improve the well being of the patient and prevent its progress when accompanied with specific drug for these diseases (Tripathi *et al.*, 2001). Antioxidants can act as pro-oxidants at higher concentrations causing cells to undergo severe oxidative stress ultimately resulting in suicidal cell death (Koshy *et al.*, 2003).

2.7.1 Antioxidant Activity of the Plants

- The glyceric extract of *Citrus paradisi*, grape fruit was demonstrated to have a good antioxidant activity (Giamperi *et al.*, 2004).
- The alcoholic extract of the bulb of *Scilla indica* is a potent antioxidant in a low dose range (Tripathi *et al.*, 2001).
- Khopde *et al.*, (2001) reported that ascorbic acid and other polyphenols present in the natural formulation of amla showed much superior antioxidant activity compared to their equivalent amounts in the pure isolated form.
- The antioxidant and free radical scavenging activities of alcoholic extract of roots of *Picrorhiza kurrooa* Royle ex Benth. was evaluated by Govindarajan *et al.*, (2003) and it was reported to possess antioxidant, antiradical and hepatoprotective activity.

2.7.2 Antimicrobial Activity of the Plants

- The aqueous extracts of seeds and fruits of *Caesalpina digyna* were tested against human pathogens and reported that the seed extract is less effective than the fruit extract (Elizabeth, 2003).
- The petroleum ether, chloroform and methanolic extracts of *Memecylon malabaricum* leaves were tested for antimicrobial activity. Only the methanolic extract showed activity against both Gram (+)ve and Gram (-)ve bacteria and fungi (Hullatti and Rai, 2004).
- Among various extracts (petroleum ether, ethylacetate and chloroform) of *Salacia beddomi*, ethylacetate extract of both leaves and stems exhibited pronounced activity against the tested microorganisms (Deepa and Bai, 2004)

Acalypha indica, (L.) (Family - Euphorbiaceae) is referred in Tamil as 'Kuppai-meni'. The plant is used for its anodyne, anthelmintic, cathartic, diuretic, emetic, expectorant and emmenagogue effect. Samy *et al.*, (1999) stated that

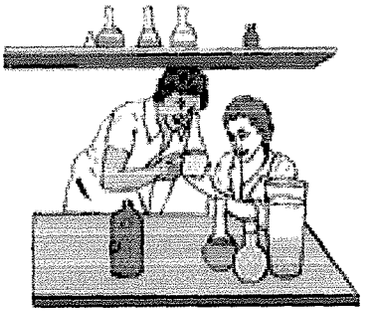
the aqueous residues of this plant showed maximum inhibition against *Aeromonas hydrophilla* and *Bacillus cerues*. Ethanolic extract of *Acalypha indica* showed antivenom activity (Shirwaiker *et al.*, 2004) and wound healing activity (Reddy *et al.*, 2002)

Eclipta prostrata (L.) (Family-Compositae) widely known as 'Karishalanganni', which can be used as alterative-antipyretic, hemostatic, laxative, nervine, rejuvenative, tonic, vulnery, purgative, *etc.*, Methonolic extracts of leaves, barks and roots of *Eclipta prostrata* exhibited the broadest spectrum of antimicrobial activity (Wiart *et al.*, 2004). The chloroform extract showed antigiardial activity (Sawangjaroen *et al.*, 2005). The butanolic and purified butanolic extracts of *Eclipta prostrata* partially inhibited the hemorrhagic activity and displayed very low anti-phospholipase A_2 activity and did not inhibit proteolytic activity of Malyan Pit Viper (MPV) venom (Pithayankal *et al.*, 2004).

Mollugo latoides (L.) O. Kuntze (Family - Ficoidae) is locally known as 'Cherruppadai'. It is used as a stimulant. This plant grows in dried pond during summer.

Leucas aspera (S.) (Family - Labiatae) is a common aromatic herb referred as 'Thumbai' in Tamil. Traditionally, the whole plant is taken for analgesic, antipyretic, antirheumatic, antiinflammatory and antibacterial treatment (Sadhu *et al.*, 2003). In the case of snake bites farmers apply the fresh juice of crushed plants in affected part (Goudgaon *et al.*, 2003).

Nelumbo nucifera (G.) (Family - Nymphaeceae) is an aquatic plant, commonly known as 'Thamari'. The flower has cooling, astringent, expectorant and sedative properties. The tuber has demulcent effect and the seeds are used for their tonic and nutrient property.



EXPERIMENTAL PROCEDURE

3.0 EXPERIMENTAL PROCEDURE

The methodology followed for the "EVALUATION OF ANTIOXIDANT POTENTIAL AND ANTIBACTERIAL EFFICACY OF SOME INDIAN MEDICINAL PLANTS" is given as follows:

3.1 COLLECTION OF PLANT MATERIAL

The whole plants namely *Acalypha indica* (L.), *Eclipta prostrata* (L.), *Mollugo latoides* (L.), *Leucas aspera* (S.) and the flowers of *Nelumbo nucifera* (G.) were collected in and around Coimbatore, Tamilnadu, India. The freshly cut plants and flowers were dried in the drying room with active ventilation and ambient temperature for 7 days. The plant materials were powdered and used for further investigation.

3.2 PREPARATION OF HERBAL MIXTURE

The powdered materials were mixed in equal amount to prepare the herbal mixture and it is believed to strengthen the body.

3.3 PREPARATION OF THE EXTRACT

a) Preparation of the extract for analyzing antioxidant potential

The herbal mixture and its components were extracted with respective buffers to analyze the enzymatic and non-enzymatic antioxidants present in them.

b) Preparation of the extract for determining antibacterial efficacy

5g of each sample was individually extracted with water and alcohol. Extraction was done by shaking conical flasks continuously for 48 hours. Then it was filtered through Whatman No.1 filter paper and the crude extracts obtained were evaporated in water bath at 40°–60°C to give a gummy solid residue. The residue was dissolved in dimethyl sulphoxide (DMSO). These crude extracts were stored in refrigerator and screened for antibacterial activity.

3.4 SOURCES OF MICROORGANISMS

The following microorganisms were selected for the study

Escherichia coli	*Proteus mirabilis*
Enterococcus faecalis	*Serratia marescens*
Enterococcus cloacaceae	*Staphylococcus aureus*
Klebsiella pneumoniae	*Salmonella typhi*
Pseudomonas aeruoginosae	*Shigella flexineri*

The organisms were obtained from Department of Microbiology, P.S.G.Institute of Medical Sciences, and Coimbatore. The bacterial strains were maintained on nutrient agar slants.

3.5 QUALITATIVE PHYTOCHEMICAL SCREENING

Plant based diet provides an array of biologically active phytochemicals that are thought to confer specific health benefits, including the prevention and mitigation of chronic diseases (Vuong et *al.*, 2004). Hence the crude aqueous and alcoholic extracts were analysed for the presence of phytochemicals by the method of Khandelwal, (2002) as described in Appendix I.

3.6 ANTIOXIDANTS

Antioxidants are either nutrients or enzymes (and sometimes both), which mop up damaging free radicals in our body.

(http://www.gnc.com/category.asp?id=52),

3.6.1 Enzymatic Antioxidants

Antioxidative enzymes provide a major defense mechanism against free radical damage either by metabolizing them to less reactive species or non-toxic products (Reiter *et al.*, 2003).

3.6.1.1 Estimation of catalase activity

Catalase is a hemoprotein localized in the peroxisomes or microperoxisomes, which catalyzes the decomposition of H_2O_2 to water and oxygen and thus protect the cell from oxidative damage by H_2O_2 (Loki and Rajamohan, 2003). Catalase activity was estimated by the method of Luck, (1974) and the detailed procedure is given in Appendix II.

3.6.1.2 Estimation of peroxidase activity

In plants, antioxidant enzymes namely catalase and peroxidase have been shown to increase when subjected to stress conditions (Rani *et al.*, 2004). The activity of peroxidase of the given sample was determined by the method of Reddy *et al.*, (1995) as described in Appendix III.

3.6.1.3 Estimation of superoxide dismutase activity

Superoxide radical generated in the respiratory chain undergoes rapid dismutation catalyzed by the presence of superoxide dismutase in mitochondrial matrix (Nohl *et al.*, 2004). SOD is a metalloprotein and is the first enzyme involved in the antioxidant defense by lowering the steady state level of $O_2^{\cdot -}$ (Sabu and Kuttan, 2004). The superoxide dismutase activity was estimated by the method described by Misra and Fridovich, (1972) and it is explained in Appendix IV.

3.6.1.4 Estimation of polyphenol oxidase activity

Polyphenol oxidase is an oxygen transferring enzyme. Besides using O_2 to catalyze the dehydrogenation of catechols to orthoquinones and the orthohydroxylation of phenols to catechols, a peroxidase activity has been reported (http://www.worthington-biochem.com/TY/default.html).

The polyphenol oxidase activity of the plant sample was estimated by Esterbauer *et al.*, (1977) method as stated in Appendix V.

3.6.1.5 Estimation of glutathione-S-transferase activity

Glutathione-S-transferase is another important enzyme known to catalyze antioxidant process of thiol compounds and in turn protect the cells from electrophiles, free radicals induced damage and oxidative stress (Singh *et al.*, 2004). The activity of GST was determined by the method proposed by Habig *et al.*, (1974) and the procedure is described in Appendix VI.

3.6.1.6 Estimation of glutathione peroxidase activity

Glutathione peroxides are able to reduce endogenously produced H_2O_2 and organic hydroperoxides. Glutathione, as a substrate for glutathione peroxidase reactions also result in GSSG formation (Zatorska *et al.*, 2003) The method of Rotruck, *et al.*, (1973) was followed to assess the activity of glutathione peroxidase and it is given in Appendix VII.

3.6.1.7 Estimation of glutathione reductase activity

The recycling of GSSG to GSH is accomplished mainly by the enzyme glutathione reductase. This enzyme uses the coenzyme NADPH as its source of electrons (Cathcart, 1985). The activity of glutathione reductase was determined by the method proposed by David and Richard, (1983), which is elaborated in Appendix VIII.

3.6.2 Non-enzymatic Antioxidants

Apart from enzymatic antioxidants a spectrum of non-enzymatic antioxidants namely vitamin A, C, E and glutathione are important in cellular system in curtailing Reactive Oxygen Species (ROS) (Karthikeyan and Rani 2003).

3.6.2.1 Estimation of ascorbic acid

Ascorbic acid is reported to be associated with better scavenging activities *in vivo* than the antioxidant enzyme, because they are present in both intracellular and extracellular fluid (Chatterjee and Nandhi, 1991). Ascorbic acid was estimated by the method of Roe and Keuther (1953) and the procedure is given in Appendix IX.

3.6.2.2 Estimation of α-Tocopherol

Vitamin E is known to be the most fat-soluble, chain breaking antioxidant (Gupta *et al.,* 2004). The antioxidant properties of tocopherol are the result of its ability to quench both singlet oxygen and peroxides (Fryer, 1992). Within the membrane, tocopherol is the only protective agent that can act against toxic effects of oxygen radicals (Suntress and Shek, 1995). This fat-soluble membrane associated antioxidant was estimated by Emmerie-Engel method as described by Rosenberg (1992). The detailed procedure is given in Appendix X.

3.6.2.3 Estimation of reduced glutathione

Glutathione (GSH), a major non-protein thiol in living organisms, plays a crucial role in coordinating the body's antioxidant defense process (Loki and Rajamohan, 2003). Reduced glutathione, which is a substrate for glutathione peroxidase, neutralizes hydroxyl radicals and singlet oxygen. Since it is present in high concentration in the cells, it protects cells from free radical damage (Selvendiran *et al.*, 2003). The content of reduced glutathione of the sample was determined by the method of Moron *et al.*, (1979) as given in Appendix XI.

3.6.2.4 Estimation of polyphenol

Phenolic compounds are widely distributed in foods of plant origin and are regarded as effective antioxidants (Liebert *et al.*, 1999). Plant phenolics

constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators. It was reasonable to determine their total amount in the selected plant extracts. The procedure of Malick and Singh (1980) was followed to estimate the total phenolic content as explained in Appendix XII.

3.6.2.5 Estimation of total carotenoids and lycopene

β-carotene in low concentration produced a pronounced antioxidant (AO) effect in the liver and myocardium (Konovalova *et al.*, 2003) Lycopene, the carotenoid possess exceptionally high antioxidant capacity compared to other carotenoids (Rana, 2004). Carotenoids and Lycopene were analysed by the method of Zakeria *et al.*, (1979) as described in Appendix XIII.

3.6.3. Estimation of Protein

All the enzymes are protein in nature, hence, the total protein of samples were estimated by Lowry *et al.*, (1951) method. The complete procedure is given in appendix XIV.

3.6.4 Estimation of Carbohydrate

The carbohydrate content of the plant samples were analyzed by the method of Hedge and Hofreiter, (1962) as given in Appendix XV.

3.6.5 Free radical Scavenging Effect of Plant Samples

3.6.5.1 *In vitro* lipid peroxidation assay

Quantitative measurement of lipid peroxidation in goat liver homogenate was performed by the method of Okhawa *et al.*, (1979). The amount of malondialdehyde was quantified by reaction with thiobarbituric acid and used as an index of lipid peroxidation (SudheerKumar *et al.*, 2004). The detailed procedure is given in Appendix XVI.

3.6.5.2 Determination of superoxide production *in vitro*

Superoxide radical ($O2^{-}$) is highly toxic species, which is generated by numerous biological photochemical reactions (Govindarajan *et al.,* 2003). The potential of plant samples (buffer, aqueous and alcoholic extracts) to inhibit the generation of superoxide was determined by Winterbourn *et al.*, (1975) method as it is explained in Appendix XVII.

3.6.5.3 Determination of nitric oxide generation *in vitro*

Aqueous solution of sodium nitroprusside spontaneously generates nitric oxide at physiological pH, which interacts with oxygen to produce nitrite ions and which is measured colorimetrically (Rekha *et al.*, 2001). Appendix XVIII explains the procedure given by Green *et al.*, (1982) which was used to estimate the extent of nitric oxide inhibition by plant samples and crude extracts.

3.7 ANTIMICROBIAL SUSCEPTIBILITY TEST

3.7.1 Antibiotic Sensitivity Test

The inocula were prepared from fresh overnight broth cultures in nutrient broth. Plates were prepared by pouring sterile nutrient agar (Himedia) into sterile petridishes previously autoclaved. Sterilized cotton swabs were dipped in the nutrient broth and squeezed, then swabbed on the agar plate. Standard antibiotic discs (10mcg - Himedia) were placed carefully on the swabbed plates and incubated overnight. Antibiotic Sensitivity Test (AST) was conducted against Ampicillin, Chloramphenicol, Erythromycin, Gentamicin and Streptomycin.

3.7.2 Antibacterial Sensitivity Test (AST)

The antibacterial activity of the crude aqueous and alcoholic and extracts was determined by the following methods:

- Disc diffusion assay (Qualitative screening)
- Microbroth dilution assay (Quantitative determination)

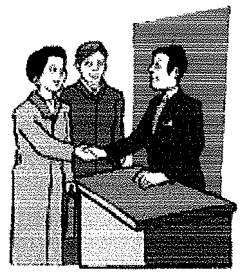
3.7.2.1 Disc diffusion assay

Plates were prepared by pouring sterile nutrient agar (Himedia) into sterile petridishes previously autoclaved. The sterilized cotton swab was dipped in bacterial subculture in nutrient broth and then swabbed on agar plate. Sterile discs (Himedia) saturated with different extracts were placed on nutrient agar seeded with test organisms. Discs fed with DMSO served as control. The plates were incubated at 37°C and observed for zones of growth inhibition after 24 hours.

3.7.2.2 Microbroth dilution assay

The MIC (Minimum Inhibitory Concentration - the lowest concentration at which no visible growth of microorganism could be detected) was determined by means of microbroth dilution assay. It is used to measure quantitatively the *in vitro* activities of the extracts against bacterial isolates.

100µl of nutrient broth was added to all the wells. 100µl of the extract was aspirated from the micropipette. 100µl of diluent is transferred and mixed with 100µl of diluent in the second row, making 4:1 dilution. This proceeds consecutively down the plate making two fold dilution in each well. An aliquot of the organism was added to the wells. The covered plates were incubated under aerobic conditions at 37°C for 24 hours.



RESULTS AND DISCUSSION

4.0 RESULTS AND DISCUSSION

Plant and plant products are known to possess excellent antioxidant properties (Ashok *et al.*, 2004). Several antioxidants of plant origin are experimentally proved and used as a protective agent against oxidative stress. When the normal level of antioxidant defense mechanism is not sufficient for the eradication of free radical induced injury, administration of antioxidants of plant origin has a protective role to play. (Rekha *et al.*, 2001). To counteract the destructive effect of activated oxygen species, cells deploy an array of enzymatic and non-enzymatic antioxidant defense (Jain *et al.*, 2004).

Problems with drug-resistant microorganisms and side effects of modern drugs and emerging diseases where no medicines are available, have stimulated renewed interest in plants as a significant source of new medicines (Patwardhan *et al.*, 2004). Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties. These substances that can inhibit pathogens and have little toxicity to host cells are considered candidates for developing new antimicrobial drugs (Aqil and Ahmad, 2003). The present study was carried out to evaluate the antioxidant potential and antibacterial activity in five different Indian medicinal plants individually and in combination.

The whole plant of *Acalypha indica* (L.), *Eclipta prostrata* (L.), *Mollugo latoides* (L.) and *Leucas aspera* (S.) and flowers of *Nelumbo nucifera* (G.) were analyzed for enzymatic (catalase, peroxidase, superoxide dismutase, polyphenol oxidase, glutathione-S-transferase, glutathione reductase and glutathione peroxidase) and non-enzymatic (ascorbic acid, α-tocopherol, reduced glutathione, polyphenols and carotenoids, lycopene) antioxidants. The protein and carbohydrate content of the samples were also evaluated.

The free radical scavenging potential *viz.*, *in vitro* lipid peroxidation, the extent of inhibition of superoxide and nitric oxide generation were determined. The antibacterial efficacy of the samples was analyzed using aqueous and alcoholic extracts. The findings of the present study are discussed under the following headings:

4.1 Phytochemical Screening of the Plant Samples
4.2 Antioxidant Potential of the Plant Samples
4.2.1 Enzymatic Antioxidants
4.2.2 Non-enzymatic Antioxidants
4.3. Protein And Carbohydrate Content
4.4 Free Radical Scavenging Effect of the Plant Samples
4.4.1 Effect of Plant Samples on *In Vitro* Lipid Peroxidation
4.4.2 Effect of Plant Samples on Superoxide Generation *In Vitro*
4.4.3 Effect of Plant Samples on Nitiric Oxide Generation *In Vitro*
4.5 Antibacterial Activity of the Plant Samples
4.5.1 Screening of Antibacterial Activity by Agar Diffusion Method
4.5.1.1 Antibiotic Sensitivity Test (AST)
4.5.1.2 Antibacterial Activity of the Aqueous Extracts
4.5.1.3 Antibacterial Activity of the Alcoholic Extracts
4.5.2 Microbroth Dilution Assay

4.1 PRELIMINARY PHYTOCHEMICAL SCREENING OF THE EXTRACTS

As a first step of analyzing the chemical nature of the compound responsible for antioxidant and antimicrobial activity in the plant samples, a preliminary phytochemical screening was done in the aqueous and alcoholic extracts of the leaves. The results obtained for the various qualitative confirmatory tests for alkaloids, phenolics and flavonoids are presented in Table III.

The observations of the phytochemical screening revealed that the plant samples including the mixture were found to possess alkaloids, phenolics and flavonoids. The data obtained revealed that the samples are good sources of phenolics and flavonoids.

TABLE III

QUALITATIVE SCREENING OF THE PLANT SAMPLES FOR THE PRESENCE OF ALKALOIDS, PHENOLICS AND FLAVONOIDS

Plant Extracts		Alkaloids			Phenolic compounds		Flavonoids		
		Mayer's Test	Dragendorff's Test	Wagner's Test	Ferric chloride Test	Lead acetate Test	Aqueous NaOH Test	Sulphuric acid Test	Schindo's Test
Aqueous extract	*A.indica*	-	-	-	+++	+++	-	+	-
	E.prostrata	-	-	+	+	-	-	++	-
	M.latoides	-	-	-	-	+	++	+	+
	L.aspera	-	+	+++	++	+++	-	+++	+++
	N.nucifera	-	++	+	+	+	-	-	-
	Mixture	-	+	+	++	+++	++	+++	++
Alcoholic extract	*A.indica*	-	-	+	+	-	-	-	-
	E.prostrata	-	-	+	+	++	-	+	-
	M.latoides	-	+	++	+++	++	-	+++	++
	L.aspera	-	-	++	+	-	-	+++	-
	N.nucifera	+	+	+++	++	-	-	+++	-
	Mixture	-	-	+++	+	-	-	+++	+

+++ Appreciable amount ; ++ Moderate amount ; + Trace amount ; - Completely absent

The concentration of alkaloids was found to be moderate. A major portion of alkaloids was found to be present in aqueous extracts of *Leucas aspera* and alcoholic extracts of *Nelumbo nucifera* and the mixture. Aqueous extracts of *Acalypha indica, Leucas aspera* and alcoholic extracts of *Mollugo latoides* and *Nelumbo nucifera* were found to be rich source of phenolic compounds. Flavonoids were found to be present in the alcoholic extracts of *Mollugo latoides* and *Nelumbo nucifera*. In the case of *Leucas aspera* and the mixture recorded the maximum flavonoid content.

Flavonoids have been documented to possess potent antioxidant and free radical scavenging effect (Shirwaikar[a] *et al.*, 2004). Flavonoids have been believed to exert many positive health effects through their antioxidative properties (Hayakawa *et al.*, 2004). Umadevi *et al.*, (2003) reported that the presence of flavonoids in the chloroform, acetone, methanol and aqueous extracts of *Andrographis echiodes* are likely to be responsible for antibacterial and antifungal activity.

