INTRODUCTION:

Diseases are born with mankind and drugs came in to existence since a very early period, to remove the pain of diseases and cure them. Thus the history of drugs is as old as mankind.

Plants provide food, clothing, shelter and medicine. Every society relied on the healing power of plants to treat illness and there has always been some magic in plants mysterious and omnipotent. An herb is a plant or plant part valued for its medicinal, aromatic or savory qualities.

Herbal medicine, referred to, as Herbalism or botanical medicine is the use of herbs for their therapeutic or medicinal value. The promise of herbalism and the firm conviction of definite therapy from herbal treatment persisted in all the countries of the world, especially Egypt, Babylon, Asian conglomeration of countries, etc. In India, the Ayurvedic system of medicines was firmly believed to have originated from the Vedas and ancient religious scripts.

Interest in medicinal plants has grown dramatically in recent years. Considerable attention is given to utilize eco-friendly and boi-friendly plant based products for the prevention and cure of different diseases.

Reasons for drug development from plants

- Nearly 25% of all medicinal prescriptions contain active principles extracted from higher plants.

Biologically active substances derived from plants may have less toxicological profiles to humans they serve as templates for synthetic modifications and structure function studies with anticipation of useful drug production.
Many secondary, highly active, plant constituents are found to be useful in studying biological systems and disease processes.\textsuperscript{278}

\textbf{Introduction to herbal research}

The main Indian traditional Indian systems of medicine namely Ayurveda, Siddha and Unani are primarily plant based systems.

The medicinal plant sector has traditionally occupied an important position in the social, cultural, spiritual and medicinal area of the rural and tribal sector of India.

And since independence India has made tremendous progress in agro technology, process technology, research and development, quality control and standardization of natural products. Now in a wider context there is a growing demand from plant based medicines, health products, food supplements, cosmetics etc in the national and International markets. Almost every modern drugs use today owes its origin to some medicinal plant or have long history of use in traditional medicine.\textsuperscript{279}

\textbf{Table no.7.1: Common name and medicinal uses of some plants}

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Common name</th>
<th>Drug</th>
<th>Medicinal uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sarpagandha</td>
<td>Ajmalcine, &amp; Reserpine</td>
<td>Anti hypertensive</td>
</tr>
<tr>
<td>2</td>
<td>Foxglove</td>
<td>Digitoxin</td>
<td>Cardiotonic</td>
</tr>
<tr>
<td>3</td>
<td>Opium poppy</td>
<td>Morphine</td>
<td>Analgesic</td>
</tr>
<tr>
<td>4</td>
<td>Periwinkle</td>
<td>Vincristine</td>
<td>Anticancer</td>
</tr>
</tbody>
</table>

\textbf{Future needs for herbal research and quality, safety and efficacy of Herbal products}

In 1995 W H O as a part of its global strategy of “Health for all” began the extensive task of reviewing the world’s scientific literature of commonly used herbal medicines. WHO monographs published as a result of this work are technical reviews
of quality, safety and efficacy of commonly used herbal medicines and are intended primarily to standardize proper use of herbal products.

Quality Safety and efficacy

The safety problems emerging with herbal medicine are due to lack of effective quality controls. They can be overcome by making no derogation with regard to necessary physic-chemical, biological and microbiological tests. Products should comply with quality standards. Herbs can also produce undesirable side effects just as other products. If the herbal product has the recognized efficacy and acceptable safety profile, it can be used without the need for the preclinical data or clinical trials. Otherwise it should be done compulsorily.

Each herb-manufacturing factory should have high standards that strictly adhere to Good Manufacturing Practices, Good Clinical Practices, and Good laboratory Practices and Good Agriculture practices.280

INTRODUCTION TO ANTIOXIDANTS:

Just as a burning fire needs oxygen, every cell in our body needs a steady supply of oxygen to derive energy from digested food. But consuming oxygen comes with a price; it also generates free radicals.281

In chemistry, radicals (often referred to as free radicals) are atomic or molecular species with unpaired electrons on an otherwise open shell configuration. These unpaired electrons are usually highly reactive so they attack healthy molecules in the body in the hope of stealing electrons to get stabilized; Damage caused by free radicals is called oxidation. "Radical" and "Free Radical" are frequently used interchangeably; the first organic free radical, was identified by Moses Gomberg in 1900 at the University of Michigan.
Free radicals play an important role in a number of biological processes, some of which are necessary for life, such as the intracellular killing of bacteria by neutrophil granulocytes. Free radicals have also been implicated in certain cell signaling processes the two most important oxygen-centered free radicals are superoxide and hydroxyl radical. They are derived from molecular oxygen under reducing conditions. However, because of their reactivity, these same free radicals can participate in unwanted side reactions resulting in cell damage. The damage done by free radicals is most injurious to DNA, results in mutations and death of cells. DNA is constantly bombarded by free radicals. However our body has enzymes and antioxidant that repair damaged DNA\textsuperscript{282}.