Tarfa *et al.*, (2004) stated that the phytochemical screening of hexane, ethylacetate and methanolic extracts of *Tapinanthus sessilifolius* showed the presence of hydrolysable tannins, saponins, flavonoids, terpenes, balsam, cardiac glycosides and nutrients such as proteins and carbohydrate. The secondary metabolites in the plant could be responsible for some of the observed antimicrobial activity.

4.2 ANTIOXIDANT POTENTIAL OF THE PLANT SAMPLES

4.2.1 Enzymatic Antioxidants

The antioxidative enzymes play a primary role in scavenging the free radicals produced by the metabolic processes. The levels of various antioxidative enzymes were determined and presented in Tables IV, V and Figure 5.

TABLE IV

ENZYMATIC ANTIOXIDANTS IN THE PLANT SAMPLES

S.No	Plants Screened	Enzymatic antioxidants (U/g)			
		Catalase	Peroxidase	Superoxide dismutase	Polyphenol oxidase
1	*Acalypha indica* (L.)	1310.600	0.640	30.567	$3.626 X10^{-3}$
2	*Eclipta prostrata* (L.)	1220.050	0.450	99.783	$2.720X10^{-3}$
3	*Mollugo latoides* (L.)	1312.160	0.467	119.090	$5.440 X10^{-3}$
4	*Leucas aspera* (S.)	1809.550	0.910	27.740	$5.440 X10^{-3}$
5	*Nelumbo nucifera* (G.)	1110.277	0.440	32.516	$2.720 X10^{-3}$
6	Mixturc	1401.407	0.403	78.930	$5.440 X10^{-3}$
	SEd	131.6575	0.0938	0.1765	2.0939
	CD (0.05)	286.8596	0.2043	0.3845	4.5622

The values are mean of triplicates.

Enzymes		Units
Catalase	-	Amount of enzyme required to decrease the optical density by 0.05 units
Peroxidase	-	1µmole of pyrogallol oxidized / min
Superoxide dismutase	-	Amount that causes 50% reduction in the extent of NBT oxidation
Polyphenol oxidase	-	Amount of enzyme that transforms 1µmole of dihydrophenol to 1µmole of quinone/min.

Catalase is an essential enzyme in the decomposition of hydrogen peroxide (Kujumdzieva *et al.*, 2002). In the present study, the estimated catalase level in different plant samples ranges widely from 1110.277 to 1809.550U/g. Plant catalases are reported to be very sensitive to environmental conditions and have a rapid turn over rate (Hartwig *et al.*, 1992). The maximum catalase activity was exhibited by *Leucas aspera* (1809.550 U/g), followed by the mixture. No appreciable change in the catalase activity was observed in *Acalypha indica* and *Mollugo latoides*. The minimum catalase activity was observed in *Nelumbo nucifera* (1110.277 U/g).

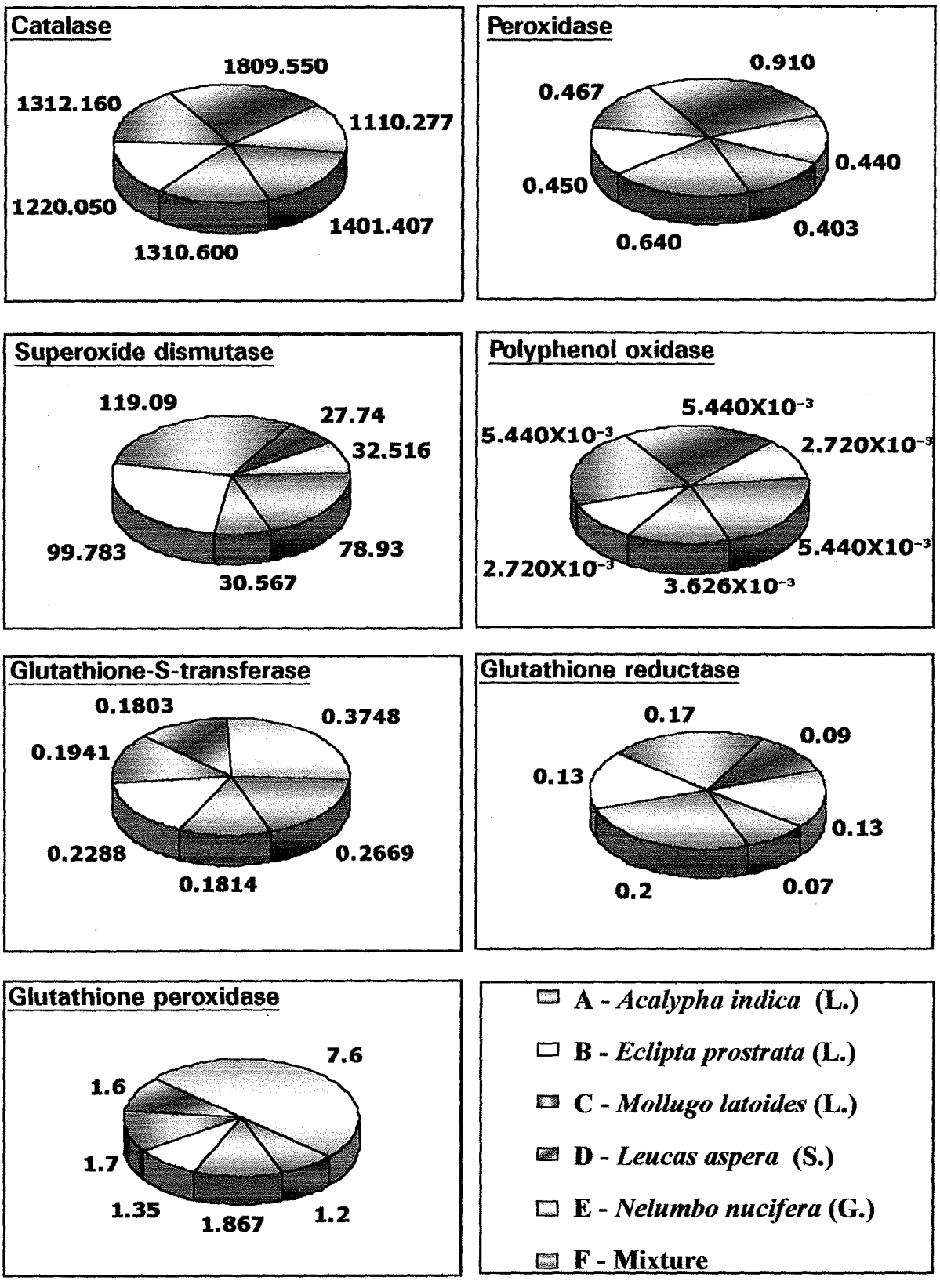
Peroxidase catalyzes the oxidation of cellular components by hydrogen peroxide or hydroperoxides (Bhatia *et al.*, 2004). *Leucas aspera* possessed the highest peroxidase activity (0.910 U/g). The decline in peroxidase activity was observed in *Acalypha indica*, *Mollugo latoides*, *Eclipta prostrata* and *Nelumbo nucifera*. The mixture was found to be the poorest source of the enzyme peroxidase (0.403 U/g). Catalase and peroxidase are the two major systems for the removal of hydrogen peroxide in plants and a fine tuning in hydrogen peroxide levels is one of the factors that contributes to efficient resistance against various biotic stresses (Gholizadeh *et al.*, 2004).

When there is an increase in oxygen consumption, superoxide radicals are produced, which are generally dismutated by superoxide dismutase (Christen, 2000). *Mollugo latoides* (119.092 U/g) was the richest source of superoxide dimutase. *Eclipta prostrata* and the mixture had increased activity of superoxide dismutase. The decreased activity was shown by *Nelumbo nucifera* and *Acalypha indica* and the lowest activity by *Leucas aspera* (27.740U/g). Superoxide dismutase protects the oxygen metabolizing cells against the harmful effects of oxygen free radicals (Poongothai *et al.*, 2004).

Enzymatic oxidation of phenolics via polyphenol oxidases accounts for most plant tissue browning, particularly that which occurs very rapidly. The regulation of enzymatic browning is important in food industry (Cheng and Crisosto, 1995). The highest polyphenol oxidase activity (5.44×10^{-3} U/g) was observed in three different samples (*Mollugo latoides*, *Leucas aspera* and the mixture). *Acalypha indica* exhibited a moderate activity. *Eclipta prostrata* and *Nelumbo nucifera* possessed the lowest activity (2.72×10^{-3}U/g).

FIGURE 5

ENZYMATIC ANTIOXIDANTS IN THE PLANT SAMPLES*



* - Enzyme activity in U/g sample

TABLE V

LEVELS OF ENZYMATIC ANTIOXIDANTS IN THE PLANT SAMPLES

S.No	Plants Screened	Enzymatic antioxidants (U/g)		
		Glutathione -S-transferase	Glutathione peroxidase	Glutathione reductase
1	*Acalypha indica* (L.)	0.1814	1.867	0.200
2	*Eclipta prostrata* (L.)	0.2288	1.350	0.130
3	*Mollugo latoides* (L.)	0.1941	1.700	0.170
4	*Leucas aspera* (S.)	0.1803	1.600	0.090
5	*Nelumbo nucifera* (G.)	0.3748	7.600	0.130
6	Mixture	0.2669	1.200	0.070
	SEd	0.1680	0.9216	0.0427
	CD (0.05)	0.3660	2.0079	0.0931

The values are mean of triplicates.

Enzymes		Units
Glutathione-S-transferase	-	µmoles of CDNB-GSH conjugate/min/g sample
Glutathione peroxidase	-	µmoles of GSH consumed /min/g sample
Glutathione reductase	-	µmoles of NADPH oxidized/min/g sample

Many investigators have suggested that glutathione-S-transferase offers protection against LPO by promoting the conjugation of toxic electrophiles with GSH (Illavarasan *et al.*, 2003). *Nelumbo nucifera* exerted the highest activity for glutathione-S-transferase (0.3748 U/g) and glutathione peroxidase (7.60 U/g). Other plant samples including the mixture showed reasonable activity for glutathione-S-transferase. In contrast, in the case of glutathione peroxidase, except *Nelumbo nucifera* all other samples recorded insignificant level of enzyme activity. Glutathione-S-transferase and glutathione peroxidase are essential for maintaining a constant ratio of reduced glutathione to oxidized glutathione in the cell (Jainu and Devi, 2004).

The maximum glutathione reductase activity was exhibited by *Acalypha indica* (0.20 U/g) followed by *Mollugo latoides*. *Eclipta prostrata* and

Nelumbo nucifera had similar level of glutathione reductase. *Leucas aspera* (0.09 U/g) and the mixture (0.07U/g) recorded very low activity.

The highest activity of catalase, peroxidase and superoxide dismutase exhibited by *Leucas aspera* can be compared with the results of Gholizadeh *et al.*, (2004) who have reported the same pattern of activity for these enzymes in antiviral proteins isolated from *Celosia cristata*. Out of different plants used in our study, *Nelumbo nucifera* possessed the highest activity of glutathione-S-transferase and glutathione peroxidase, which involve in active regeneration of reduced glutathione conferring *Nelumbo nucifera* as an effective source of antioxidants.

4.2.2 Non-enzymatic Antioxidants

The enzymatic and non-enzymatic antioxidants act synergistically to scavenge free radicals produced in the normal metabolic pathways or in pathological conditions. Most of the non-enzymatic antioxidants are vitamins in nature. These non-enzymatic antioxidants were evaluated and given in Table VI. The pictorial representation of the data is shown in Figure 6.

It is evident from the Table VI that the levels of ascorbic acid (29.043 mg/g), α-tocopherol (1.092 mg/g), reduced glutathione (7.545mg/g) and total phenol (13.706mg/g) were found to be maximum in *Leucas aspera* followed by the mixture which recorded an increased value of ascorbic acid and α-tocopherol. The rest of the plants showed significant decrease in the content of ascorbic acid and α-tocopherol but the decrease was found to be drastic in the case of ascorbic acid.

The level of the pigments carotenoids (0.139 mg/g) and lycopene (0.035mg/g) were maximum in *Eclipta prostrata* followed by *Acalypha indica*. In all the samples, the level of carotenoids was higher than lycopene. The lowest level of carotenoids was observed in *Mollugo latoides* (0.013mg/g) and lycopene

TABLE VI

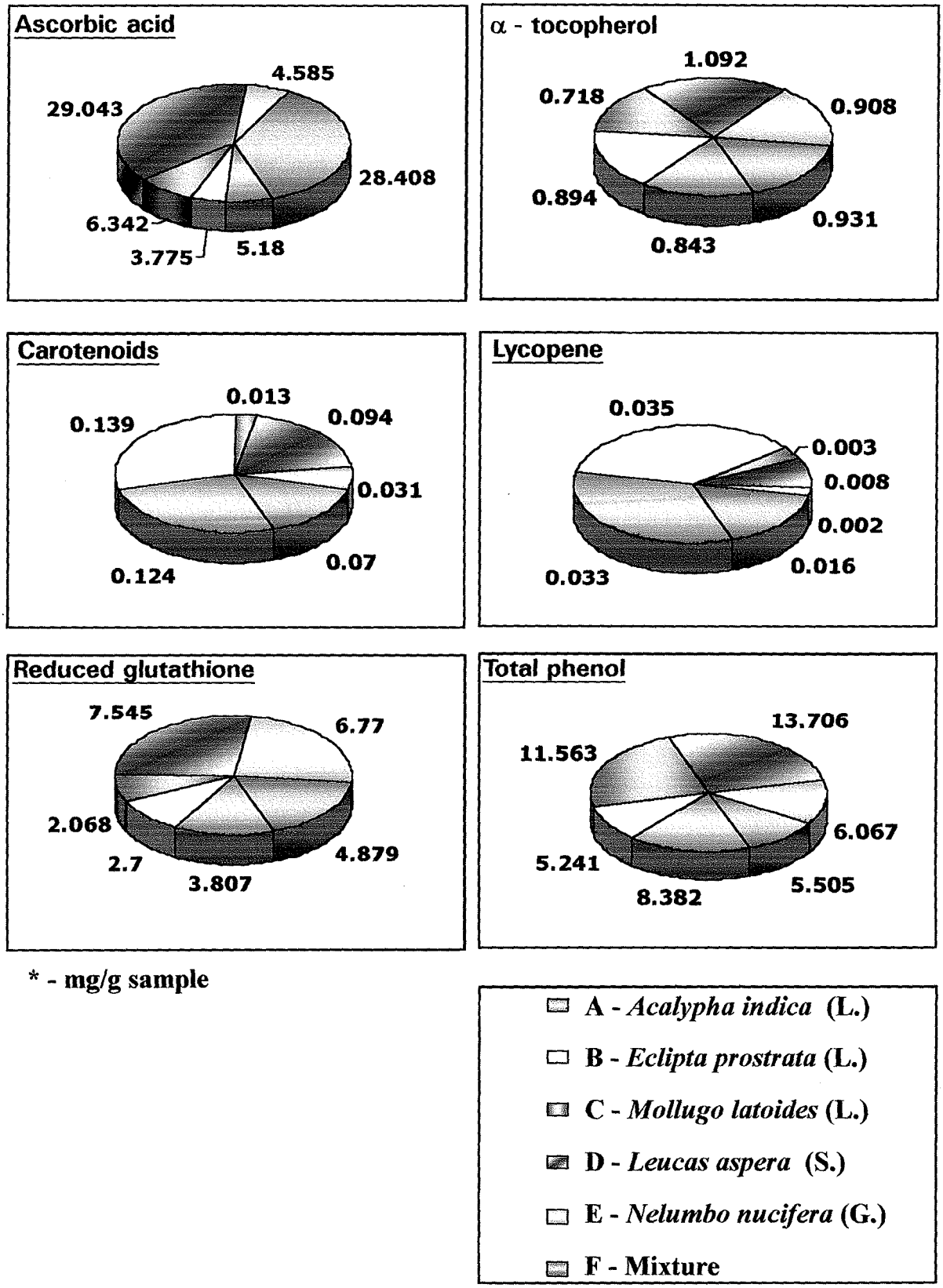
NON-ENZYMATIC ANTIOXIDANTS IN THE PLANT SAMPLES

S.No	Plants Screened	Non - enzymatic antioxidants (mg/g)					
		Ascorbic acid	α-tocopherol	Reduced glutathione	Total phenol	Carotenoids	Lycopene
1	*Acalypha indica* (L.)	5.180	0.843	3.807	8.382	0.124	0.033
2	*Eclipta prostrata* (L.)	3.775	0.894	2.700	5.241	0.139	0.035
3	*Mollugo latoides* (L.)	6.342	0.718	2.068	11.563	0.013	0.003
4	*Leucas aspera* (S.)	29.043	1.092	7.545	13.706	0.094	0.008
5	*Nelumbo nucifera* (G.)	4.585	0.908	6.770	6.067	0.031	0.002
6	Mixture	28.408	0.931	4.879	5.505	0.073	0.016
	SEd	1.6097	0.0021	0.3825	0.2092	0.0024	0.0005
	CD (0.05)	3.5072	0.0045	0.8335	0.4557	0.0052	0.0011

The values are mean of triplicates

FIGURE 6

NON-ENZYMATIC ANTIOXIDANTS IN THE PLANT SAMPLES



in *Nelumbo nucifera* (0.02mg/g) suggesting the poorest source of pigments. The highest values recorded by *Leucas aspera* and *Acalypha indica* pinpoint the antioxidant potential of the two different plants.

The tocopheroxyl radicals formed during the conversion of oxyradicals to hydroperoxides are reduced back to tocopherol by ascorbic acid-glutathione cycle. Ascorbic acid, α-tocopherol and reduced glutathione can act synergistically in the reduction of free radicals formed during any of the stress conditions (Jain *et al.*, 2004). Oxidative biomarker studies indicate that vitamin C protects against *in vivo* oxidation of lipids and DNA in humans (Carr and Frei, 1999). Prevention of lipid peroxidation is thought to be one of the major functions of α-tocopherol (Huang and May, 2003). Phenolic compounds are regarded as effective antioxidants (Liebert *et al.*, 1999). Antioxidative properties of polyphenols arise from (i) their high reactivity as hydrogen donors; (ii) from the ability of polyphenol-derived radical to stabilize or delocalize the unpaired electron (chain-breaking function) and (iii) from their ability to chelate transition metal ions (Rice-Evans *et al.*, 1997).

4.3 PROTEIN AND CARBOHYDRATE CONTENT

Proteins and carbohydrates are important cellular constituents. The plant samples were quantitatively analyzed for protein and carbohydrate content and the results are depicted in Table VII and Figure 7.

Protein content was found to be maximum in the mixture (40.950 mg/g) followed by *Eclipta prostrata.* All other plant samples except *Mollugo latoides* showed increased protein content. The highest carbohydrate content was recorded by *Acalypha indica* (119.999 mg/g) followed by *Eclipta prostrata.* The carbohydrate content of the mixture and *Leucas aspera* was found to be lesser when compared to *Acalypha indica* and *Eclipta prostrata* but the difference was not statistically significant.

TABLE VII

PROTEIN AND CARBOHYDRATE IN THE PLANT SAMPLES

S.No	Plants Screened	Protein (mg/g)	Carbohydrate (mg/g)
1	*Acalypha indica* (L.)	31.815	119.999
2.	*Eclipta prostrata* (L.)	40.535	118.313
3	*Mollugo latoides* (L.)	28.337	59.238
4	*Leucas aspera* (S.)	37.937	100.095
5	*Nelumbo nucifera*(G.)	38.440	74.317
6	Mixture	40.950	113.493
	SEd	0.5809	10.8904
	CD (0.05)	1.2657	23.7283

The values are mean of triplicates.

In contrast, *Mollugo latoides* and *Nelumbo nucifera* recorded decreased carbohydrate content and the decrease was found to be statistically significant.

4.4 FREE RADICAL SCAVENGING EFFECT OF THE PLANT SAMPLES

4.4.1 Effect of Plant Samples on *In Vitro* Lipid Peroxidation

Table VIII shows the inhibitory effect of the plant samples on *in vitro* lipid peroxidation (Figure 8).

TABLE VIII

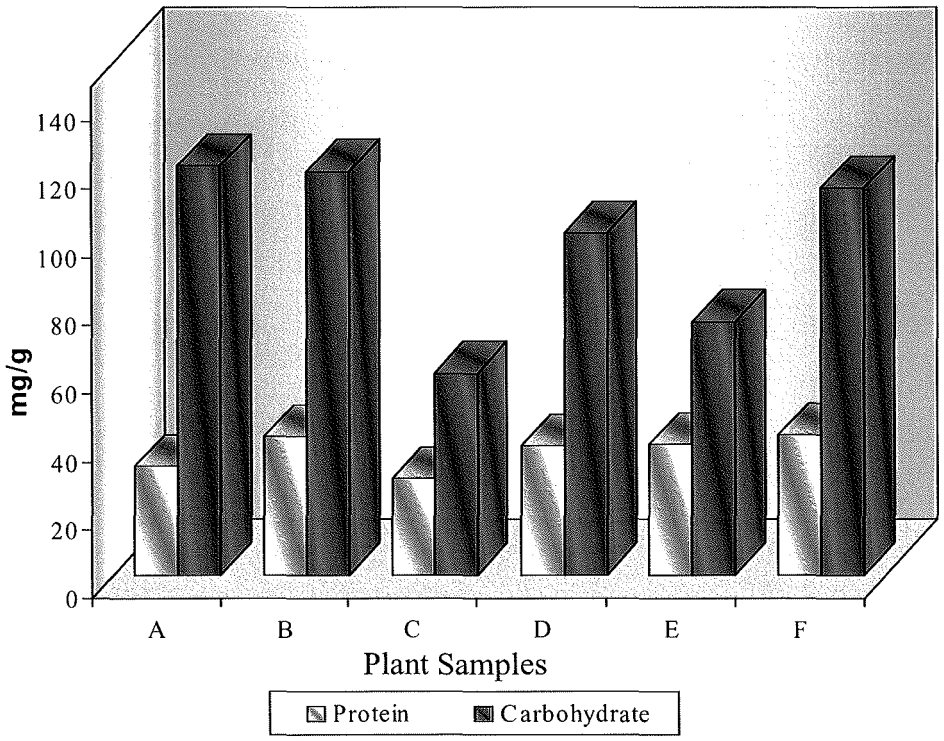
EFFECT ON *IN VITRO* LIPID PEROXIDATION

S.No	Plants Screened	Percentage Inhibition
1	*Acalypha indica* (L.)	62.500
2.	*Eclipta prostrata* (L)	45.833
3	*Mollugo latoides* (L.)	25.000
4	*Leucas aspera* (S.)	45.833
5	*Nelumbo nucifera*(G.)	50.000
6	Mixture	50.000
	SEd	12.2663
	CD (0.05)	26.7263

The values are mean of triplicates.