There are several different forms of reactive oxygen free radicals. Reactive oxygen species or ROS are species such as super oxide, hydrogen peroxide, and hydroxyl radical and are associated with cell damage\textsuperscript{283}.

**Generation of Free Radicals\textsuperscript{284}**

1. **Internally**: Primary source of free radical formation in body is during energy production in the mitochondria, by normal metabolism of fat, carbohydrates and proteins. And other factors like endocrine hormones, low blood supply during heart attacks, stroke and constant stress leads to the formation of free radicals internally.

2. **Externally**: Environmental contaminations such as pollution and cigarette smoking, ionizing and UV radiations such as over exposure to sunlight and prolonged exposure to X-rays, over exercising, exposure to toxic chemicals in pesticides and insecticides, use of petroleum based products, and junk foods are reasons for free radical damage externally.
1. **Super oxide (O$_2$):**

Super oxide anion O$_2^-$ may be generated by direct auto oxidation of O$_2$ during mitochondrial electron transport reaction Alternatively, O$_2^-$ is produced enzymatically by xanthine oxidase and cytochrome P$_{450}$ in the mitochondria or cytosol. O$_2^-$ so formed is catabolised to produce H$_2$O$_2$ by Super oxide dismutase (SOD).

2. **Hydrogen peroxide (H$_2$O$_2$):**

H$_2$O$_2$ is reduced to water enzymatically by catalase (in the peroxisome) and glutathione peroxidase GSH (both in the cytosol and mitochondria).

3. **Hydroxyl radical (OH-):**

OH- radical is formed by 2 ways in biological processes: by radiolysis of water and by reaction of H$_2$O$_2$ by (Fe++) ferrous ions; the latter process is termed as Fenton Reaction. Besides O$_2^-$, H$_2$O$_2$ and OH, nitric oxide (NO) generated by various body cells (endothelial cells, neurons, macrophages etc) can as a free radical.
Mechanisms of formation of Free Radicals

**O$_2$**
- Auto oxidation
- P450

**enzymatic (xanthine oxidase cytochrome)**

**O$_2^-$**
- SOD
- $2H_2O_2$
- Catalase GSH
- $2H_2O + O_2$
- Radiolysis
- $O_2^-$

**OH•**
**Fenton reaction**

Mechanism of Cell damage

- Lipid peroxidation
- Protein oxidation
- DNA damage
- Osteoskeletal damage

**CELL DEATH**

**PUFA**
i) Lipid peroxidation:

Polyunsaturated fatty acids (PUFA) of membrane are attacked repeatedly and severely by oxygen-derived free radicals to yield highly destructive PUFA radicals—lipid hypoperoxy radicals and lipid hypoperoxidase. This reaction is termed as lipid Peroxidation. The lipid peroxidase is decomposed by transition metals such as iron. Lipid peroxidation is propagated to other sites causing widespread membrane damage and destruction of organelles.

i) Oxidation of proteins: Oxygen derived free radicals cause cell injury by oxidation of protein macromolecules of the cells, cross linking of labile amino acids as well as by fragmentation of polypeptides directly. The end result is degradation of cytosolic neutral protease and cell destruction.

ii) DNA damage: Free radicals cause breaks in the single strands of the nuclear and mitochondrial DNA. This results in the cell injury; it may also cause malignant transformation of cells.

iii) Cytoskeleton Damage: Reactive oxygen species are also known to interact with cytoskeletal elements and interfere in mitochondrial aerobic phosphorylation and thus cause ATP depletion.

Diseases associated with free radicals: When the formation of free radicals overwhelms the body’s natural defense system, a chain reaction take place that generates even more free radicals the result is cellular damage. This damage ultimately affects several body systems and has been implicated in the development of multitude of disease.
Table No.: 7.2: Diseases associated with body system

<table>
<thead>
<tr>
<th>Body system affected</th>
<th>Associated diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Integumentary</td>
<td>Skin diseases, rheumatoid arthritis</td>
</tr>
<tr>
<td>Neuromuscular</td>
<td>Alzheimer's disease, amyotrophic lateral sclerosis, parkinson's disease</td>
</tr>
<tr>
<td>Endocrine</td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>Respiratory</td>
<td>Acute respiratory distress syndrome (ARDS), lung cancer</td>
</tr>
<tr>
<td>Cardio vascular</td>
<td>Aertirothrombosis, heart failure</td>
</tr>
<tr>
<td>Other</td>
<td>Prostate cancer, renal cancer, Human Immunodeficiency Virus (HIV)</td>
</tr>
</tbody>
</table>

Markers used for assessment of free radicals

Oxidative stress is an imbalance between the production of reactive oxygen species (ROS) and the body's defense system against the free radicals. The following markers are used to assess patients for oxidative stress and the production of ROS. Further research is needed to determine which marker are the most specific and sensitive.

SERUM: Ascorbic acid (vitamin C), Glutathione (GSH), Gamma – glutamyl transferase (GGT), Catalase (CAT), Superoxide dismutase (SOD), Selenium, Vitamin A, d-alpha – tocopherol (vitamin E).

URINE: Ascorbic acid (vitamin C), Glutathione (GSH), Gamma – glutamyl transferase (GGT), Catalase (CAT), Superoxide dismutase (SOD), Selenium, Vitamin A, d-alpha – tocopherol (vitamin E, N-acetyl-beta-D-glucosaminidase (NAG), F2-isoprostanes, Isoprostane 8-isoprostaglandin F.

Antioxidants and management of oxidative stress: 285

Antioxidants are compounds that help to inhibit the many oxidation reactions caused by free radicals thereby preventing or delaying damage to the cells and tissues of living organisms.
The body's complex antioxidant enzyme system is influenced by dietary intake of antioxidant vitamins and minerals. Without sufficient amount of these vitamins, an overproduction of ROS could occur, leading to cellular damage. Diets lacking adequate selenium or zinc can also result in abnormally low antioxidant enzyme activity. On the other hand, over consumption on dietary antioxidant can lead to depletion of free radicals, which are essential to certain normal body function.

**Types of antioxidants:**

Antioxidants can be either synthetic, in the form of dietary supplements, or natural, in the form of endogenous enzymes or from medicinal plants sources.

**SYNTHETIC (DIETARY SUPPLEMENTS)**

Vitamin C: Ascorbic acid is a water-soluble vitamin. The RDA recommended daily allowance is 60 mg per day. Intake above 2000 mg may be associated with adverse side effects in some individuals. A major and very potent anti-oxidant; helps protect against many forms of cancer by counteracting the formation of nitrosamines (Cancer-causing substances); aids in the production of "anti-stress" hormones; is needed for healthy adrenal function; helps expel heavy metals and other toxic substances from the body.

**Vitamin E:** d-alpha tocopherol. A fat-soluble vitamin Current (RDA) is 15 IU per day for men and 12 IU per day for women. Slows the progression of Alzheimer's disease; significantly strengthens the immune system; improves muscle strength and stamina.

**Beta-carotene:** a precursor to vitamin A (retinal) Beta-carotene is converted to vitamin A by the body. It guards against heart disease and stroke; lowers cholesterol levels; protects against air pollutants and counteracts night blindness and weak eye sight; builds resistance to infections; slows the progression of osteoarthritis and cataracts.

**NATURAL (ENZYMES)**

Superoxide dismutase (SOD) SOD is an enzyme that converts O$_2^-$ to H$_2$O.

Catalase: converts H$_2$O$_2$ to H$_2$O and O$_2$

Glutathione peroxidase & Glutathione reductase: also convert H$_2$O$_2$. 
PREVENTIVE ANTIOXIDANT PROTEINS

The body has certain proteins, which try to prevent the formation of free radicals e.g.: Transferin, Albumin, Myoglobin, Ceruloplasmin.

REPAIR ENZYMES

Certain enzymes (e.g.: DNA repair enzymes, methionine sulfoxide reductase) try to repair the damaged caused to macromolecules by free radicals. In the normal circumstances, there occurs a continuous generation of free radicals which are effectively scavenged by the antioxidant system of the body. Health complications occur when there is overproduction and/or impaired scavenging of free radicals produced.

MEDICINAL HERBS

Many medicinal plants contain large amount of anti-oxidants such as polyphenols, which play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.

**Indian Medicinal Plants with Antioxidant Property**

Aloe vera, Alstonia scholaris, Andrographis paniculata, Asparagus racemoss, Azadirachta indica, Bacopa monnieri, Bauhinia racemosa Benincasa hispida

Brassica oleracea, Butea monosperma, Caesalpinia bonduc, Camellia sinensis

Cannabis sativa, Capsicum annum, Carica papaya, Cassia fistula, Cassia tora

Celastrus paniculatus, Centella asiatica, Chenopodium album, Cinnamomum verum

Coccinia grandis, Commiphora wighti, Coriandrum sativum, Cucumis melo

Cucurbita pepo, Curcuma longa, Cynodon dactylon, Desmodium gangeticum.