FIGURE 7

PROTEIN AND CARBOHYDRATE IN THE PLANT SAMPLES



A - *Acalypha indica* (L.)

B - *Eclipta prostrata* (L.)

C - *Mollugo latoides* (L.)

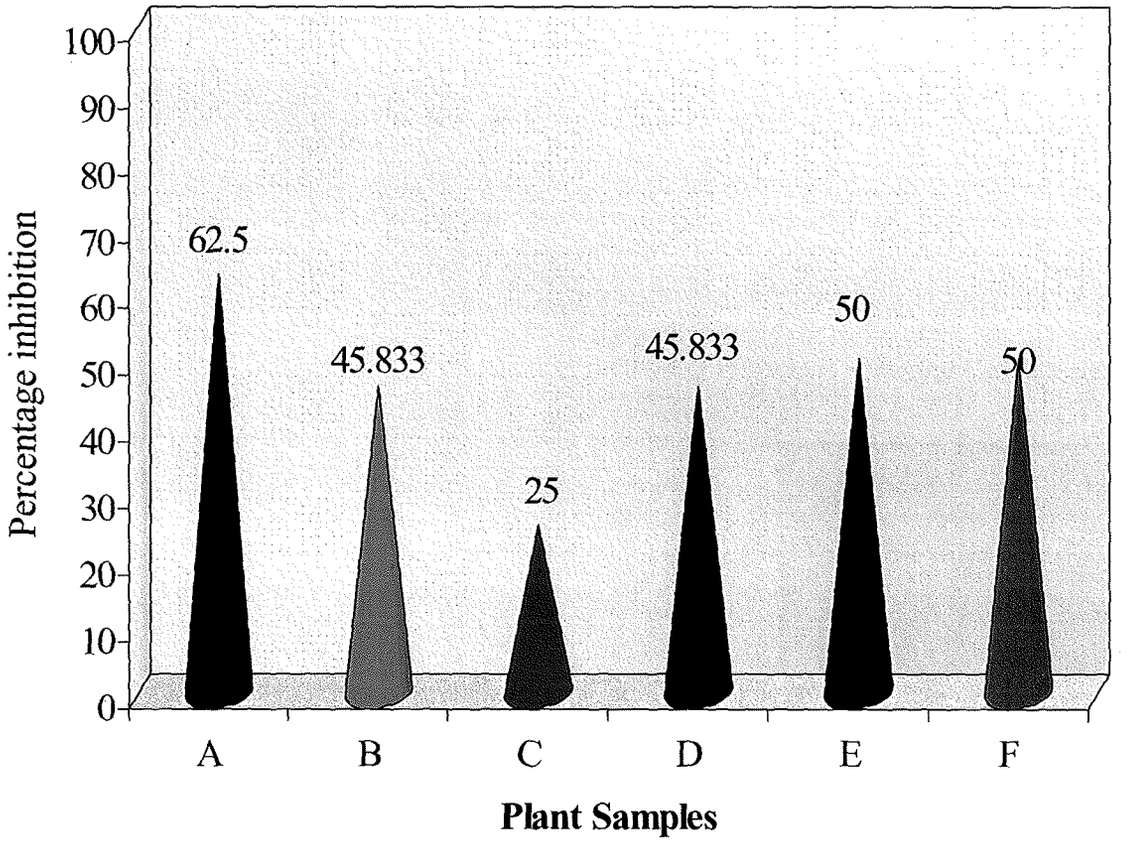
D - *Leucas aspera* (S.)

E - *Nelumbo nucifera* (G.)

F - Mixture

FIGURE 8

EFFECT OF PLANT SAMPLES ON PERCENTAGE INHIBITION OF *IN VITRO* LIPID PEROXIDATION



A - *Acalypha indica* (L.)

B - *Eclipta prostrata* (L.)

C - *Mollugo latoides* (L.)

D - *Leucas aspera* (S.)

E - *Nelumbo nucifera* (G.)

F - Mixture

The maximum inhibition was given by *Acalypha indica* (62.5 %) followed by *Nelumbo nucifera* (50%) and the mixture (50%). The inhibitory effect offered by *Mollugo latoides* is significantly low (25%). This protection could be due to several mechanisms such as (a) chelation of iron, (b) conversion of Fe^{2+} to Fe^{3+} (c) increased level of reduced glutathione, (d) by scavenging hydroxyl radicals, superoxide radicals and other reactive oxygen molecules, which are responsible for lipid peroxidation (Tripathi *et al.*, 2001).

The high level of inhibitory effect exerted by *Acalypha indica*, *Nelumbo nucifera* and the mixture may be due to increased level of enzymatic and non-ezymatic antioxidants. The inhibition of *in vitro* lipid peroxidation by the plant extracts observed in the present study can be attributed to the presence of known enzymatic and non-enzymatic antioxidants in the extracts. The observed effects could also be due to the presence of secondary metabolites in the plant samples. They are effective in curtailing lipid peroxidation. The efficiency of plant extracts in inhibiting lipid peroxidation *in vitro* is a very good measure of assessment of antioxidant potential (Karthikeyan and Rani, 2003).

Peroxidation *in vivo* is influenced by the activity of superoxide dismutase, catalase and glutathione peroxidase. The evaluation of antioxidants as suppressors of chemically induced lipid peroxidation provides a scope to select natural free radical scavengers, which on co-administration *in vivo* may reduce the toxic effects of the chemicals (Anilakumar *et al.*, 2004). Acarya *et al.*, (2004) reported that the crude, boiled and ethanolic extracts of *Auricularia auricula* showed inhibition on lipid peroxidation. The alcoholic extract of the rhizomes of *Smilex china* protects the induction of lipid peroxidation induced by $FeSO_4$ (Tripathi *et al.*, 2001).

4.4.2 Effect of Plant Samples on Superoxide Generation *In Vitro*

Plant samples were found to scavenge the superoxide radical generated

by the photoreduction of riboflavin. The extent of inhibition of superoxide generation by plant samples was measured and presented in Table IX.

TABLE IX

PERCENTAGE INHIBITON OF SUPEROXIDE GENERATION *IN VITRO*

S.No	Plants Screened	Percentage inhibition		
		Buffer extract	**Aqueous extract**	**Alcoholic extract**
1	*Acalyhpa indica* (L.)	46.667	26.667	66.667
2.	*Eclipta prostrata* (L)	26.667	46.667	66.667
3	*Mollugo latoides* (L.)	46.667	46.667	46.667
4	*Leucas aspera* (S.)	73.333	26.667	26.667
5	*Nelumbo nucifera* (G.)	73.333	46.667	46.667
6	Mixture	46.667	66.667	46.667
	SEd	3.8490	5.4433	9.4280
	CD (0.05)	7.8070	11.0407	19.1231

The values are mean of triplicates.

The highest inhibition (73.333%) was given by buffer extracts of two different plant samples *Leucas aspera* and *Nelumbo nucifera* followed by the increased superoxide scavenging effect of aqueous extracts of the mixture and alcoholic extracts of *Acalypha indica* and *Eclipta prostrata*. A moderate inhibitory effect was given by buffer extracts of *Acalypha indica*, *Mollugo latoides* and the mixture, aqueous extracts of *Eclipta prostrata*, *Mollugo latoides* and *Nelumbo nucifera* and alcoholic extracts of *Mollugo latoides*, *Nelumbo nucifera* and the mixture. The poor source of inhibition (26.667%) was the buffer extract of *Eclipta prostrata*, aqueous and alcoholic extract of *Leucas aspera*.

The extent of inhibiton of superoxide generation exerted by different plant samples ranged from 26.667 % to 73.333 %. Though the maximum inhibition was caused by the buffer extract of *Leucas aspera*, the aqueous and alcoholic extracts recorded the lowest percentage of inhibition. Similarly the extent of inhibition of superoxide generation was found to be maximum with the buffer extract of *Nelumbo*

nucifera and moderate with aqueous and alcoholic extracts. These results emphasize that among the three different extracts buffer extract was found to be more efficient in inhibiting superoxide generation.

The results revealed that the extracts of all the plant samples contained antioxidants either enzymatic or non-enzymatic that can inhibit the generation of superoxide.

4.4.3 Effect of Plant Samples on Nitric Oxide Generation *In Vitro*

Nitiric oxide radicals generated from sodium nitroprusside at physiological pH were found to be inhibited by the plant samples (Rekha *et al.*, 2001). The reduction of nitrate by buffer, aqueous and alcoholic extracts of the samples was measured and shown in Table X and Figure 9.

TABLE X

PERCENTAGE INHIBITION OF NITRIC OXIDE GENERATION *IN VITRO*

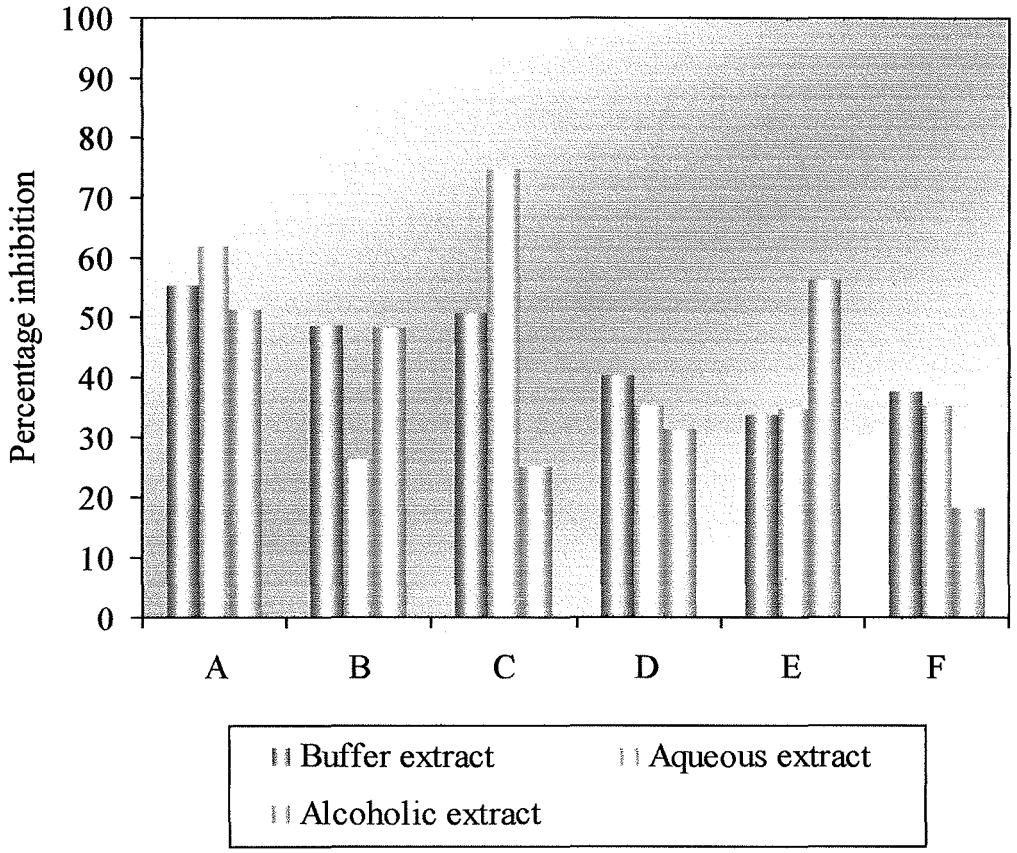
S.No	Plants Screened	Percentage inhibition of NO• generation		
		Buffer extract	**Aqueous extract**	**Alcoholic extract**
1	*Acalypha indica* (L.)	55.412	61.856	51.454
2.	*Eclipta prostrata* (L.)	48.711	26.174	48.322
3	*Mollugo latoides* (L.)	50.515	74.533	25.134
4	*Leucas aspera* (S.)	40.463	35.458	31.319
5	*Nelumbo nucifera*(G.)	33.762	34.787	56.487
6	Mixture	37.794	35.346	18.232
	SEd	0.0850	0.1202	0.2083
	CD (0.05)	0.1725	0.2439	0.4225

The values are mean of triplicates.

Aqueous extract of the *Mollugo latoides* had a maximum potential (74.533%) on inhibtion of nitric oxide generation, followed by aqueous extract of

FIGURE 9

PERCENTAGE OF INHIBTION OF NITRIC OXIDE GENERATION *IN VITRO*



A - *Acalypha indica* (L.)

B - *Eclipta prostrata* (L.)

C - *Mollugo latoides* (L.)

D - *Leucas aspera* (S.)

E - *Nelumbo nucifera* (G.)

F - Mixture

Acalypha indica. An increased nitric oxide scavenging effect was shown by buffer extracts of *Acalypha indica* and *Mollugo latoides*, alcoholic extract of *Acalypha indica* and *Nelumbo nucifera*. The least inhibition (18.232%) was given by alcoholic extract of the mixture.

Shirwaikar *et al.*, (2004) observed that free radicals were scavenged by test compounds in a concentration dependent manner. The maximum percentage of inhibition in all the models *viz.*, nitric oxide, lipid peroxidation and superoxide were found to be 73.64, 51.61 and 51.71 % respectively. The results of the present investigation are in agreement with the above report and suggest that the plant samples are the best source of antioxidants, which exhibit inhibitory action in any one of the extracts.

4.5 ANTIBACTERIAL ACTIVITY OF THE EXTRACTS AGAINST BACTERIAL ISOLATES

The antibacterial activity of the extracts was determined by two different methods, agar diffusion assay for a qualitative antibacterial screening and the microbroth dilution methods for a quantitative assay. Ten different bacterial isolates were used to screen the possible antibacterial activities of crude aqueous and alcoholic extracts.

4.5.1 Screening of Antibacterial Activity by Agar Diffusion Method

4.5.1.1 Antibiotic Sensitivity Test (AST)

Table XI and Figure 10 represent the sensitivity levels of the microorganisms against standard antibiotics in terms of growth inhibition zone.

TABLE XI

ANTIBIOTIC SENSITIVITY TEST (AST) USING DIFFERENT ANTIBIOTICS

S.No	Microorganisms	Zone of inhibition (mm)				
		Ampicillin	Chloram phenicol	Erythro mycin	Genta micin	Strepto mycin
1	*E.coli*	35	22	25	25	22
2	*E.faecalis*	11	18	NZ	15	16
3	*E.cloacaceae*	NZ	NZ	NZ	16	20
4	*K.pneumoniae*	32	20	28	27	20
5	*P.aeruoginosae*	30	29	28	25	25
6	*P.mirabilis*	30	29	28	25	25
7	*S.marescens*	21	27	23	27	22
8	*S.aureus*	21	25	25	24	23
9	*S.typhi*	25	29	27	28	24
10	*S.flexineri*	ND	ND	ND	ND	ND

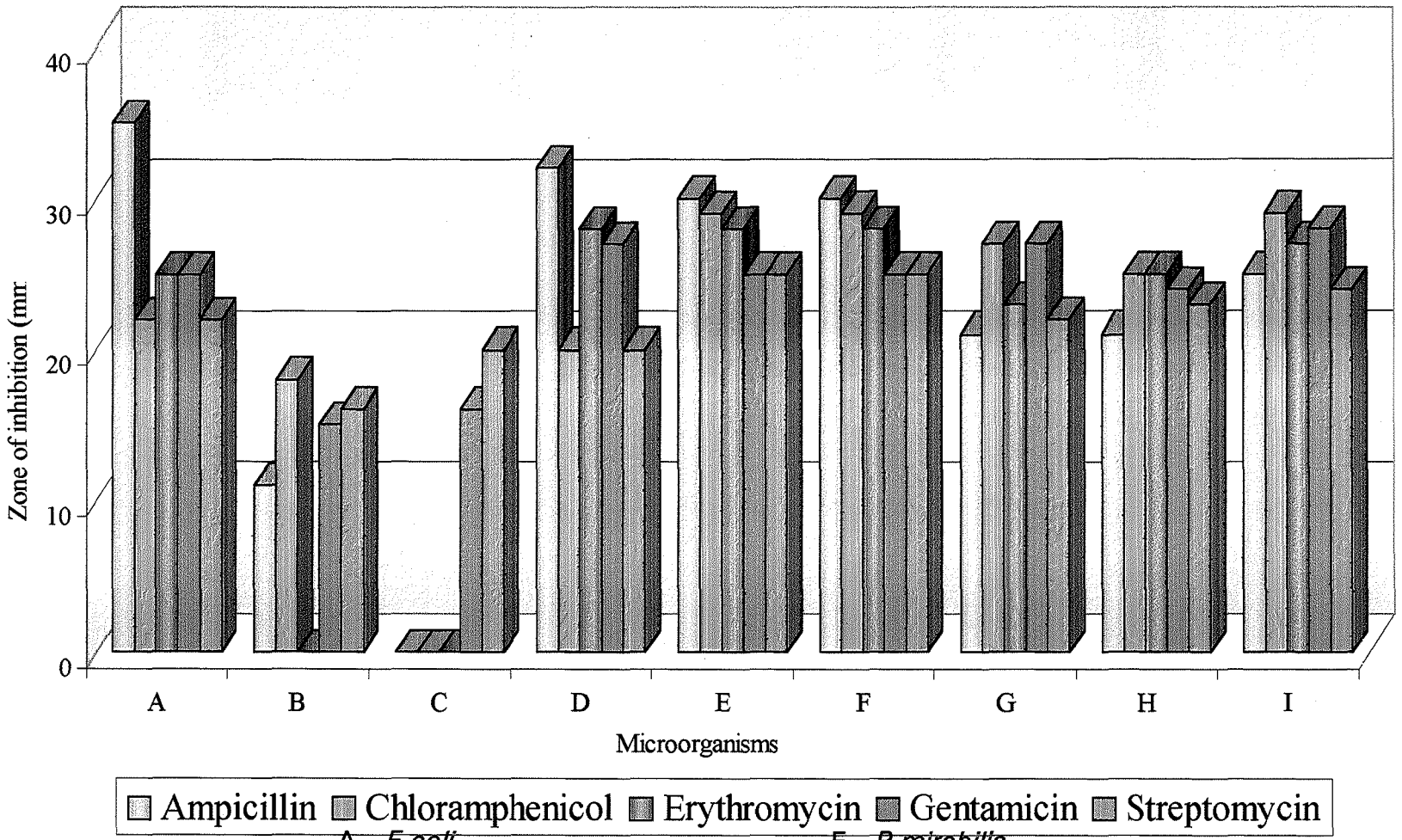
The values are mean of triplicates

NZ – No Zone; ND – Not Determined

E.coli was found to be the most sensitive organism to the antibiotic Ampicillin, followed by *K.pneumoniae*. The least sensitive organism for Ampicillin was *E.faecalis*. Chloramphenicol inhibits the growth of *P.aeruoginosae*, *P.mirabilis* and *S.typhi* at the highest level and *E.faecalis* was poorly inhibited. *K.pneumoniae*, *P.aeruoginosae*, *P.mirabilis* and *S.typhi* were highly sensitive to Erythromycin. Gentamicin showed its increased inhibitory effect on *S.typhi*, *K.pneumoniae*, *S.marescens*, *E.coli*, *P.aeruoginosae* and *P.mirabilis*. Growth of all the organisms used was inhibited by Streptomycin. The same inhibitory pattern was exhibited by *P.aeruoginosae* and *P.mirabilis* against the standard antibiotics used in our study.

The findings showed that *E.cloacaceae* used in the study was multidrug resistant showing resistance to many of the antibiotics. *E.faecalis* was

FIGURE 10
ANTIBIOTIC SENSITIVITY TEST (AST)



resistant to the action of Erythromycin and it was less sensitive to all the antibiotics used. All the antibiotics showed an increased or moderate activity on the other organisms. The results of the present study are in agreement with Das *et al.*, (2003), who have indicated the susceptibility of *S.typhi* and *E.coli* towards Ampicillin, Erythromycin and Chloramphenicol. Umadevi *et al.*, (2003) have showed the sensitivity of *S.aureus, K.pneumoniae, E.coli* and *P.aeruoginosae* to Erythromycin.

4.5.1.2 Antibacterial activity of the aqueous extracts

The aqueous extracts of the plant samples were tested for antibacterial activity in terms of growth inhibition zone and the values are given in Table XII.

TABLE XII

ANTIBACTERIAL ACTIVITY OF THE AQUEOUS EXTRACTS

S.No	Microorganisms	Zone of inhibition (mm)					
		Acalypha indica (L.)	*Eclipta prostrata* (L.)	*Mollugo latoides* (L.)	*Leucas aspera* (S.)	*Nelumbo nucifera* (G.)	Mixture
1	*E.coli*	9	NZ	10	NZ	NZ	NZ
2	*E.faecalis*	NZ	NZ	NZ	NZ	NZ	NZ
3	*E.cloacaceae*	NZ	NZ	NZ	NZ	NZ	NZ
4	*K.pneumoniae*	NZ	NZ	NZ	NZ	NZ	NZ
5	*P.aeruoginosae*	NZ	NZ	NZ	NZ	NZ	NZ
6	*P.mirabilis*	NZ	NZ	NZ	NZ	NZ	NZ
7	*S.marescens*	NZ	NZ	NZ	NZ	NZ	NZ
8	*S.aureus*	NZ	NZ	NZ	NZ	NZ	NZ
9	*S.typhi*	NZ	NZ	NZ	NZ	NZ	NZ
10	*S. flexineri*	NZ	NZ	NZ	NZ	NZ	NZ

The values are mean of triplicates. NZ – No Zone

Of all the microorganisms tested, only the growth of *E.coli* was inhibited by the aqueous extracts of *Acalypha indica* (9mm) and *Mollugo latoides* (10mm). Other organisms were resistant to all other aqueous extracts. The study revealed the fact that the inhibitory effect of *Acalypha indica* and *Mollugo latoides*

may be due to the presence of adequate amount of water extractable antimicrobial compounds in the extracts.

The aqueous extracts of plant samples were inert in inhibiting the growth of the microbes. No inhibition of growth by aqueous extracts might be due to absence or low concentration of water extractable antimicrobial compounds. Umadevi *et al.*, (2003) reported the inefficiency of aqueous extracts of *Andrographis echiodes* towards the tested organisms namely *S.aureus, B.subtilis, B.pumilus, M.luteus, K.pneumoniae, E.coli* and *P.aeruoginosae.*

4.5.1.3 Antibacterial activity of the alcoholic extracts

The zone of inhibition formed by the alcoholic extracts against the bacterial isolates is shown in Table XIII and Figure 11.

TABLE XIII

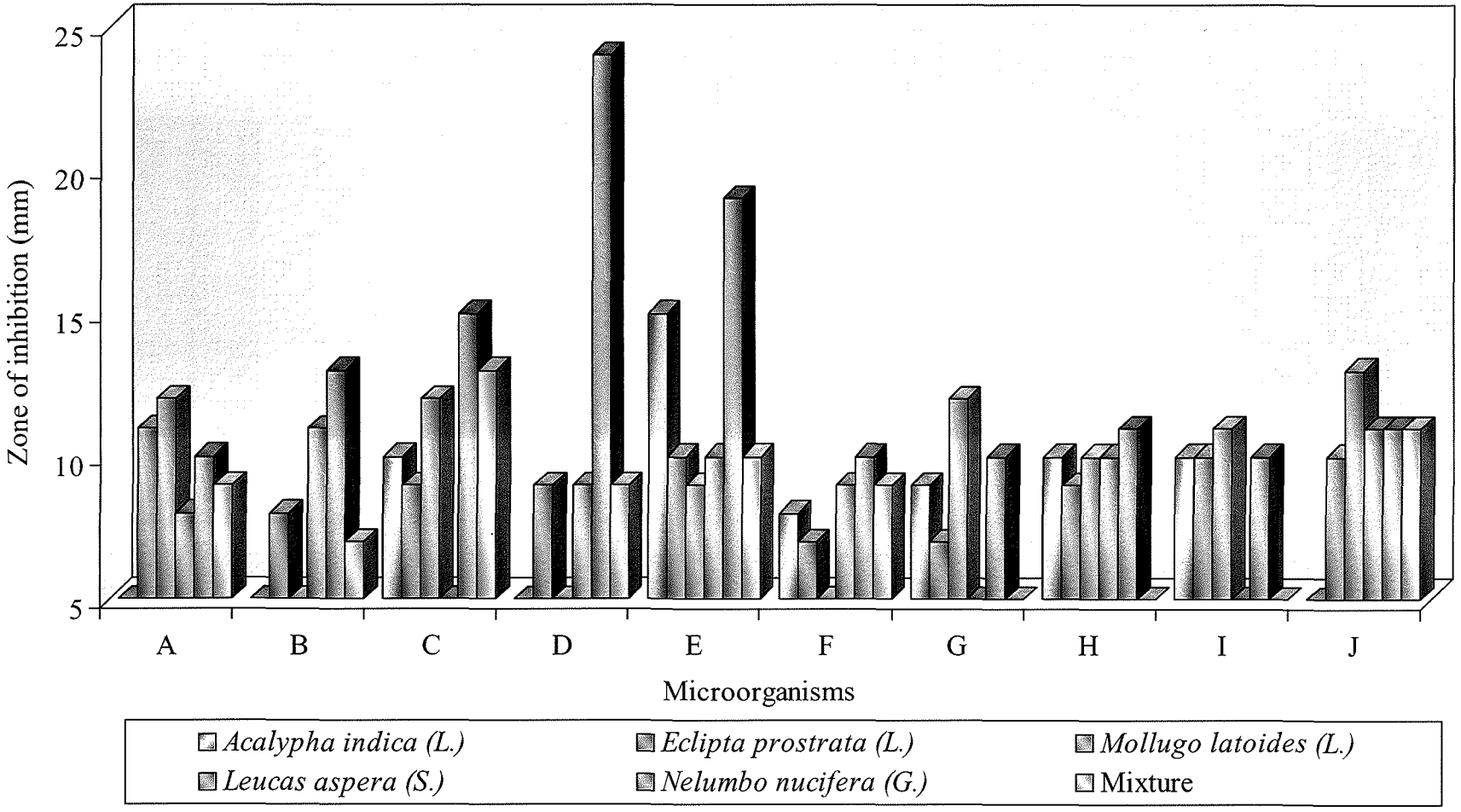
ANTIBACTERIAL ACTIVITY OF THE ALCOHOLIC EXTRACTS

S.No	Microorganisms	Zone of inhibition (mm)					
		Acalypha indica (L.)	*Eclipta prostrata* (L.)	*Mollugo latoides* (L.)	*Leucas aspera* (S.)	*Nelumbo nucifera* (G.)	Mixture
1	*E.coli*	NZ	11	12	8	10	9
2	*E.faecalis*	NZ	8	NZ	11	13	7
3	*E.cloacaceae*	10	9	12	NZ	15	13
4	*K.pneumoniae*	NZ	9	NZ	9	24	9
5	*P.aeruoginosae*	15	10	9	10	19	10
6	*P.mirabilis*	8	7	NZ	9	10	9
7	*S.marescens*	9	7	12	NZ	10	NZ
8	*S.aureus*	10	9	10	10	11	NZ
9	*S.typhi*	10	10	11	NZ	10	NZ
10	*S. flexineri*	NZ	10	13	11	11	11

The values are mean of triplicates; NZ – No Zone

From the figure 11, it is evident that all the alcoholic extracts showed inhibitory response to 60 - 70% of the microorganisms tested. The alcoholic extracts of *Eclipta prostrata* and *Nelumbo nucifera* showed inhibitory effect on all the

FIGURE 11
ANTIBACTERIAL ACTIVITY OF CRUDE ALCOHOLIC EXTRACTS



A - *E.coli*
B - *E.faecalis*
C - *E.cloacaceae*
D - *K.pneumoniae*
E - *P.aeruoginosae*
F - *P.mirabilis*
G - *S.marescens*
H - *S.aureus*
I - *S.typhi*
J - *S.flexineri*

sufficient antibacterial activity, Minimum Inhibitory Concentration (MIC) was determined using the bacterial isolates. Microbroth dilution assay was performed to measure quantitatively the *in vitro* activities of the extracts against the bacterial isolates. The concentration of the extracts used for the assay ranged from 0.390 to 200 mg/ml.

The concentration at which microbial growth does not occur on plates indicates the extract concentration adequate to inhibit the growth of microorganisms whereas that at which it does occur on plates indicates the extract concentration inadequate to inhibit the growth.

The Minimum Inhibitory Concentration (MIC) was determined for all the aqueous and alcoholic extracts against the bacterial isolates used for agar diffusion assay and the results are presented in Tables XIV and XV.

TABLE XIV

EFFECT OF THE AQUEOUS EXTRACTS ON BACTERIAL ISOLATES

S.No	Microorganisms	Minimum inhibitory concentration (mg / ml)					
		Acalypha indica (L.)	*Eclipta prostrata* (L)	*Mollugo latoides* (L.)	*Leucas aspera* (S.)	*Nelumbo nucifera* (G.)	Mixture
1	*E.coli*	12.5	>200	6.25	>200	>200	>200
2	*E.faecalis*	>200	>200	>200	>200	>200	>200
3	*E.cloacaceae*	>200	>200	>200	>200	>200	>200
4	*K.pneumoniae*	>200	>200	>200	>200	>200	>200
5	*P.aeruoginosae*	>200	>200	>200	>200	>200	>200
6	*P.mirabilis*	>200	>200	>200	>200	>200	>200
7	*S.marescens*	>200	>200	>200	>200	>200	>200
8	*S.aureus*	>200	>200	>200	>200	>200	>200
9	*S.typhi*	>200	>200	>200	>200	>200	>200
10	*S.flexineri*	>200	>200	>200	>200	>200	>200

From the Table XIV, it is evident that the aqueous extracts of *Acalypha indica* and *Mollugo latoides* were found to be effective in inhibiting the growth of

E.coli at a concentration of 12.5 mg/ml and 6.25mg/ml respectively. All other extracts are required in a high concentration (>200mg/ml) to inhibit the growth of other microbes indicating the resistance towards aqueous extracts. MIC of aqueous extract of *Cassia siamea* was 1.9mg/ml for *P.mirabilis* (Chandrasekaran and Venkatasalu, 2004).

TABLE XV

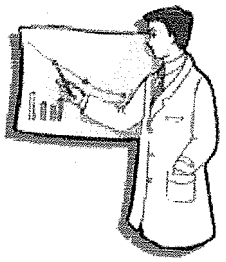
EFFECT OF THE ALCOHOLIC EXTRACTS ON BACTERIAL ISOLATES

S.No	Microorganism	Minimum inhibitory concentration (mg / ml)					
		Acalypha indica (L.)	*Eclipta prostrata* (L)	*Mollugo latoides* (L.)	*Leucas aspera* (S.)	*Nelumbo nucifera* (G.)	Mixture
1	*E.coli*	> 200	6.25	1.562	6.25	6.25	6.25
2	*E.faecalis*	>200	25	>200	1.562	3.125	6.25
3	*E.cloacaceae*	12.5	12.5	1.562	>200	3.125	0.781
4	*K.pneumoniae*	>200	12.5	>200	3.125	0.390	6.25
5	*P.aeruoginosae*	6.25	12.5	3.125	1.562	0.781	3.125
6	*P.mirabilis*	25	25	>200	3.125	6.25	6.25
7	*S.marescens*	25	25	1.562	>200	6.25	>200
8	*S.aureus*	12.5	6.25	3.125	3.125	6.25	>200
9	*S.typhi*	12.5	12.5	1.562	>200	6.25	>200
10	*S.flexineri*	>200	6.25	0.781	1.562	6.25	1.562

It is clear from Table XV, the MIC of alcoholic extract of *Nelumbo nucifera* was found to be 0.390mg/ml for *K.pneumoniae*. MIC of 0.781mg/ml was observed in the case of *Mollugo latoides* against *S. flexineri, Nelumbo nucifera* against *P.aeruoginosae* and the mixture against *E.cloacaceae*. The alcoholic extract of *Mollugo latoides* inhibited the growth of *E.coli* at a concentration of 1.562 mg/ml. A concentration of 6.25 mg/ml of other extracts retarded the growth of *E.coli* except *Acalypha indica* which required in a higher concentration

(>200mg/ml). The microbes P.*mirabilis and S.marescens* were sensitive to the alcoholic extracts at a concentration ranging from 3.125 to >200 mg/ml. The alcoholic extracts of *Nelumbo nucifera* and *Eclipta prostrata* exhibited good bacteriostatic effect against all the test organisms.

The alcoholic extracts of bark and leaf (100mg/ml and 300mg/ml) of *Xylocarpus grantum* were found to be having significant antimicrobial activity against *B.subtilis, B.pumilus*, *S.aureus*, *E.coli, C.albicans* and *S. cereviseae* (Rao *et al.*, 2003).



SUMMARY AND CONCLUSION

5.0 SUMMARY AND CONCLUSION

Plants as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times. In aerobic life, carbohydrates, lipids, nucleic acids and proteins can be oxidized by free radical mediated reactions. Antioxidants play a major role in combating the effects of free radicals. Nowadays use of natural antioxidants as food additives for inactivating free radicals receives a lot of attention, not only for their scavenging property, but also for their non-synthetic nature and their appreciation by consumers at large.

Increase of microbial resistance is a world health problem. Development of new antibacterial principles as substitutes for inefficient ones is a major weapon to combat the problem. Traditionally used medicinal plants produce a variety of compounds that can inhibit pathogens and have little toxicity to host cells. These substances are considered as candidates for developing new antimicrobial drugs.

The present study has been designed with an aim to analyze the antioxidant potential and antibacterial efficacy of five Indian medicinal plants individually and in combination. The results pertaining to the study are summarized below:

The whole plant of *Acalypha indica* (L.), *Eclipta prostrata* (L.), *Mollugo latoides* (L.), and *Leucas aspera* (S.) and flowers of *Nelumbo nucifera* (G.) were shade dried and powdered. The powder was extracted with respective buffers and analyzed for enzymatic antioxidants such as catalase, peroxidase, superoxide dismutase, polyphenol oxidase, glutathione-S-transferase, glutathione reductase and glutathione peroxidase and non-enzymatic antioxidants like ascorbic acid, α-tocopherol, reduced glutathione, total phenol, carotenoids and lycopene.

The powdered plant samples were separately extracted with water and alcohol The crude aqueous and alcoholic extracts were tested for phytochemicals and antimicrobial activity.

The aqueous and alcoholic extracts of the samples were analyzed for the presence of phytochemicals such as alkaloids, phenolics and flavonoids. A major portion of alkaloids was found to be present in the aqueous extract of *Leucas aspera* and alcoholic extract of *Nelumbo nucifera*. Aqueous extracts of *Acalypha indica, Leucas aspera* and alcoholic extracts of *Mollugo latoides, Nelumbo nucifera* and the mixture were found to be the rich source of phenolic compounds. Flavonoids were found to be present in high concentration in the alcoholic extracts of *Mollugo latoides* and *Nelumbo nucifera*. In the case of *Leucas aspera* and the mixture both aqueous and alcoholic extracts recorded the maximum flavonoid content.

Leucas aspera was found to be the richest source of enzymatic antioxidants such as catalase (1809.550 U/g) and peroxidase (0.910 U/g). The maximum activity of superoxide dismutase (119.090U/g) was given by *Mollugo latoides*. The highest activity of polyphenol oxidase was exhibited by three of the plant materials namely *Mollugo latoides*, *Leucas aspera* and the mixture. The enzymes that metabolize glutathione in the cell includes glutathione-S-transferase, glutathione peroxidase and glutathione reductase. The peak activity of glutathione-S-transferase (0.374U/g) and glutathione peroxidase (7.60U/g) was observed in *Nelumbo nucifera* and glutathione reductase (0.200U/g) in *Acalypha indica*.

The non-enzymatic antioxidants such as ascorbic acid (29.043mg/g), α-tocopherol (1.092mg/g), reduced glutathione (7.545mg/g) and total phenol (13.706mg/g) were found to be at maximum level in *Leucas aspera*. *Eclipta prostrata* possessed the highest content of carotenoid (0.139mg/g) and lycopene

(0.035mg/g). Mixture and *Acalypha indica* were found to be the rich source of protein (40.950mg/g) and carbohydrate (119.999mg/g) respectively.

Acalypha indica inhibited lipid peroxidation (62.5%) at the highest level. Both *Leucas aspera* and *Nelumbo nucifera*, the rich sources of enzymatic and non-enzymatic antioxidants prevented the *in vitro* generation of superoxide (73.333%) at maximal level. *Mollugo latoides* was found to be effective in inhibiting *in vitro* nitric oxide generation (74.533%).

The observations of the present study suggested that the plant sample are good sources of phytochemicals and antioxidants. The mixture exhibited a moderate activity suggesting the fact that antioxidants may act as pro-oxidants at higher concentration. This implies the need for identification and isolation of active principles in the mixture.

The antibacterial activity of the aqueous and alcoholic extracts was determined by two different methods. The initial screening was done by agar diffusion method. Microbroth dilution assay was carried out to measure the antibacterial activity quantitatively. The microorganisms *Escherichia coli*, *Enterococcus faecalis*, *Enterococcus cloacaceae*, *Klebsiella pneumoniae*, *Pseudomonas aeruoginosae*, *Proteus mirabilis*, *Serratia marescens*, *Staphylococcus aureus*, *Salmonella typhi* and *Shigella flexineri* were used to detect the antibacterial activity of the extracts. The control studies were performed with DMSO concurrently and antibiotic sensitivity test was done with standard antibiotics.

Antibiotic Sensitivity Test (AST) using different antibiotics was carried out and the findings showed that the highest inhibition zone was formed by Ampicillin (35mm) against *E.coli*. *E.cloacaceae* was found to be a multi-drug resistant strain.

The aqueous extracts of *Acalypha indica* (9mm) and *Mollugo latoides* (10mm) were effective in inhibiting the growth of only *E.coli* and inert to other microbes. No inhibition in the growth by aqueous extracts might be due to the absence or low concentration of water extractable antimicrobial compounds in the extracts. All the alcoholic extracts showed inhibitory response to 60-70% of the microorganisms tested. The alcoholic extracts of *Eclipta prostrata* and *Nelumbo nucifera* showed inhibitory effect on all the organisms producing maximum zone of inhibiton against *E.coli* (11mm) and *K.pneumoniae* (24mm) respectively. The findings of the present study showed that the most of the microbes were found to be sensitive to the alcoholic extracts of the samples whereas the aqueous extracts were inactive.

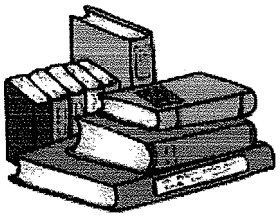
The Minimum Inhibitory Concentration (MIC) was determined to confirm the activity of the extracts against the clinical isolates. The MIC value ranged from 0.390-200mg/ml. The lowest MIC of 6.25 mg/ml was observed in the aqueous extract of *Mollugo latoides* against *E.coli. Acalypha indica* inhibited the growth of *E.coli* at a concentration of 12.5 mg/ml. Other samples did not have any effect on retarding the growth of bacterial isolates used. MIC of 0.390 mg/ml was shown by the alcoholic extract of *Nelumbo nucifera* against *K.pneumoniae. A*mong the various alcoholic extracts, *Eclipta prostrata* and *Nelumbo nucifera* exhibited a good bacteriostatic effect.

The results of the present study revealed that the plant samples used are rich sources of antioxidants and phytochemicals, which offer a promising avenue for the development of phytomedicine and therapeutic compounds against many diseases. Susceptibility of various microbes to the alcoholic extracts of the plant sample in our study suggest and immense scope for developing antimicrobial natural herbal agents. On the basis of the present investigation, it can be highlighted that

the promising antibacterial properties of several of the plant extracts could be exploited in herbal preparations against bacterial infections justifying their use in traditional medicine.

FUTURE PROSPECTS

- Active principles in the plants can be isolated, identified, characterized and screened for their safety and efficacy.
- Plant samples can be extracted with other organic solvents and tested against other infectious agents.
- Effect of the plant samples against various diseases can be studied *in vivo.*



BIBLIOGRAPHY

BIBLIOGRAPHY

Abel, C. and Busia. K. (2005), An exploratory ehtanobotanical study of the practice of herbal medicine by the Akan peoples of Ghana, Altern Med. Rev., 10, 112-122.

Acharya, K., Samui, K., Rai, M., Dutta, B.B. and Acharya, R. (2004), Antioxidant and nitric oxide synthase activation properties of *Auticularia auricula*, Ind. J. Exp. Biol., 42, 538-540.

Afaq, S.H., Shehbaz, A, Masood, A.K., Owasis, M. and Nadeem, A. (2004), Antibiotic screening of certain unani medicinal plants, Indian Drugs, 41, 236-239.

Agarwal, A., Choudhary, D., Upreti, M., Rath, P.C. and Kale, R.K. (2001), Radiation induced oxidative stress: L-studies in Ehrlich solid tumor in mice, Molecular and Cellular Biochemistry, 223, 71 –80.

Ahmad[a], I. (2001), Catalase, Spring, 77: 222, 1-10.

Ahmad[b], I. (2001), Hydrogen peroxide (H_2O_2), Spring, 77: 222, 1-10.

Akgul, C. and Kaya, I. (2004), Potent antibacterial activity of oligo-3-aminopyridine against *Staphylococcus aureus* and *Enterococcus faecalis*, Biochem. and Biophys., 41, 120-122.

Akinyemi, K.O., Oladapo, O., Okwara, C.E., Ibe, C.C. and Fasure, K.A. (2005), Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant *Staphylococcus aureus* acitivity, BMC Complementary and Alternative Medicine, 5:6, 1-7.

Andrasevic, A.T. (2004), Antibiotic resistance bacteria fight back, Acta Med. Creation, 58, 245-250.

Anilakumar, K.R., Khanum, F., Sudarshanakrishna, K.R. and Santhanam, K. (2004) Effect of amaranth leaves on dimethylhydrazine-induced changes in multicomponent antioxidant system of rat liver, Ind. J. Exp. Biol., 42, 595-600.

Antosiewicz, J., Popinigis, J., Wozniak, M., Damiani, E., Carloni, P. and Greci, L. (1995), Effects of indolinic and quinolinic aminoxyls on protein and lipid peroxidation of rat liver microsomes, Free Radicals Biol.Med., 18, 931-917.

Aqil, F. and Ahmad, I. (2003), Broad-spectrum antibacterial and antifungal properties of certain traditionally used Indian medicinal plants, World J. Microbiol., 19, 653-657.

Ashok, G., Raghu, C., Dhanaraj, S.A., Suresh, B. and Vijayan, P. (2004), Free radical scavenging activity of selected essential oils, Indian Drugs, 41, 244-245.

Athar, M. (2002), Oxidative stress and experimental carcinogenesis, Ind. J. Exp. Biol., 40, 656-667.

Bafna, P.A. and Balaraman, R. (2004), Anti-ulcer and antioxidant activity of Normacid , a herbomineral formulation, Ind. J. Exp. Biol., 42, 674-680.

Baraczyk-kuzma, A., Kuzma, M., Gutowicz, K. and Sawicki, J. (2004), , Glutathione-S-transferase pi as a target for tricyclic antidepressants in human brain, Acta Biochimica Polonica, 51, 207-212.

Behrend, L., Henderson, G. and Zwacka, R.M. (2003), Reactive oxygen species in oncognic transformation, Biochemical Society, 31, 1441-1444.

Beyer, R.E. (1994), The role of ascorbate in antioxidant protection of biomembranes: interaction with Vitamin E and Coenzyme Q, J. Bioenerg. Biomembr., 26, 349-358.

Bhatia, S., Kapoor, H.C. and Lodha, M.L. (2004), Modification of Antioxidant status of host cell in response to *Bougainvillea* Antiviral proteins, J. Plant Biochem. and Biotech., 13, 113-118.

Botha, J., Witkowski, E.T.F. and Shackleton, M. (2004), Market profiles and trade in medicinal plants in the low level, South Africa, Environmental Conservation, 31, 38-46.

Boynes, J.W., (1991), Role of oxidative stress in development of complications in diabetes, Diabetes, 40, 405-411.

Bukan , N., Sancak , B., Yavuz. O., Koca , C., Tutkun, F ., Ozcelikay , A.T. and Altan, N. (2003), Lipid peroxidation and scavenging enzyme levels in the liver of Streptozotocin-induced diabetic rats, Ind. J. Biochem. Biophys., 40, 447-450.

Carr, A.C. and Frei, B. (1999), Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans, Am. J. Clin. Nutri., 69, 1086-1107.

Castello, M., Phatak, A., Chandra, N. and Sharon, M. (2002), Antimicrobial activity of crude extracts from plant parts and corresponding calli of *Bixa rellana*, Ind. J. Exp. Biol., 40, 1378-1381.

Cathcart, M.K. (2004), Regulation of superoxide anion production by NADPH oxidase in Monocytes/Macrophages, Atherosclerosis, Thrombosis and Vascular Biology, 24 : 23.

Cathcart, R.F. (1985), Vitamin C : the nontoxic nonrate-limited, antioxidant, free radical scavenger, Med Hypotheses, 18, 61-77.

Chandrasekeran, M. and Venkatasalu, V. (2004), Antibacterial activity of bark of *Cassia Siamea*, Indian Drugs, 41,298-299.

Chatterjee, I., Chakravarthy, A.K. and Gomes, A. (2004), Ind. J. Exp. Biol., 42, 468-475.

Chatterjee, I.B. and Nandhi, A. (1991), Ascorbic acid: A scavenger of oxyradicals, Ind. J. Biochem. and Biophys., 28, 233-239.

Cheng, G.W. and Crisosto, H.C. (1995), Browning potential, Phenolic composition and Polyphenol oxidase Activity of Buffer Extracts of Peach and Nectarine Skin Tissue, J. Amer. Hort. Sci., 120, 835-838.

Christen, Y. (2000), Oxidative stress and Alzheimer's disease, Am. J. Clin. Nutr., 71 (Suppl.) 621S - 629S.

Clement, Y.N., Williams, A.F., Aranda, D., Chase, R., Watson, N., Mohammed, R., Stubbs, O. and Williamson, D. (2005), Medicinal herb use among asthamatic patients attending a specialty care facility on Trinidad, BMC Complementary and Alterntive Medicine, 5:3,1-8.

Cowan, M.M. (1999), Plant Products as antimicrobial agents, Clin. Microbiol. Rev., 12, 564-582.

Czuczejko, J., Zachara, A.B., Staubach-Topczewska, E., Halota, W. and Kedziora, J. (2003), Selenium, glutathione and glutathione perixidase in blood of patients with chronic liver diseases , 50, 1147-1154.

Das, S., Prakash, R. Devaraj, S.N. (2003), Antidiarrhoeal effects of methanolic root xtract of *Hemidesmus indicus* (Indian Sarasaparilla) – An *in vitro* study, Ind. J.Exp.Biol., 41, 363-366.

Datta, S. (2004), Yesterday's wisdom, Today's Science, Science Reporter, 37.

David, M. and Richard, J.S. (1983), Methods of enzymatic analysis, 3, Bergmeyyer, J. and Marriane, G.B.(ed), Verlag Chemic Weinheina Dec, Field Beach Floride Based, 358.

Deepa, M.A. and Bai, V.N. (2004), Antibacterial activity of *Salacia beddomei*, Fitoterapia, 75, 589-591.

Derda, M., Wandurska-Nowak, E. and Hada, E. (2004), Changes in the level of antioxidants in the blood from mice infected with *Trichinella spiralis*, Springer-Verlag, 10, 1093-1099.

Deshphande, U.R., Joseph, L., Patwardhan, U.N. and Samuel, A.M. (2003), Effect of antioxidants (Vitamin C, E. and turmeric extract) on Methimazole induced hypothyroidism in rats, Ind. J. Exp. Biol., 40, 735-738.

Devasagayam, T.P.A. and Kamat, J.P. (2002), Biological significance of singlet oxygen, Ind. J. Exp. Biol., 40, 680-692.

Dixon, D.P., Cuimmins, I., Cole, D.J. and Edwards, R. (1998), Glutathione-medicated detoxification system in plants, Curr. Opin. Plant Biol., 1, 256.

Edreva, A. (2005), Generation and scavenging of reactive oxygen species in chloroplast : a submolecular approach, Agriculture, Ecosystem and Environment, 106, 199-213.

Eftekhar, F., Yousefzadi, M. and Borhani, K. (2004), Antibacterial activity of essential oil from *Ferula gummosa* seed, Fitoterapia, 75, 758-759.

Elizabeth, K.M. (2003), Antimicrobial activity of *Caesapina digyna* on some human pathogenic microorganisms, Ind. J. Microbiol., 43, 73-74.

Esterbauer, H., Schwarlz, E. and Hayan, M. (1997), A rapid assay for Catechol Oxidase and laccase using 2-nitro-5-thio benzoic acid, Anal. Biochem, 77, 489-494.

Everekilioglu, C., Er, H., Doganay, S., Cekmen, M., Turkoz, Y., Otlu, B. and Ozerol, E. (2003), Nitric oxide and lipid peroxidation are increased and associated with decreased antioxidant enzyme activities in patients with age-related macular degeneration, Documenta Opthalmologica, 106, 129-136.

Fryer, M.J. (1992), The antioxidant effects of thylakoid Vitamin E (α-tocopherol), Plant Cell Environ., 15, 381-387.

Furtado, J., Siles, X. and Campos, H. (2004), Carotenoid concentrations in vegetables and fruits common to the Costa Rican diet, Int. J. Food Sci. and Nutr., 55, 101-113.

Gamel-Eldeen, A.M., Kawashty, S.A., Ibrahim, L.F., Shabana, M.M. and El-Negoumy, S.I. (2004), Evaluation of antioxidant, anti-inflamatory and antinociceptive properties of aerial parts of *Vicia sativa* and its flavonoids, J. Natural Remedies, 4/1, 81-96.

Geetha, S., Ram, M.S., Mongia, S,S., Singh, V., Ilavazhagan, G., and Sawhney, R.C. (2003), Evaluation of antioxidant activity of leaf extract of seabuckthorn (*Hippophae rhamnoides* L.) on chromium (VI) induced oxidative stress in albino rats, J. Ethanopharmacol., 87, 247-257.

Gholizadeh, A., Kumar, M., Balasubramaniyam, A., Sharma, S., Narwal, S., Lodha, M.L. and Kapoor, H.C. (2004), Antioxidant activity of antiviral proteins from *Celosia cristata* (L.), J. Plant Biochem. Biotech., 13, 13-18.

Giamperi, L., Fratermale, D., Bucchini, A. and Ricci, D. (2004), Antioxidant activity of *Citrus paradesi* seeds glyceric extracts, Fitoterapia, 221-224.

Gitanjali, P., Devi, Y., and Prakash, M.S. (2004), Effect of cooking on total phenolic content and antioxidant activity in selected cereals, millets and pulses, The Ind. J. Nutr. Dietet., 41, 337-345.

Gogtay, N.J., Bhatt, H.A., Dalvi, S.S. and Kshirsagar, N.A. (2002), The use and safety of non –allopathic Indian medicines, Drug Saf., 25, 1005-1019.

Gopinathan, N., Srinivasan, K.K. and Mathew, J.W. (2004), Free radical scavenging properties of the ethanol extract of *Saccharum spontaneum*, Indian Drugs, 41, 633-635.

Goudgaon, N.M., Basavaraj, N.R. and Vijaylakshmi, A. (2003), Antiinflammatory activity of different fractions of *Leucas aspera*, Spreng, Ind. J. Pharmacol., 35, 397-398.

Govindarajan, R., Vijayakumar, M., Rawat, A.K.S. and Mehrotra, S. (2003), Free radical scavenging potential of *Piocrorhiza kurrooa* Royle ex Benth., Ind. J. Exp. Biol., 41, 875-879.

Green, L.C., Wagner, D.A., Glogowski. J., Skipper, P.L., Wishnok, J.S. and Tannenbaum, S.R. (1982), Analysis of nitrate, nitrite and (^{15}N) nitrate in biological fluids, Anal. Biochem., 126, 131-138.

Gumieniczek, A., Hopkla, H., Wojtowicz, Z. and Nikolajuk, J. (2002), Changes in antioxidant status of heart muscle tissue in experimental carcinogenesis, Acta Biochimica Polonica, 49, 529-535.

Gupta, S., Reddy, M.V.R. and Harinath, B.C. (2004), Role of oxidative stress and antioxidants in aetiopathogenesis and management of oral submucous fibrosis Ind. J. Clinc. Biochem., 19, 138-141.

Habig, W.H., Pabst, M.J. and Jacoby, W.B. (1974), Glutathione-S- Transferases: The first enzymatic step in mercapturic acid formation, J. Biol. Chem., 249, 7310-7339.

Halliwell.B. and Gutteridge, T.M.C. (1999), Free radicals in Biology and Medicine, 3rd ed-New York, Oxford University Press, 134-140.

Hartwig, B., Sten, P. and Feierabend, J. (1992), Light dependence of catalase synthesis and degradation in leaves and the influence of interfering stress conditions, Plant Physiol., 100, 1547.

Hayakawa, F., Ishizu, Y., Hoshino, N., Yamaji, A., Ando, R. and Kimura, T. (2004), Peroxidative activities of tea catechins in the presence of Cu^{2+}, Biosci. Biotechnol. Biochem., 68, 1825-1830.

Hedge, J.E. and Hofreiter, B.T. (1962), Carbohydrate Chemistry, 17th Edition, (Wistler, R.L. and B. Miller., J.N (ed)), Academic Press, NewYork.

Hore, A. (2004), The cost of free radicals, Science and Culture, 70, 249-254.

http://www.gnc.com/category.asp?id=52

http://www.dojindo.com/newsletters/review_vol2.html.

http://www.chem.admu.edu.ph/~nina/rosby/main.htm.

http://www.acris-online.de/sheets/lf-p00 24.pdf.

http://en.wikipedia.org/wiki/Peroxidase.

http://genox.com.why.htm

http://holisticonline.com/cancer/cancer_lycopene.html.

http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/R/ROS.html

http://www. rice.edu/~jenkly/sports/antiox.html.

http://www.genox.com.what.htm.

http://www.infoaging.org/b_oxdam_9_age.html

http://www.sigmaaldrich.com/Area_of _Interest/Life_Science/Cell_Signalling/ Scientific_Resources/Pathway_Slides_Charts/Oxidative_Stress.html.

http://www.worthington.biochem.com/TY/default.html

http://www.friedii.com/herbs/intro.html.

Huang, J. and May, J.M. (2003), Ascorbic acid spares α- tocopherol and prevents lipid peroxidation in cultured H4IIE liver cells, Molecular and Cellular Biochemistry, 247, 171-176.

Huang, T.H., Kota, B.P., Roznvski, V. and Roufogalis, B.D. (2005), Herbal or natural medicine as modulators of peroxisome proliferator activated receptors and related nuclear receptors for therapy of metabolic syndrome, Basic Clin. Pharmacol. Toxicol., 96, 3-14.

Hullattai, K.K. and Rai, V.R. (2004), Antimicrobial activity of *Memecylon malabaricum* leaves, Fitoterapia, 75, 409-411.

Huntley, A. (2004), Drug-herb interactions with herbal medicines for menopause, J. Br Menopause Soc., 10, 162-165.

Illavarasan, R., Vasudevan, M., Anbazhagan, S. and Venkataraman, S. (2003), Antioxidant activity of *Thespesia populnea* bark extracts against carbon tetrachloride-induced liver injury in rats, J. Enthanopharmacol., 87, 227-230.

Indrayan, A.K., Yadav, V., Tyagi, P.K. and Kumar, M. (2004), Antibacterial activity of the dye from rhizome of *Arnebia nobilis* (Ratanjot), Ind. J. Microbiol., 44, 69-71.

Irshad, M. and Chaudhuri, P.S. (2002), Oxidant-antioxidant system: Role and significance in human body, Ind. J. Exp. Biol., 40, 1233-1239.

Jacqueline, M.S., Jongsson, L. and Paul, F.T. (1997), Tumor necrosis factor-α-induced insulin resistance in 3T3-L1 adipocytes is accompanied by a loss of insulin receptor substrate-1 and GLUT4 expression without a loss of insulin receptor–mediated signal transduction, J. Biol. Chem., 272, 971-976.

Jain, K., Kataria, S. and Guruprasad, K.N. (2004). Oxyradicals under UV-B stress and their quenching by antioxidants, Ind. J. Exp. Biol., 42, 884-892.

Jainu, M. and Devi, C.S.S. (2004), Antioxidant effect of methanolic extract of *Solanium nigrum* berries on aspirin induced gastric mucosal injury, Ind. J. Clin. Biochem., 19, 57-61.

Jayashree, G., Muraleedhare, G.H., Sudarslal, S. and Jacob, V.B. (2003), Anti-oxidant activity of *Centella asiatica* on lymphoma-bearing mice, Fitoterapia, 74, 431-434.

Jenner, P. (1994), Oxidative damage in neurodegenerative disease., Lancet, 32, 796-798.

Kala, C.P., Farooque, N.A. and Dhar, U. (2004), Priortization of medicinal plants on the basis of available knowledge, existing practices and use value status in Uttaranchal, India, Biodiversity and Consercation, 13, 453-469.

Kamboj, V.P. (2000), Herbal medicine, Curr. Sci., 78, 35

Karanath, J., Kumar, R. and Jeevaratnam, K. (2004), Response of antioxidant system in rats to dietary fat and physical activity, Ind. J. Physiol. Pharmacol., 48, 446-452.

Karthikeyan, J. and Rani, P. (2003), Enzymatic and non-enzymatic antioxidants in selected *Piper* species, Ind. J. Exp. Biol., 41, 135-140.

Khandelwal, K.R. (2002), Practical Pharmocognosy-Techniques and experiments, IX edition, Nirali Prakashan Publishers, 183-184.

Khopde, S.M., Priyadarsini, K.I., Mohan, H., Gawnadi, V.B., Satav, J.G., Yakhmi, J.V., Banavaliker, M.M., Biyani, M.K. and Mittal, J.P. (2001), Characterizing the antioxidant, Curr. Sci., 81, 185-190.

Klapcinska, B., Derejczyk. J., Wieczorowska-Tobis, K., Sobczak, A., Sadowska-Krepa, E. and Danch, A. (2000), Antioxidant defence in centenarians (a preliminary study), Acta Biochimica Polonica, 47, 281-292.

Klewala, N.S. and Ananthanarayan, L. (2004), Antioxidant activity of selected food stuffs, Int. J. Food Sci. Nutr., 55, 511-516.

Klotz, L.O., Holbrook, N.J. and Sies, H. (2001), UVA and singlet oxygen inducers of cutaneous signalling events, Oxidants and Antioxidants in Cutaneous Biology Thiele, J. and Elsner, P. (ed)), 95.

Kobiela, J., Krajewski, J., Kalinska-Blach, B. and Stefaniak, T. (2002), Selectivity of oxidative stress targeting in estrogen-induced experimental nephrocarcinogenesis, Acta Biochimica Polonica, 49, 51-58.

Konovalova, G.G., Lisina, M.O., Tikhaze, A.K. and Lankin , V.Z. (2003), A complex of antioxidant vitamins effectively inhibits free-radical oxidation of LDL phosphlipids in blood plasma and membrane structures of the liver and myocardium, Bull. Exp. Biol. and Medicine, 143-146.

Koshy, L., Dwarakanath, B.S., Raj, H.G., Chandra, R. and Mathew, T.L. (2003), Suicidal oxidative stress induced by certain antioxidants, Ind. J. Exp. Biol., 41, 1273-1278.

Krawiec, Z., Bilinski, T., Schuller, C. and Ruis, H. (2000), Reactive oxygen species as second messengers? Induction of the expression of yeast catalase T gene by heat and hyperosmotic stress does not require oxygen, Acta Biochimica Polonica,47, 201-207.

Kujumdzieva, A., Rasheva, T.V. and Petrova, V. (2002), Catalase enzyme in mitochondria of *Saccharomyces cerevisiae*, EJP Electronic J. Biotech., 5, 1-3.

Lakshmi, B., Tilak, J.C., Adhikari, S., Devasagayam, T.P.A. and Janardhanan, K.K. (2005), Inhibition of lipid peroxidation induced by γ-radiation and AADH in rat liver and brain mitochondria by mushrooms, Curr. Sci., 88, 484-488.

Latha, M. and Pari, L. (2003), Preventive effects of *Cassia auriculata* L. flowers on brain lipid peroxidation in rats treated with Streptozotocin, Molecular and Cellular Biochemistry, 243, 23-28.

Lawler J.M., Camela, C.C., Zheutu, S. and Coastr, R., (1997),Am. Physiol. Soc., 201-207.

Lee, S.B., Nam, K., Lee, S.J., Lee, J.H., Inoye, K. and Park, K.H. (2004), Antioxidative effects of Glycosyl-ascorbic acid syunthesized by maltogenic amylase to reduce lipid oxidation and volatiles producing in cooked chicken meat, Biosci. Biotechnol. Biochem., 68, 1601-1604.

Liebert, M., Licht, U., Bohm, V. and Bitsch, R. (1999), Antioxidant properties and total phenolics content of green and black tea under different brewing conditions, Z Lebensm Unters Forsch A, 208, 217-220.

Lima, D.C., Abreu, P.A., Freitas, C.C., Santos, D.O., Borges, R.O., Santos,T.C., Cabral, L.M., Rodriguess, C.R. and Castro, H.C. (2005), Snake venom: Any Clue for Antibitics and CAM?, eCAM, 2, 39-47.

Ljubuncic, P., Azaizeh, H., Portnaya, I., Cogan, V., Said ,O., Saleh, K.A., and Bomzon, A. (2005), Antioxidant activity and cytotoxicity of eight plants used in traditional Arab medicine in Israel, J. Ethnopharmacol., 99, 43-47.

Lohr, J.B. and Browning, J.A. (1995), Free radical involvement in neuro-psychiatric illnesses, Psychopharmacol. Bull., 31, 159-165.

Loki, A.L. and Rajamohan, T. (2003), Hepatoprotective and antioxidant effect of tender coconut water on Carbon tetrachloride induced liver injury in rats, Ind. J. Biochem. Biophys., 40, 354-357.

Lovkova, M.Ya., Buzuk, G.N., Sokolova, S.M. and Kliment'eva, N.I. (2001), Chemical features of medicinal plants (Review), Applied Biochemistry and Microbiology, 37, 229-237.

Lowry, O.H., Rosebrough, N.J. Farr., A.L. and Randall, R.J. (1951), J. Biol. Chem., 193, 265.

Luck, H. (1974), Methods of enzymatic analyis, Academic Press, 885-894.

Malarvannan, L. and Devaki, T. (2003), Protective effect of *Indigofera tinctoria* on tissue antioxidant defence system against D-galactosamine and endotoxin-induced hepatopathy in rats, J. Natural Remedies, 3/1, 49-53.

Malick, C.P. and Singh, M.B. (1980), Plant enzymology and Histoenzymology, Kalyani Publishers, 286.

Manoharan, S., Kolanjiappan, K. and Kayalvizhi, M. (2004), Enhanced lipid peroxidation and impaired enzymic antioxidant activities in the erythrocytes of patients with cervical carcinoma, Cellular and Molecular Biology letters, 9, 699-707.

Mares, J.A. (2004), High-dose antioxidant supplementation and cataract risk, Nutrition Reviews, 62, 28-32.

Martin, K.W. and Ernst, E. (2003), Herbal medicines for treatment of bacterial infections: a review of controlled clinical trails, J. Antiimicrob. Chemother., 31, 241-246.

Mary, N.K., Acuthan, C.R., Babu, B.H. and Padikkala, J. (2003), *In vitro* antioxidant and antithrombotic activity of *Hemidesmus indicus* (L.) R. Br., J. Ethanopharmacol., 87, 187-191.

Maurya, R., Srivastava, S., Kulshreshta, D.K. and Gupta, C.M. (2004), Traditional Remedies for Fertility Regulation, Current Medicinal Chemistry, 11, 1434-1450.

May, M.J., Vernoux, T., Leaver, C., Van-Motagu, M. and Inze, D. (1998), Glutathione homeostasis in plants: implications for enviromental sensing and plant development, J. Exp. Bot., 49, 649-667.

Milliauskas, G., Venskutonins, P.R. and Beek, T.A., (2004), Screening of radical scavenging activity of some medicinal and aromatic plant extracts, Food Chemistry, 85, 231-237.

Misra, M.P. and Fridovich, I. (1972), The role of superoxide anion in the autoxidation of epinephrine and simple assay for superoxide dismutase, J. Biol. Chem., 247, 3170.

Moller, P. and Loft, S. (2002), Oxidative DNA damage in human white blood cells in dietary antioxidant intervention studies, Am. J. Clin. Nutr., 76, 303-310

Moron, M.S., Bepierre, J.W. and Mannerwick, B. (1979), Levels of glutathione reductase and glutathione-S-transferase in rat lung and liver, Biochem. Biophys. Acta, 582.

Noctor, G. and Foyer, C.H. (1998), Ascorbate and glutathione: Keeping active oxygen under control, Ann. Rev . Plant Physiol., Plant Mol. Biol., 49, 249-279.

Nohl, H., Gille, L. and Staniek, K. (2004), The mystery of reactive oxygen species derived form cell respiration, Acta Biochimca Polonica, 51, 223-229.

Okhawa, H., Oshisi, N. and Yagi, K. (1979), Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction, Anal Biochem., 95, 351-358.

Padmaja, S. and Raju, T.N. (2004), Antioxidant effect of curcumin in selenium induced cataract of Wistar rats, Ind.J.Exp.Biol., 42, 601-603.

Padmavathi, B., Rath, P.C., Rao, A.R. and Singh, R.P. (2005), Roots of *Withania somnifera* inhibit forestomach and skin carcinogenesis in mice, eCAM, 2, 99-105.

Panwala, P.T., Naik, S.R. and D'Mello, P.M. (2004), Antihyuperlipidemic and antioxidant activity of *Syzygium cumini*, Indian Drugs, 41, 345-349.

Patwardhan, B., Vaidya, A.D.B. and Chorghade, M. (2004), Ayurveda and natural products drug discovery, Curr. Sci., 86, 789-799.

Percy, C., Pat, B., Poronik, P. and Gobe, G. (2005), Role of oxidative stress in age associated chronic kidney pathologies, Adv Chronic Kidney Dis., 12, 78-83.

Philips, I., Casewell, M., Cox, T., Groot, B.D., Friis, C., Jones, R, Nightingale, C., Preston, R. and Waddell, J. (2004), Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data, J.Antimicrob. Chemother., 53, 25-52.

Picaud.T. and Desbois, A. (2002), Glutathione Reducatse (Human), J. Biol. Chem., 277, 31715-31721.

Pithayankal, P., Lavachirasuwan, S., Bavovado, R., Pakmance, N. and Suttisri, R. (2004), Anti-venom potential of butanloic extract of *Eclipta prostrata* against Malyan pitviper venom, J. Ethanopharmacol., 90, 347-352.

Poongothai, A.R., Vishnupriya, S. and Ragunathan, D. (2004), Quantitative variation of superoxide dismutase levels in leukaemias, Ind.J.Human Genetics, 10, 9-11.

Pradhan, A.K., Shukla, A.K., Reddy, M.V.R. and Garg, N. (2004), Assessment of oxidative stress and antioxidant status in age related cataract in rural population, Ind. J. Clin. Biochem., 19, 83-87.

Radha, R., Latha, T. and Rajendran, N.N. (2003), Antimicrobial activity of crude extracts of *Heliotropium marifolium* Ratz., J. Natural Remedies, 3, 208-211.

Rajeswari, M. and Subbulakshimi, G. (2004), What are phytochemicals, Food & Nutrition World, 13-18.

Rana, M.K. (2004), Fortifying foods, Science Reporter, 47-49.

Rana, P., Vadhera, S. and Soni, G. (2004), *In vivo* antioxidant potential of Rice Bran Oil (RBO) in albino rats, Ind. J. Physiol. Pharmacol., 48, 428-436.

Rani, P., Unni, K.M. and Karthikeyan, J. (2004), Evaluation of antioxidant properties of berries, Ind. J. Clin. Biochem., 19, 103-110.

Rao, P.R., Trilochana, Y. and Chaitanya, K.K. (2003), Antidiarrheal and antimicirobial activities of bark and leaf extracts of *Xylocarpus granatum* Koenig, J. Natural Remedies, 3/2, 155-160.

Rao[a], K.V.K., Schwartz, S.A., Nair, H.K., Aalinkeel, R., Mahajan, S., Chawda, R. and Nair., M.P.N. (2004), Plant derived products as a source of cellular growth inhibitory phytochemicals on PC-3M, DU-145 and LNCaP prostate cancer cell lines, Curr. Sci., 87, 1585-1588.

Rao[b], M.R., Palada, M.C. and Becker, B.N. (2004), Medicinal and aromatic plants in Agroforestery systems, 61, 107-122.

Ray, G. and Husain, S.A. (2002), Oxidants, antioxidants and carcinogenesis, Ind. J. Exp. Biol., 40, 1213-1232.

Reddy, J.S., Rao, P.R. and Reddy, M,S. (2002), Wound healing effects of *Heliotropium indicum, Plumbago zaylanicum* and *Acalypha indica* in rats, J. Ethanopharmacol., 79, 249-251.

Reddy, M.B. and Clark, B.S.L. (2004), Iron, Oxidative Stress and Disease Risk, Nutrition Reviews, 62, 120-124.

Reddy, Y.N., Murthy, S.V., Krishna, D.R. and Prabhakar, M.C. (2004), Role of free radicals and antioxidants in tuberculosis patients, Ind. J. Tuberc., 51,213-218.

Reddy. K.P., Subhani, S.M., Khan, P.A. and Kumar, K.B. (1995), Effect of light and bezyladenine are dark treated growing leaves, Changes in the peroxidase activity, Cell Physiol., 26, 984.

Reiter, R.J., Tan, D., Mayo, J.C., Sainz, R.M., Leon, J. and Czarnocki, Z. (2003), Melatonin as an antioxidant: biochemical mechanisms and pathophysiological implications in humans, Acta Biochimica Polonica, 50, 1129-1146.

Rekha, P.S., Kuttan, G. and Kuttan, R. (2001), Antioxidant activity of Brahma rasayana, Ind. J. Exp. Biol., 39, 447-452.

Rice-Evans, C.A., Miller, N.J. and Paganga, G (1997), Antioxidant properties of phenolic compounds, Trends Plant Sci., 2, 152-159.

Roe, J.H. and Keuther, A. (1953), The determination of ascorbic acid in whole blood and wine through 2, 4-dinitropheryl hydrazine derivative of Dehydroascorbic acid, J. Biol. Chem., 147, 399-404.

Rose, P.A. (2001), Superoxide, Spring, 77: 222, 1-10.

Rosenberg, H.R. (1992), Chemistry and Physiology of the Vitamins. Interscience publishers, Inc., New York, 452-453.

Rana, P., Vadhera, S. and Soni, G. (2004), *In vivo* antioxidant potential of Rice Bran Oil (RBO) in albino rats, Ind. J. Physiol. Pharmacol., 48, 428-436.

Rani, P., Unni, K.M. and Karthikeyan, J. (2004), Evaluation of antioxidant properties of berries, Ind. J. Clin. Biochem., 19, 103-110.

Rao, P.R., Trilochana, Y. and Chaitanya, K.K. (2003), Antidiarrheal and antimicirobial activities of bark and leaf extracts of *Xylocarpus granatum* Koenig, J. Natural Remedies, 3/2, 155-160.

Rao[a], K.V.K., Schwartz, S.A., Nair, H.K., Aalinkeel, R., Mahajan, S., Chawda, R. and Nair., M.P.N. (2004), Plant derived products as a source of cellular growth inhibitory phytochemicals on PC-3M, DU-145 and LNCaP prostate cancer cell lines, Curr. Sci., 87, 1585-1588.

Rao[b], M.R., Palada, M.C. and Becker, B.N. (2004), Medicinal and aromatic plants in Agroforestery systems, 61, 107-122.

Ray, G. and Husain, S.A. (2002), Oxidants, antioxidants and carcinogenesis, Ind. J. Exp. Biol., 40, 1213-1232.

Reddy, J.S., Rao, P.R. and Reddy, M,S. (2002), Wound healing effects of *Heliotropium indicum*, *Plumbago zaylanicum* and *Acalypha indica* in rats, J. Ethanopharmacol., 79, 249-251.

Reddy, M.B. and Clark, B.S.L. (2004), Iron, Oxidative Stress and Disease Risk, Nutrition Reviews, 62, 120-124.

Reddy, Y.N., Murthy, S.V., Krishna, D.R. and Prabhakar, M.C. (2004), Role of free radicals and antioxidants in tuberculosis patients, Ind. J. Tuberc., 51,213-218.

Reddy. K.P., Subhani, S.M., Khan, P.A. and Kumar, K.B. (1995), Effect of light and bezyladenine are dark treated growing leaves, Changes in the peroxidase activity, Cell Physiol., 26, 984.

Reiter, R.J., Tan, D., Mayo, J.C., Sainz, R.M., Leon, J. and Czarnocki, Z. (2003), Melatonin as an antioxidant: biochemical mechanisms and pathophysiological implications in humans, Acta Biochimica Polonica, 50, 1129-1146.

Rekha, P.S., Kuttan, G. and Kuttan, R. (2001), Antioxidant activity of Brahma rasayana, Ind. J. Exp. Biol., 39, 447-452.

Rice-Evans, C.A., Miller, N.J. and Paganga, G (1997), Antioxidant properties of phenolic compounds, Trends Plant Sci., 2, 152-159.

Roe, J.H. and Keuther, A. (1953), The determination of ascorbic acid in whole blood and wine through 2, 4-dinitropheryl hydrazine derivative of Dehydroascorbic acid, J. Biol. Chem., 147, 399-404.

Rose, P.A. (2001), Superoxide, Spring, 77: 222, 1-10.

Rosenberg, H.R. (1992), Chemistry and Physiology of the Vitamins. Interscience publishers, Inc., New York, 452-453.

Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G. and Hoeckstra, W.G. (1973), Selenium: Biochemical role as a component of glutathione peroxidase, Science, 179, 588 – 590.

Roy-Byrne, P.P., Bystritsky, A., Russo, J., Craske, M.G., Sherbourne, C.D. and Stein, M.B. (2005), Use of herbal medicine in primary care patients with mood and anxiety disorders, Psychosomatics, 46, 117-122.

Sabu, M.C. and Kuttan, R. (2004), Antidiabetic activity of *Aegle marmelos* and its relationship with its antioxidant properties, Ind. J. Physiol. Pharmacol., 48, 81-88.

Sadhu, S.K., Okuyama, E., Fujimoto, H. and Ishibashi, M. (2003), Seperation of *Leucas aspera*, a medicinal plant of Bangladesh, guided by prostaglandin inhibitory and antioxidant activity, Chem. Pharm. Bull. (Tokyo), 51,595-598.

Safari, M., Rezaic, M. and Sheikh, N. (2003), Effects of some flavonoids on the susceptability of low-density lipoprotein to oxidative modification, Ind. J. Exp. Biol., 40, 358-361.

Samuni, A.M., DeGraff, W., Krishna, M.C. and Mithchell, J.B. (2002), Nitroxides as antioxidants : Tempol protects against E09 Cytotoxicity, Molecular and Cellular Biochemistry, 234/235,327-333.

Samy, P.R., Ignacimuthu, S. and Raja, D.P. (1999), Preliminary screening of ethanomedicinal plants from India, J. Ethanopharmacol., 66, 235-240.

Sanchez-Amat, A. and Solano, F. (1997), A pluripotent polyphenols oxidase from the melanogenic marine alteromonas shares catalytic capabilities, Biochem. Biophys. Res. Comm., 240, 787-792.

Satheesh, M.A. and Pari, L. (2004), Antioxidant effect of *Boerhavia diffusa* L. in tissues of alloxan induced diabetic rats, Ind. J. Exp. Biol., 42, 989-992.

Sawangjaroen, N., Subhadirasakul, S., Phongpaichit, S., Sripanth, C., Jamijaroen, K. and Sawagnjaroen, K. (2005), The *in vitro* anti-giardial activity of extracts from platns that are used for self-medication by AIDS patients in sourthern Thailand, Parasitol Res., 95, 17-21.

Selvendiran, K., Singh, J.P.V., Krishnan, K.B. and Sakthisekaran, D. (2003), Cytoprotective effect of piperine against benzo[a]pyrine induced lung cancer with reference to lipid peroxidation and antioxidant system in Swiss albino mice, Fitoterapia, 74, 109-115.

Sen, C.K. (2001), Antioxidant and redox regulation of cellular signalling: Introduction, J. Medicine and Science in Sports and Exercise, 33, 368- 370.

Seth, S.D. and Sharma, B. (2004), Medicinal plants in India, Ind. J. Med. Res, 120, 9-11.

Shi, C., Dai,Y., Xia, B., Xu, X., Xie, Y. and Liu, Q. (2001), The purification and spectral properties of polyphenol oxidase I from *Nicotiana tobacum*, Plant Molecular Biology Reporter, 19,381a – 381h.

Shirwaikar, A., Shenoy, R., Udupa, A.L. and Shetty, S. (2003), Wound healing property of ethanolic extract of leaves of *Hyptis suaveloens* with supportive role of antioxidant enzymes, Ind. J. Exp. Biol., 41, 238-241.

Shirwaikar[a], A., Rajendran, K. and Kumar, D. (2004), *In vitro* antioxidant studies on *Annona squamosa* Linn leaves, Ind. J. Exp. Biol., 42, 803-807.

Shirwaikar[b], J.S., Rajendran, K., Bodla, R. and Kumar, C.D. (2004), Neutralization potential of Viper russelli russelli (Russell's viper) venom by ethanol leaf extract of *Acalypha indica*, J. Ethanopharmacol., 94, 267-273.

Singh, B., Kala, R.K. and Rao, A.R. (2004), Modulation of antioxidant potential in liver of mice by kernel oil of cashew nut (*Anacardium occidentale*) and its lack of tumour promoting ability in DMBA induced skin papillomagenes is, Ind. J. Exp. Biol., 42, 373-377.

Singh, K. and Pushpa, A. (2005), Alteration in some antioxidant enzymes in cardiac tissue upon monosodium glutamate (MSG) administration adult male mice, Ind. J. Clin. Biochem., 20, 43-46.

Snyman, T., Stewart, M.J., Grove, A. and Steenkamp, V. (2005), Adulteration of South African traditional herbal remedies, Ther. Drug Moint., 27, 86-89.

Sohi, K.K. and Khanduja, K.L. (2003), Nimesulide affects antioxidant status during acute lung inflammation in rats, Ind. J. Biochem. Biophys., 40,238-245.

Srinivasan, V. (2002), Melatonin oxidative stress and neurodegenerative diseases, Ind. J. Exp. Biol., 40, 668-679.

SriRam, K.I. and Lakshmi, C. H. J. (2001), Curr. Sci., 80,921-923.

Stroz, P. (2005), Reactive oxygen species in tumor progression, Front Biosci., 10, 1881-1896.

Suchalatha, S., Thiruganasambandam, P., Maheswaran, E. and Shyamala Devi, C.S. (2004), Role of *Arogh*, a polyherbal formulation to mitigate oxidative stress in experimental myocardial infarction, Ind. J. Exp. Biol., 42,224-226.

Sudha, K., Rao, A.V., Rao, S.N., and Rao, A. (2004), Oxidative damage and plasma antioxidants in cerebrovascular accident, Ind. J. Physiol. Pharmacol., 48, 489-492.

SudheerKumar, M., Reddy, B.S., Babu, S.K., Bhilegaonkar, P.K., Shirwaikar, A. and Unnikrishnan, K.K. (2004), Antiinflammatory and antiulcer activities of phytic acid in rats, Ind. J. Exp. Biol., 42, 179-185.

Sugimoto, M., Furui, S. and Suzuki, Y. (1997), Molecular cloning of a cDNA encoding putative phospholipid hydroperoxide glutathione peroxidase from spinach, Biosci. Biotech. Biochem., 61, 1379-1381.

Suk, K. (2005), Regulation of neuroinflammation by herbal medicine and its implications for neurodegenerative diseases, Neruosignals, 14, 23-33.

Sulochana, K.N., Punitham, R. and Ramakrishnan, S. (2002), Effect of cigarette smoking on cataract: Antioxidant enzymes and constituent minerals in the lens and blood of humans, Ind. J. Pharmacol., 34, 428-431.

Suntress, Z.A. and Shek, P.N. (1995), Prevention of lung injury by (α- tocopherol) liposome, J. Drug Target, 3, 201-205.

Tandon, R., Khanna, H.D., Dorababu, M. and Goel, R.K. (2004), Oxidative stress and antioxidant status in peptic ulcer and gastric carcinoma, Ind. J. Physiol. Pharmacol., 48, 115-118.

Tarfa, F.D., Obodozie, O.O., Mshelia, E., Ibrahim, K. and Temple, V.J. (2004) Evaluation of phytochemical and anitmicrobial properties of leaf extract of *Tapinanthus sessilifolius,* P. Becauv van Tiegh, Ind.J.Exp. Biol., 42, 326-329.

Tiwari, A.K. (2004), Antioxidants : New-generation therapeutic base for treatment of polygenic disorders, Curr. Sci., 86, 1092-1102.

Tripathi, Y.B. (1998), Free radicals in Ayurveda, Ancient Science of life, 17, 158-168.

Tripathi, Y.B., Upadhyay, A.K. and Chaturvedi, P. (2001), Antioxidant property of *Smilex china* Linn, Ind. J. Exp. Biol., 39, 1176-1179.

Tsumori, M., Tanaka, J., Koshimura, K., Kawaguchi, M., Murakami, Y. and Kato, Y. (2002), Cytotoxic effect of nitric oxide on human hematological malignant cells, Acta Biochimica Polonica, 49, 139-144.

Umadevi, S., Mohanta, G.P., Chelladurai, V., Manna,P.K. and Manavalan,R. (2003), Antibacterial and antifungal activity of *Andrographis echiodes*, J. Natural Remedies, 3/2, 185-188.

Valenzuela, A., Sanhyueza, J., Alonso, P., Corbari, A. and Nieto, S. (2004), Inhibitory action of conventional food-grade natural antioxidants and of natural antioxidants of new development on the thermal-induced oxidation of cholesterol, Int. J. Food Sci. and Nutr., 55, 155-162.

Velaquez, E., Touriner, H.A., Buschiazzo, P.M., Saavedra, G. and Schimella, G.R. (2003), Antioxidant activity of Paraguayan plant extracts, Fiterapia, 91-97.

Vendemiale, G., Grattagliano, L. and Altomare, E. (1999), An Update on the role of free radicals and antioxidant defense in human disease, Ind. J. Clin. Lab. Res., 29, 49-55.

Venu, T., Vishwanadham, D., Jayashree, P.R. and Kumar, P.R.M. (2003), Ethanolic extract of *Bacopa mohanniera* (Bramhi) induces shortening of cell-cycle durations in naturally synchronous *Physarum polycephalum.* Curr. Sci., 85, 245-247.

Verma, S.K., Singh, M., Jain, P. and Bordia, A. (2004), Protective effect of ginger, (*Zingiber officinale*) Rose on experimental atherosclerosis in rabbits, Ind. J. Exp. Biol., 42, 736-738.

Vuong, L.T., Buchholz, B.A., Lame, M.W. and Ducker, S.R. (2004), Phytochemical research using accelerator Mass Spectrometry, Nutrition Reviews, 62, 375-388.

Whiteside, M.A., Heimburger, D.C. and Johanning, G. L. (2004), Micronutrients and Cancer Threapy, Nutrition Reviews, 62, 142-147.

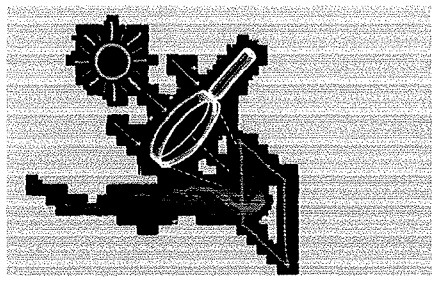
Wiart, C., Mogana, S., Khalifah, S., Mahan, M., Ismaill, S., Buckle, M., Narayana, A.K. and Sulaiman, M. (2004), Antimicrobial screening of plants used for traditional medicine in the state of Perak, Peninsular Malaysia, 75, 68-73.

Winterbourn, C.C., Hawkins, R.E., Brian, M. and Carrell, R.W. (1975), The estimation of red cell superoxide dismutase activity, J. Lab. Clin. Med., 85, 337-341.

Yoshimoto, M., Kurata-Azuma, R., Fujii, M., Hou, D., Ileda, K., Yashidome, T. and Osako. M. (2004), Phenolic composition and radical scavenging activity of sweetpotata-derived shochu distillery by-products treated with koji, Biosci. Biotechnol. Biochem., 68, 2477-2483.

Zakeria, H., Simpson, K., Brown, P.R. and Krutulovic, A. (1979), Use of reverse phase HPLC analysis for the determination of provitamin α- Carotenoids in tomatoes, J. Chromatography, 176, 109-117.

Zatorska, A., Maszewski, J. and Jozwiak, Z. (2003), Changes in GSH-antioxidant system induced by daunorubicin in human normal and diabetic fibroblasts, Acta Biochimica Polanica, 50, 825-835.



APPENDICES

APPENDIX - I

QUALITATIVE PHYTOCHEMICAL ANALYSIS

(Khandelwal, 2002)

The aqueous and alcoholic extracts prepared were tested for the presence of alkaloids, phenolics and flavonoids.

DETECTION OF ALKALOIDS

a) Mayer's test: A fraction of the extract was treated with Mayer's reagent (1.36g of mercuric chloride and 5g of potassium iodide in 100ml of distilled water) and observed for the formation of cream coloured precipitate.

b) Dragendroff's test: A fraction of the extract was treated with Dragendroff's reagent and observed for the formation of reddish orange coloured precipitate.

c) Wagner's test: A fraction of the extract was treated with Wagner's reagent (1.27g of iodine and 2g of potassium iodide in 100ml-distilled water) and observed for the formation of reddish brown coloured precipitate.

DETEECTION OF PHENOLIC COMPOUNDS

a) **Ferric chloride test:** A fraction of the extract was treated with 5% $FeCl_3$ reagent and observed for the formation of deep blue-black colour.

b) **Lead acetate test:** A fraction of the extract was treated with 10% lead acetate solution and observed for the formation of white precipitate.

DETECTION OF FLAVONOIDS

a) **Aqueous NaOH test:** A fraction of the extract was treated with 1N aqueous NaOH reagent and observed for the formation of yellow orange colouration.

b) **H_2SO_4 test:** A fraction of the extract was treated with conc.H_2SO_4 and observed for the formation of orange colour.

c) **Schinodo's test:** A fraction for the extract was treated with a piece of magnesium turning followed by a few drops of conc. HCl and heated slightly. Observed for the formation of dark pink colour.

APPENDIX II

ESTIMATION OF CATALASE ACTIVITY

(Luck, 1974)

Catalase has double function as it catalyzes the following reactions:

a) Decomposition of hydrogen peroxide (H_2O_2) to give water and oxygen

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

b) Oxidation of hydrogen donors for example methanol, formic acid, phenol with the consumption of one mole of peroxide

$$ROOH + AH_2 \xrightarrow{\text{Catalase}} H_2O + ROH + A$$

Principle

The UV light absorption of hydrogen peroxide solution can be easily measured between 230 and 250nm. On decomposition of hydrogen peroxide by catalase, the absorption decreases with time. The enzyme activity could be arrived at from this decrease.

Reagents

1. Phosphate buffer (0.067M, pH 7.0)
   Dissolved 3.522g of KH_2PO_4 and 7.628g of Na_2HPO_4. $2H_2O$ in distilled water and made up the volume to one litre.
2. H_2O_2 - Phosphate buffer
   Diluted 0.16ml of H_2O_2 (10% w/v) to 100ml with phosphate buffer. Prepared fresh. The absorbance of the solution should be about 0.5 at 240nm with a 1cm light path.

Procedure

Enzyme extract

Homogenized the plant tissue in a blender with M/150 phosphate buffer (assay buffer diluted 10 times) at 1-4^{O}C and centrifuged. Stirred the sediment with cold phosphate buffer, allowed to stand in the cold with occasional shaking and then repeated the extraction once or twice. The extraction should not take greater than 24 hours. The combined supernatants were used for the assay.

Assay

Read against a control cuvette containing the enzyme solution as in the experimental cuvette, but containing H_2O_2 free phosphate buffer (M/15).

Pipetted out 3ml of H_2O_2 - phosphate buffer into the experimental cuvette, added 0.01-0.04ml of the sample and mixed with a glass or plastic rod flattened at one end. Noted the time (Δt) required for a decrease in absorption from 0.45 to 0.40. This value was used for the calculations. If 't' is more than 60 seconds, the measurements have to be repeated with a more concentrated solution of the sample.

Calculation

Calculated the concentration of H_2O_2 using the extinction co-efficient 0.036 per μmol per ml.

APPENDIX III
ESTIMATION OF PEROXIDASE ACTIVITY
(Reddy *et al*, 1995)

Principal

In the presence of a hydrogen donor (Pyrogallol or dianisidine) peroxidase converts H_2O_2 to water and oxygen. The oxidation of pyrogallol or dianisidine to a coloured product called purpurogalli is measured colorimetrically

$$\text{Pyrogallol} + H_2O_2 \longrightarrow \underset{\text{(Coloured)}}{\text{Oxidised pyrogallol}} + H_2O + O_2$$

Reagents

1. Pyrogallol
   0.05M in 0.1M phosphate buffer (pH 6.5)
2. 1% H_2O_2
3. Enzyme extract
   Macerated one part of plant tissue with 5 parts (w/v) of 0.1M phosphate buffer (pH 6.5) in a homogeniser, centrifuged the homogenate at 500g for 15 minutes, used the supernatants as the enzyme source. All procedures were carried out at 0-5^{O}C.

Procedure

Pipetted out 3ml of 0.05M pyrogallol solution and 0.02ml of enzyme extract in a test tube. Adjusted the spectrophotometer to read '0' at 430nm. Added 0.5ml of 1% H_2O_2 in the cuvette. Recorded the change in absorbance for every 30 seconds upto 3 minutes.

Calculations

Change in absorbance /min at 430nm	=	X
Weight of the plant material taken	=	300mg
Volume of the extract taken for the assay	=	0.02ml
Change in absorbance for 0.02ml	=	X
Change in absorbance for 1.5 ml extract	=	(X/0.02) x 1.50 = Y
(i.e) Peroxidase activity in 300mg plant tissue	=	Y
Peroxidase activity /gram of plant tissue	=	Y x (1000/300) Units

APPENDIX IV

ESTIMATION OF SUPEROXIDE DISMUTASE ACTIVITY

(Misra and Fridovich, 1972)

Principle

Superoxide dismutase used the photochemical reduction of riboflavin as oxygen generating system and catalyses the inhibition of NBT reduction, the extent of which can be assayed spectrophotometrically at 600nm.

Reagents

1. 50mM Potassium phosphate buffer (pH 7.4)
2. 45mM Methionine
3. 5.3mM Riboflavin
4. 84mM NBT
5. 20mM Potassium cyanide

Procedure

The incubation medium contained 300µl of each reagent (50mM potassium phosphate buffer, 45mN methionine, 5.3mM riboflavin, 84mM, NBT and 20mM potassium cyanide). To the test 300µl of sample was added. The final volume was made up to 3ml with water. The tubes were well placed in an aluminum foil lined box maintained at 25^{O}C and equipped with 15W fluorescent lamps. Reduced NBT was measured spectrophotometrically at 600nm after exposure of light for 10 minutes. The maximum reaction was evolved in the absence of enzyme.

One unit of enzyme activity is defined as the enzyme reaction, which gave 50% inhibition of NBT reduction in one minute under the assay conditions and expressed on specific activity in units.

APPENDIX V

ESTIMATION OF POLYPHENOL OXIDASE ACTIVITY

(Esterbauer *et al.*, 1977)

Principle

Phenol oxidases are copper proteins of wide occurrence in nature, which catalyze the aerobic oxidation of certain phenolic substrates to quinones, which are autoxidized to dark brown pigments generally known as melanins. The polyphenol oxidases (PPO) comprise catechol oxidase and laccase.

One unit of either catechol oxidase or laccase in defined as the amount of enzymes that transforms 1µmol of dihydrophenol to 1 of µmol of quinone per minute under the assay conditions.

Reagents

1. 50mM Tris-HCl (pH 7.2)

2. 0.4M Sorbitol
3. 10mM Sodium chloride
4. 0.1M Phosphate buffer (pH 6.5)
5. 0.01M Catechol solution

Preparation of enzyme extract

Ground about 5g of the plant tissue and made upto 20ml with the medium containing 50mM Tris-HCl (pH 7.2), 0.4M sorbitol and 10mM NaCl. Centrifuged the homogenates at 2000g for 10 minutes and used the supernatant for the assay.

Procedure

Added 2.5ml of 0.2M phosphate buffer (pH 6.5), 0.3 ml of catechol solution (0.01m) into the cuvette and set the spectrophotometer at 495 nm. Now added the enzyme extract (0.2ml) and started recording the change in absorbance for every 30 seconds upto 5 minutes.

Enzyme units in the test = K x (Δ /Min)
K for catechol oxidase = 0.272
K for laccase = 0.242

APPENDIX VI
ESTIMATION OF GLUTATHIONE-S-TRANSFERASE ACTIVITY
(Habig *et al* ., 1974)

Principle

The enzyme activity is measured by its ability to conjugate glutathione (GSH) and 1-chloro-2,4-dinitrobenzene (CDNB), the extent of conjugation causing a proportionate change in absorption at 340nm.

Reagents

1. 1mM CDNB in ethanol
2. 1mM Glutathione (GSH)
3. 0.1M Phosphate buffer
4. 50mM Tris-HCl buffer pH (7.2)

Procedure

Enzyme extract

The sample was homogenized with Tris –HCl buffer (pH 7.2). The homogenate was centrifuged at $4^{O}C$ for 30 minutes at 8500rpm. The supernatant was used as the enzyme source.

Assay

The assay was done at 25°C under conditions giving activities linear with respect to incubation times and protein concentrations for atleast 3 minutes.

The enzyme activity was determined by monitoring the change in absorbance at 340nm in a spectrometer. 0.1ml of both substrates (GSH and CDNB) were taken in 0.1M phosphate buffer pH 6.5 at room temperature to make a volume of 2.9ml.The reaction was started by adding 0.1ml of sample to this mixture and the readings were recorded against distilled water blank for a minimum for 3 minutes. The complete assay mixture without the sample served as the control to monitor non-specific binding of substrates. Care was taken to ensure that final concentration of ethanol in mixture was always less than 4 per cent.

Calculation

GST activity was calculated using the extinction co-efficient of the product formed and the values have been expressed as nanomoles of CDNB conjugated per minute per g sample.

APPENDIX VII

ASSAY OF GLUTATHIONE PEROXIDASE ACTIVITY

(Rotruck et al., 1973)

A known amount of enzyme preparation was used to react with H_2O_2 in the presence of GSH for a specified time period. Then the remaining GSH was measured by the method of Ellman.

$$2GSH + H_2O_2 \xrightarrow{Se - GPx} GSSG + 2H_2O_2$$

Reagents

1. 0.4M Tris buffer
2. 10mM Sodium azide
3. 10% TCA
4. 0.4mM EDTA
5. 10mM Hydrogen peroxide
6. 2mM Glutathione

Procedure

To 2ml of Tris buffer, 0.2ml of EDTA, 0.1ml of sodium azide and 0.5ml of plant extract were added. 0.2ml of glutathione followed by 0.1ml of hydrogen peroxide were added to the mixture, mixed well and incubated at 37°C for 10 minutes along with a tube containing all the reagents except sample. After 10 minutes the reaction was arrested by the addition of 0.5ml of

10 % TCA centrifuged and the supernatant was assayed for glutathione by the method of Ellman.

The activities are expressed as µg GSH consumed /minute/mg protein.

APPENDIX VIII

ESTIMATION OF GLUTATHIONE REDUCTASE ACTIVITY

(David and Richard, 1983)

Principle

Glutathione reductase catalyses the conversion of oxidized glutathione to reduced glutathione employing NADPH as a substrate. The amount of NADPH utilized is a direct measure of enzyme activity.

Reagents

1. 0.12M Phosphate buffer (pH 7.2)
2. 15mM EDTA
3. 10mM Sodium azide
4. 6.3mM Oxidized glutathione
5. 9.6mM NADPH

Procedure

1g of sample was homogenized with 0.12M phosphate buffer centrifuged at 500rpm for 10 minutes and supernatant was used as the source of enzyme.

Assay

The assay system contained 1ml of 0.12M potassium phosphate buffer, 0.1ml EDTA, 0.1ml of 10mM Sodium azide, 0.1ml of 0.3mM oxidized glutathione and 0.1ml of enzyme source and made up to 2.0ml with water. Kept for 3 minutes at room temperature. Then 0.1ml of NADPH was added. The absorbance at 340nm was recorded at an interval of 15 seconds for 2-3 minutes. Control was carried out which contain water instead of oxidized glutathione. The enzyme activity was expressed as µmoles of NADPH oxidized/minute/g sample.

APPENDIX IX

ESTIMATION OF ASCORBIC ACID

(Roe and Keuther, 1953)

Principle

Ascorbate is converted to dehydroascorbate by treatment with activated charcoal or bromine. Dehydroascorbic acid then reacts with 2,4-dinitrophenylhydrazine to form osazones, which dissolve in sulphuric acid to

give an orange coloured solution whose absorbance can be measured spectrophotometrically at 540nm.

Reagents

1. 4% Trichloro acetic acid
2. 9N Sulphuric acid
3. 2% 2, 4-dinitrophenylhydrzine reagent (DNPH)
   Dissolved 2g of DNPH in 100ml of 9N sulphuric acid.
4. 10% Thiourea
5. 85% Sulphuric acid
6. Stock standard solution
   Dissolved 100mg of ascorbic acid in 100ml of 4% TCA
7. Working standard solution
   Diluted 10ml of stock solution to 100ml with 4% TCA

Procedure

Ground 1g of the sample and homogenized in 4% TCA. Made upto 10ml and centrifuged at 2000rpm for 10 minutes. The supernatant obtained was treated with a pinch of activated charcoal, shaken well and kept for 10 minutes. Centrifuged once again to remove the charcoal residue. Noted the volume of clear supernatant obtained.

0.5 and 1ml aliquots of this supernatant were taken for the assay. The assay volume was made upto 2.0ml with 4% TCA. 0.2 to 1.0ml of working standard solution containing 20-100µg of ascorbate respectively was pipetted out into clean dry test tubes and the volume was made upto 2.0 ml with 4% TCA.

Added 0.5ml of DNPH reagent to all the tubes followed by 2 drops of 10% thiourea solution. Incubated at $37^{O}C$ for 3 hours. The osazone formed were dissolved in 2.5ml of 85% sulphuric acid, in cold, drop by drop with no appreciable rise in temperature. To the blank alone, DNPH reagent and thiourea were added after the addition of sulphuric acid. After incubation for 30 minutes at room temperature. The absorbance was read spectrophotometrically at 540nm.

Calculated the content of ascorbic acid in the plant sample using the standard graph.

APPENDIX X

ESTIMATION OF α-TOCOPHEROL

(Emmerie -Engel method 1938 as described by Rosenberg, 1992)

Principle

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

Reagents

1. Absolute alcohol
2. Xylene
3. 2,2'- dipyridyl
   1.2g in one litre of n-propanol
4. Ferric chloride solution
   1.2g of Fe Cl_3. $6H_2O$ in one litre ethanol. Stored in a brown bottle
4. Standard solution of D, L -α-Tocopherol
   100mg/100ml of α-Tocopherol in absolute alcohol. 91mg of α-Tocopherol equivalent to 100mg of tocopherol acetate.

Extraction of plant tissue

The sample was homogenized in a blender. Weighed accurately 2.5g of the homogenized tissue into a conical flask. Added 50ml of 0.1N sulphuric acid slowly without shaking. Stoppered and allowed to stand overnight. The next day contents of the flask were shaken vigorously and filtered through Whatman No.1 filter paper discarding the initial 10-15 ml of filtrate. Aliquots of the filtrate were used for the estimation.

Procedure

Into three stoppered centrifuge tubes (test, standard and blank) pipetted out 1.5ml of sample extract, 1.5ml of standard and 1.5ml of water respectively. To the test and blank added 1.5ml of ethanol and to the standard, added 1.5ml of water. Added 1.5ml of xylene to all the tubes stoppered, mixed well and centrifuged.

Transferred 1.0ml of xylene layer into another stoppered tube, taking care not to include any ethanol or protein. Added 2 ml of 2,2'-dipyridyl reagent to each tube stoppered and mixed. Pipetted out 1.5ml of the mixture into the spectrophotometer cuvette and read the extinction of the test and the standard against the blank at 460nm. Then in turn, beginning with the blank, added

0.33ml of ferric chloride solution. Mixed well and after exactly 15 minutes read test and standard against the blank at 520nm. The amount of vitamin E can be calculated using the formula,

$$\text{Amount of tocopherol in } \mu g = \frac{\text{Reading at 520nm} - \text{Reading at 460nm}}{\text{Reading of standard at 520nm}} \times 0.24 \times 15$$

APPENDIX XI
ESTIMATION OF REDUCED GLUTATHIONE
(Moron *et al.*, 1979)

Principle

Reduced glutathione is measured by its reaction with 5,5'-dithio-bis (2-nitro benzoic acid) (DTNB) to give a compound that absorbs at 412nm.

Reagents

1. 5 % TCA
2. Sodium phosphate buffer (0.2M, pH 8.0)
3. DTNB solution
   0.6mM DTNB in 0.2M phosphate buffer
4. Standard glutathione
   Dissolved 10mg of reduced glutathione in 100ml of 5% TCA

Procedure

1g sample was homogenized in 5% TCA to give a 20% homogenate. The precipitated protein was centrifuged down at 100rpm for 10 minutes. The homogenate was cooled on ice and 0.1ml of supernatant was taken for the estimation. The volume of the aliquot was made upto 1ml with 0.2M sodium phosphate buffer (pH 8.0). 2ml of freshly prepared DTNB solution was added to all the tubes and the intensity of yellow colour formed was read at 412nm in a spectrometer after 10minutes.

A standard curve of GSH was prepared using concentrations ranging from 2 to 10nanomoles of GSH in 5% TCA.

APPENDIX XII
ESTIMATION OF POLYPHENOL
(Malick and Singh, 1980)

Phenols, the aromatic compounds with hydroxyl groups are widespread in plant kingdom. They occur in all parts of the plants. Phenols are said to offer resistance to diseases and pests in plants. Grains containing high amount of polyphenols are resistant to bird attack. Phenols include an array of compounds

like tannins, flavonols *etc.*, Total phenol estimation can be carried out with Folin-Ciocalteau reagent.

Principle

Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium and produce blue coloured complex (Molybdenum blue)

Reagents required

1. 80% Ethanol
2. Folin-Ciocalteau reagent
3. 20% Sodium carbonate
4. Stock standard solution

   Dissolved 100mg catechol in 100ml of distilled water in a standard flask
5. Working standard solution

   Diluted 10 ml of the stock solution to 100ml with distilled water 1.0 ml of this solution contains 100µg of catechol.

Procedure

Weighed exactly 0.5 to 1.0 g of the plant sample and ground it with a mortar and pestle and in the volume of 80% ethanol. Centrifuged the homogenate at 10,000rpm for 20 minutes. Saved the supernatant. Re-extracted the residue with five times the volume of 80% ethanol, centrifuged and pooled the supernatants. Evaporated the supernatant to dryness. Dissolved the residue in a known volume of distilled water. Pipetted out different aliquots (0.2-2.0ml) into test tubes. Made up the volume in each tube to 3.0ml with water. Added 0.5ml of Folin-Ciocalteau reagent. After 3 minutes, added 2.0ml of 20% sodium carbonate solution to each tube. Mixed thoroughly placed the tubes in a boiling water bath for exactly 1 minute cooled and measured the absorbance at 650nm against a reagent blank.

APPENDIX XIII

ESTIMATION OF TOAL CAROTEINOIDS AND LYCOPENE

(Zakaria *et al.*, 1979)

Principle

The total carotenoids in the sample were extracted in petroleum ether. The total carotenoids were estimated in UV /visible spectrophotometer at 450nm.

Lycopene has the absorption maximum at 473nm and 503nm. A rapid method for the estimation of lycopene in plant product is based on the movement of absorption of the petroleum ether extract of the total carotenoids at 503nm. After measuring the total carotenoids at 450nm, the same extract can

be used for estimating lycopene at 503nm. At 503nm lycopene has a large absorption while carotenoids have only negligible absorbance.

Reagents

1) Petroleum ether
2) Anhydrous sodium sulphate
3) Calcium carbonate
4) 12% Alcoholic KOH (ice cold)

Procedure

Weighed 5-10g of the sample. Saponified for about 30 minutes in a shaking water both at $37^{O}C$ after extracting the sample in 12% alcoholic KOH. Transferred the saponified extract into a separating funnel (packed with glass cool and calcium carbonate) containing 10-15ml of petroleum ether and mixed gently. Taken up the carotenoid pigments into the petroleum ether layer. Transformed the lower aqueous phase into another separating funnel and the petroleum ether extract containing carotenoid pigments to an amber-coloured bottle. Repeated the extraction of the aqueous phase similarly with petroleum ether, until it become colourless. Discarded the aqueous phase. To the petroleum ether extract added a small quantity of anhydrous sodium sulphate to remove turbidity. Noted the final volume of petroleum ether extract and diluted if needed by a known dilution factor.

The absorbance of the extract at 450nm and 503nm was noted in a spectrophotometer using petroleum ether as a blank.

For total carotenoids

$$\text{Amount of total carotenoids present} = \frac{Px4xVx100}{W}\ \mu g$$

Where, P - Optical density of the sample
V - Volume of thee sample
W - Weight of the sample

For lycopene

$$\text{mg of lycopene per 100g sample} = \frac{3.1206 \times \text{OD of the sample} \times \text{Volume made up} \times \text{dilution } 100}{1x \text{ Weight of the sample } x1000}$$

APPENDIX XIV
ESTIMATION OF PROTEIN
(Lowery *et al.*, 1951)

Principle

The blue colour developed by the reduction of phosphomolybic-phosphotungstic components in the Folin-Ciocalteau reagent by the amino tyrosine and tryptophan present in the protein plus the colour developed by the biuret reaction of the protein with the alkaline cupric tartarate are measured in the Lowry's method.

Reagents

1. Solution A
   1% Copper sulphate
2. Solution B
   2% sodium potassium tartarate
3. Solution C
   2% sodium Carbonate in 0.1 N NaOH
4. Solution D
   Mixed just before use 1 ml of solution A 1 ml of solution B and 100ml of solution C.
5. 1N Folin-Ciocalteau reagent
   Mix equal volumes of commercially available reagent and distilled water just prior to use. Store protected from light.
6. Standard BSA
   50mg BSA in 50ml of 0.1N NaOH. Diluted 1:10 working standard.

Procedure

Aliquots of standard protein solution (40-200μg) were taken and the leaf samples were also taken and made up to 1ml with 0.1N NaOH. Shook well to treat the protein with alkali. Added 5 ml of solution D mixed well and incubated at 37^{O}C for 10 minutes. Added 0.5 ml of Folin-Ciocalteau reagent mixed well and incubated for 30 minutes at 37^{O}C. Read the colour developed at 670nm against a reagent blank.

APPENDIX XV
DETERMINATION OF TOTAL CARBOHYDRATE BY ANTHRONE METHOD
(Hedge and Hofreiter, 1962)

Carbohydrates are the important components of storage and structural materials in the plants. They exist as free sugars and polysaccharides. The basic units of carbohydrates are the monosaccharides, which cannot be split by

hydrolysis into simpler sugars. The carbohydrate content can be measured by hydrolysing the polysaccharides into simple sugars by acid hydrolysis and estimating the resultant monosaccharides.

Principle

Carbohydrates are first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium, glucose in dehydrated to hydorxymethyl furfural. This compound forms with anthrone a green coloured product with an absorption maximum at 630nm.

Reagents

1. 2.5N HCl
2. Anthrone Reagent
   Dissolve 200mg of anthrone in 100ml of the cold 95% H_2SO_4
3. Stock standard
   Dissolve 100mg of glucose in 100ml of water.
4. Working Standard
   10ml of stock diluted to 100ml with distilled water. Store refrigerated after adding few drops of toluene.

Procedure

Weighed 100mg of the sample into a boiling tube hydrolysed by keeping it in boiling water bath for 3 hours with 5ml of 2.5N HCl and cooled to room temperature. Neutralized it with solid sodium carbonate until the effervescence ceases. Made up the volume to 100ml and centrifuged, used aliquots for analysis.

Prepared standards and made up the volume to 1ml with distilled water. 4ml of anthrone reagent was added. Heated for 8 minutes in boiling water bath cooled rapidly and read the absorbance at 630nm.

APPENDIX XVI

EXTENT OF INHIBITION OF *IN VITRO* LIPID PEROXIDATION (Okhawa *et al.*, 1979)

An *in vitro* model of goat liver homogenate was used for induction of lipid peroxidation mediated by $FeSO_4$ as a pro-oxidant. Application of the relevant plant tissue extract in the medium was tried with an objective of assessing the extent of inhibition of *in vitro* lipid peroxidation by measurement of Thio Barbituric Acid Reactive Substances (TBARS) in the experimental mixtures. TBARS were measured spectrophotometrically at 535nm.

Reagents

1. Tris Buffered saline (TBS, pH 7.4)
   10mM Tris, 0.15M, NaCl

2. Ferrous sulphate
   Ferrous sulphate was prepared fresh in TBS and added at 10μmoles final concentration in the assay medium
3. 1% Thio Barbituric Acid
   1g TBA in 100ml hot water or TBS
4. 70% alcohol
5. Acetone
6. 5% Goat liver homogenate prepared in TBS (cold)

Procedure

A 5% liver homogenate was prepared in TBS (cold). 50μl of it was used in the assay. 0.5g of fresh plant tissue was weighed accurately and homogenized in 1ml of cold TBS. 50μl of it was used in the assay. Ferrous sulphate at a final concentration of 10μmoles was added in the assay medium to induce oxidation. The final volumes in the test tubes were made upto 500μl with cold TBS.

Controls were prepared for each sample containing the respective plant extract (50μl), liver homogenate (50μl) and TBS to make up the final volume to 500μl. Pro-oxidant was not added to the control tubes. A blank containing 50μl of ferrous sulphate and 450μl of TBS was also prepared.

An assay medium corresponding to 100% oxidation was prepared by adding all other constituents except leaf extracts. The experimental medium corresponding to auto oxidation contained only the liver homogenate and TBS to make up the final volume to 500μl . All the tubes were incubated at $37^{O}C$ for one hour.

At the end of incubation period, 500μl of 70 per cent alcohol was added to all the tubes to stop the LPO reaction. 1ml of 1 per cent TBA was added to all the tubes and heated in a boiling water bath for 20minutes. After cooling to room temperature, the tubes were centrifuged. To the clear supernatants collected, 500μl of acetone was added and measured the TBARS at 535nm in a spectrophotometer.

APPENDIX XVII

DETRMINATION OF SUPEROXIDE PRODUCTION *IN VITRO*

(Winterbourn *et al.*, 1975)

The extent of superoxide generation was studied on the basis of inhibition in the production of superoxide ion by the plant sample, which was measured colorimetrically at 560nm.

Reagents

1. 0.1m EDTA containing 1.5mg sodium cyanide/100ml
2. 1.5mM Nitroblue tetrozolium (NBT)
3. 0.12mM Riboflavin
4. 0.067M Phosphate buffer (pH 7.8)

Procedure

The assay tubes contained 0.02ml the plant sample (20mg concentration-100% extract), 0.2ml EDTA, 0.1ml nitroblue tetrazolium, 0.05ml riboflavin and 2.55ml phosphate buffer. Control tubes were set up without leaf extracts. The initial optical densities of the solutions were recorded at 560nm and the tubes were illuminated uniformly with a fluorescent lamp for 30 minutes. A_{560} was measured again and difference in OD taken as the quantum of superoxide production. The percentage inhibition by the leaf samples was calculated by comparing with the OD of the control tubes.

APPENDIX XVIII
DETERMINATION OF NITRIC OXIDE GENERATION *IN VITRO*
(Green *et al.*, 1982)

Aqueous solution of sodium nitroprusside spontaneously generates nitric oxide (NO) at physiological pH, which interacts with oxygen to produce nitrite ions which is measured colorimetrically.

Reagents

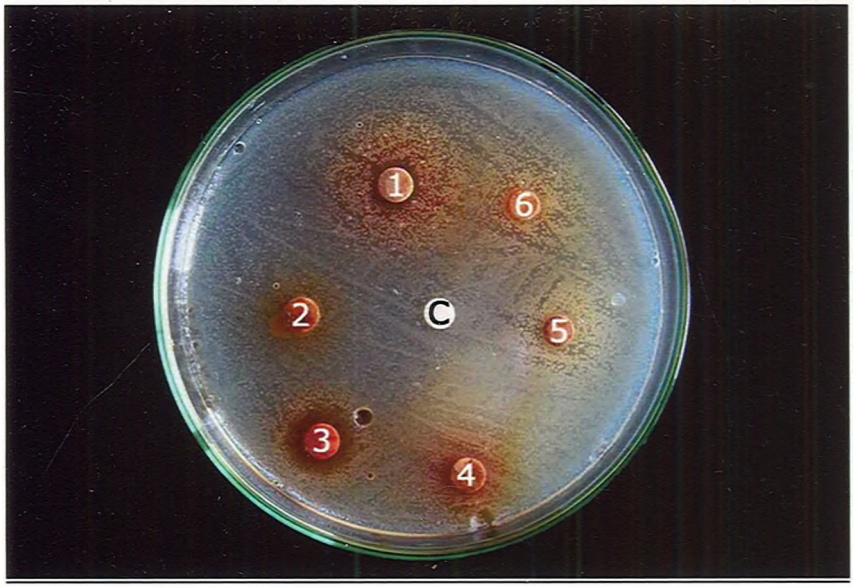
1. Phosphate buffered saline (pH 7.2) :
   0.88% NaCl, 0.02% KCl, 0.02% KH_2PO_4 and 0.115% Na_2HPO_4
2. 100mM Sodium nitroprusside
3. Griess reagent
   1% sulfanilamide, 2% H_3PO_4 and 0.1% naphthalene diamine dihydrochloride

Procedure

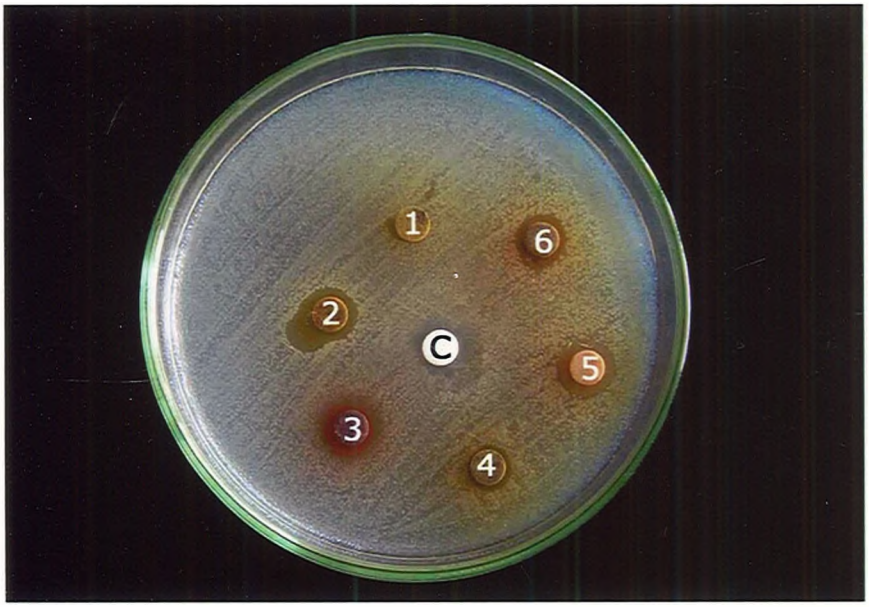
The reaction mixture containing 0.3ml sodium nitroprusside, 2.68ml PBS and 20µl of leaf extract (20mg concentration-100% extract) was incubated at 25°C for 15 minutes. Control tubes (100% generation) were prepared without leaf extracts. After incubation 0.5ml of the reaction mixture was removed and 0.5ml of Griess reagent was added to it. The absorbance of the chromophore formed, indicative of the quantum of NO generated was read at 546nm.

Plate 4

Antibacterial activity of aqueous extracts aganist *E.coli*



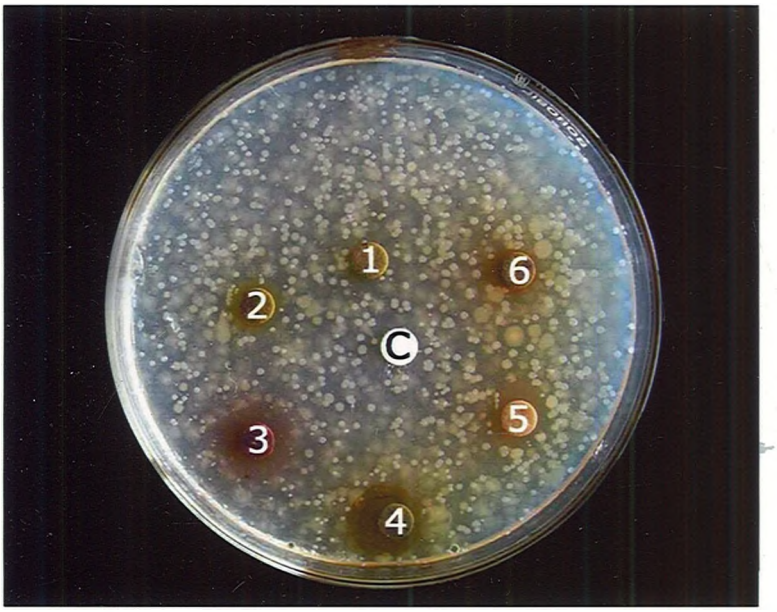
Antibacterial activity of alcoholic extracts against *E.coli*



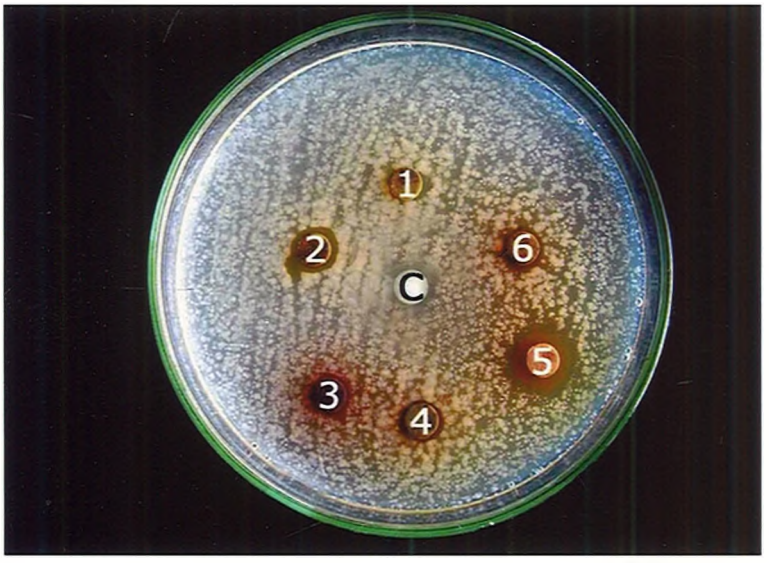
1 - *Acalypha indica* (L.)
2 - *Eclipta prostrata* (L.)
3 - *Mollugo latoides* (L.)
4 - *Leucas aspera* (S.)
5 - *Nelumbo nucifera* (G.)
6 - Mixture
C - Control

Plate 5

Antibacterial activity of alcoholic extracts against *S.flexineri*



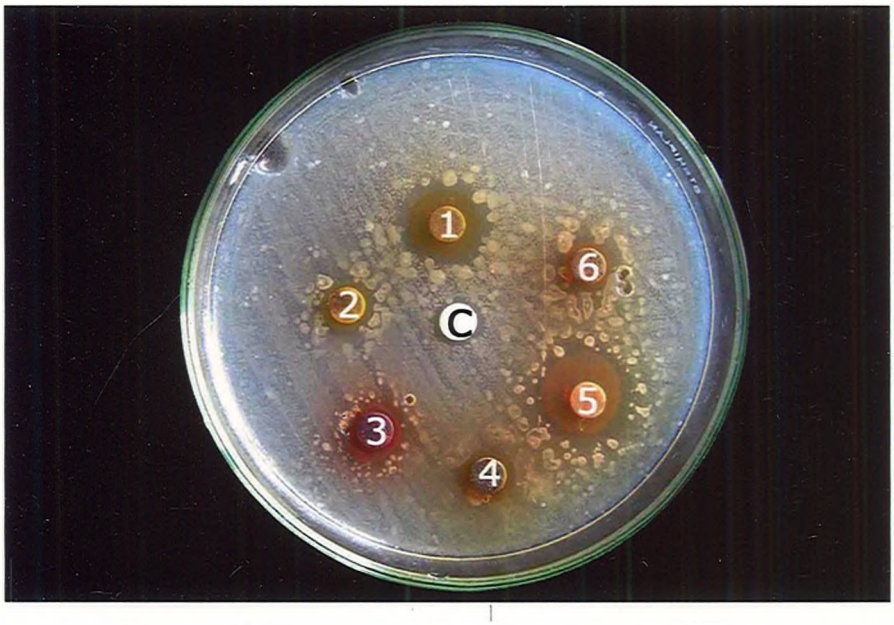
Antibacterial activity of alcoholic extracts against *K.pneumoniae*



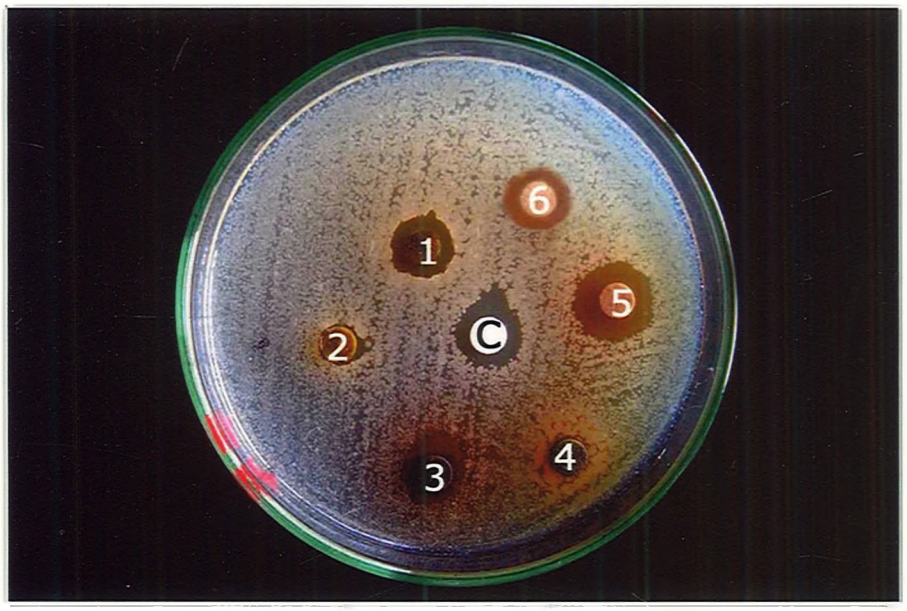
1 - *Acalypha indica* (L.)
2 - *Eclipta prostrata* (L.)
3 - *Mollugo latoides* (L.)
4 - *Leucas aspera* (S.)
5 - *Nelumbo nucifera* (G.)
6 - Mixture
C - Control

Plate 6

Antibacterial activity of alcoholic extracts against *P.aeruoginosae*



Antibacterial activity of alcoholic extracts against *E.cloacaceae*



1 - *Acalypha indica* (L.)
2 - *Eclipta prostrata* (L.)
3 - *Mollugo latoides* (L.)
4 - *Lecus aspera* (S.)
5 - *Nelumbo nucifera* (G.)
6 - Mixture
C - Control

organisms producing the maximum zone of inhibition against *E.coli* (11mm) and *K.pneumoniae* (24mm) respectively.

E.coli, *E.faecalis*, *K.pneumoniae* and *S.flexineri* were resistant to the crude alcoholic extracts of *Acalypha indica*. The extract of *Mollugo latoides* was inactive against *E.faecalis*, *K.pneumoniae* and *P.mirabilis*. Similarly the alcoholic extracts of *Leucas aspera* and the mixture had negative effect on the growth of some bacteria.

The alcoholic extract of bark of *Xylocarpus grantum* showed better antimicrobial activity (Rao *et al.*, 2003). The findings of the present study revealed a similar inhibitory pattern against *K.pneumoniae* (9mm) by *Eclipta prostrata*, *Leucas aspera* and the mixture. This observation is in par with the report of Indrayan *et al.*, (2004), who have stated the same inhibitory zone (9mm) against *K.pneumoniae* by the methanolic root extract of *Arnebia nobilis*. The essential oil of *Ferula gummosa* had low antibacterial activity against *P.aeruoginosae* (Eftekhar *et al.*, 2004), which is in correlation with our results in which *Mollugo latoides* showed poor response to *P.aeruoginosae*.

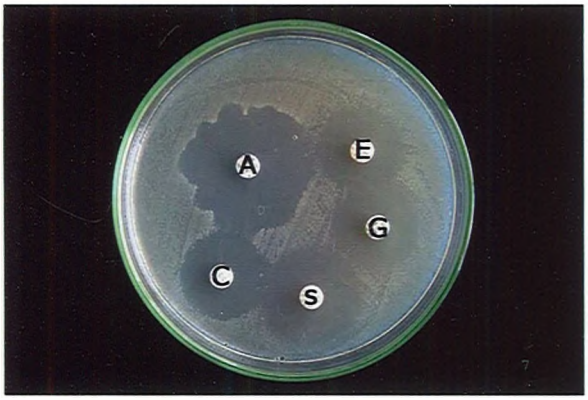
The alcoholic extracts of the plant sample exerted greater antibacterial activity than the corresponding aqueous extracts. The basis of varying degree of sensitivity of test organisms may be due to the intrinsic tolerance of microorganism and the nature and combinations of phytocompounds present in the extracts (Aqil and Ahmad, 2003). This observation has been substantiated by Akinyemi *et al.*, (2005) who have suggested that the stronger extraction capacity of alcohol could have produced greater number of active constituents responsible for antimicrobial activity. They have also implicated that the nature of biological active components would be enhanced in the presence of alcohol.

4.5.2 MICROBROTH DILUTION ASSAY

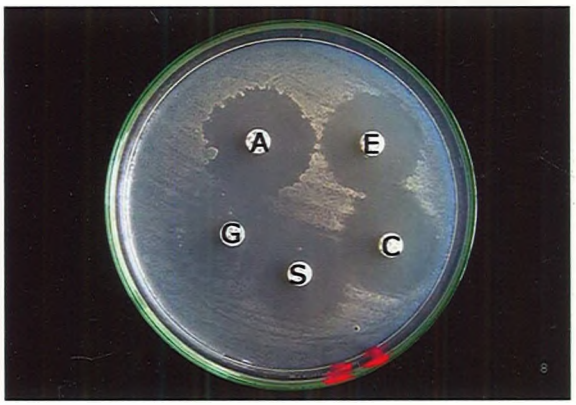
To ensure whether the extracts tested in disc diffusion method have

Plate - 3

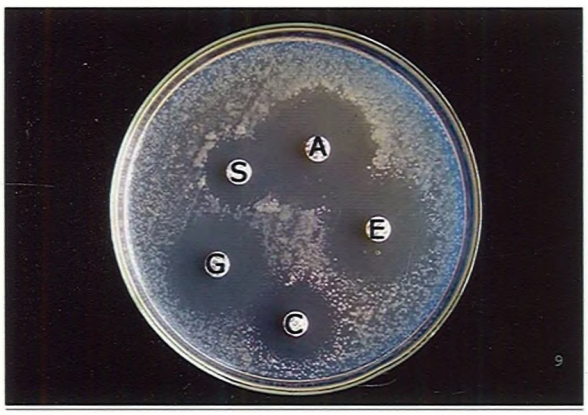
Antibiotic Sensitivity Test (AST) using different antibiotics



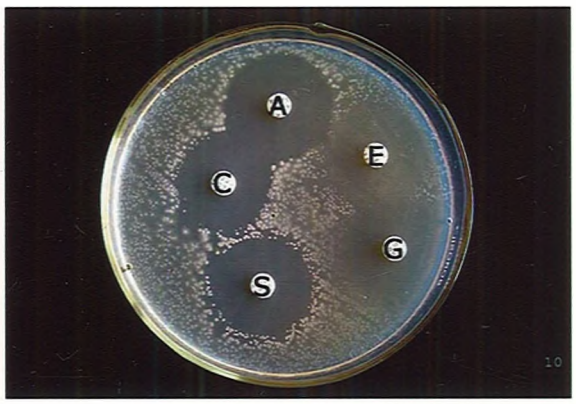
E.coli



P.aeruoginosae



K.pneumoniae



P.mirabilis

A - Ampicillin
E - Erythromycin
S - Streptomycin
C - Chloramphenicol
G - Gentamicin

Plate 1

FRESH AND POWDERED SAMPLES

***Acalypha indica* (L.)**

***Eclipta prostrata* (L.)**

Powdered Sample of
***Mollugo latoides* (L.)**

Plate 2

FRESH AND POWDERED SAMPLES

Leucas aspera (S.)



Nelumbo nucifera (G.)



Mixture