SYNTHETIC DRUGS:

- **N-acetylcystein**: is chemically similar to glutathione. In one study it improves oxygenation lung mechanics in patients with acute lung injury.

  Allopurinol: inhibits the enzyme xanthine oxidase, which generates free radicals during reperfusion injury. Some studies have found that it decrease the formation of myocardial free radicals, while others have no therapeutic effect.
Protective mechanism of antioxidants: Antioxidants are compounds that help to inhibit the many oxidation reactions caused by free radicals thereby preventing or delaying damage to the cells and tissues.

Their mechanism of action include

- Scavenging reactive oxygen and nitrogen free radicals species.
- Decrease the localized oxygen concentration there by reducing molecular oxygen oxidation potential.
- Metabolizing lipid peroxidase to non-radical products.
- Chelating metal ions to prevent the generation of free radicals.

Generally there are two principle mechanisms proposed for antioxidants. The first is a chain breaking mechanisms, by which the primary antioxidants act. The second mechanism involves removal of ROS/RNS initiators (secondary antioxidants) by quenching chain initiating catalysts.

Primary antioxidants are compounds, which are able to donate hydrogen atom rapidly to a lipid radical, forming a new radical, more stable than the initial one. Primary antioxidants such as flavonoids, tocopherol and ascorbic acid, can stop chain reaction by donating an electron to the peroxyl radical of the fatty acids, and thus stop the propagation steps. Enzymes such as glutathione peroxidase (GPx) act by reducing oxidized lipids and phospholipid hydroxide (RCOOH and PL-OOH) to their corresponding alcohols (ROH, PL-OH).

Any compound, which can react with the initiating radical (or inhibit the initiating enzyme), or reduce the oxygen level (without generating reactive radical species), can be considered as a secondary antioxidant. Secondary antioxidants can retard the rate of radical initiation reaction by means of initiator elimination. This can be accomplished by deactivation of high energy species (e.g.- singlet oxygen), absorption of VU light, scavenging of oxygen and thus reducing its concentration, cheatings of metals catalyzing free radical reaction, or by inhibition of peroxidase such as NADPH oxidase, xanthine oxidase, dopamine-hydroxylase or lypoxigenase.
INTRODUCTION TO FLAVONOIDS:

Flavonoids are calls of phenolic compounds widely distributed in plants. They occur either as free molecules or as glycosides. Over thousand individual types are known, and the list is constantly expanding. The term flavonoids is used to include all the pigments that possess structure based upon C₆-C₃-C₆ carbon skeleton found in flavones, chalcones, anthocyanins, etc.²⁸⁹

Examples of yellow Flavanoids (chalcone, aurones and yellow flavanols) and red, blue and purple anthocyanins. When they are not directly visible they contribute to the color by acting as pigments.

Flavanoids have shown potential health benefits arising from the antioxidative effect of these phytochemicals whose properties are attributed to phenolic hydroxyl group attached to the Flavonoid structure.²⁹⁰

Chemistry of flavonoids

The flavonoids are phenolic compounds possessing 15 carbon atoms; two benzene rings joined by a linear three-carbon chain.²⁹¹

Flavanoids constitute one of the most characteristic classes of compounds in higher plants. Mainly Flavanoids are easily recognized as flower pigments in most angiosperm families (flowering plants). However, their occurrence is not restricted to flowers but include all parts of the plant. The chemical structure of Flavonoids are based on C₁₅ skeleton with a CHROMANE ring bearing a second aromatic ring B in position 2, 3 or 4.
AURONES (2-benzyl-coumarone)

The oxygen bridge involving the central carbon atom (C2) of the 3C - chain occurs in a rather limited number of cases, where the resulting heterocyclic is of the FURAN type.\textsuperscript{292}

CLASSIFICATION OF FLAVONOIDS:

Various subgroups of Flavonoids are classified according to the substitution pattern of ring C. both the oxidation state of the heterocyclic ring and the position of ring B is important in the classification. Example of six major subgroups is:

1. CHALCONES:

Chalcones do not have a central heterocyclic nucleus and are characterized by the presence of three-carbon chain with a ketone function and as α, β unsaturation. Substitution on the A ring are most often identical to those other flavonoids, where as the B ring is fairly often Unsubstituted. Isoprenyl and pyranochalcones seem rather common, especially in the Fabaceae. Aurones are characterized by a 2-benzyllidenocoumarone structure.

2. FLAVONE:

(Generally found in herbaceous families, eg. Labiatae, Umbelliferae, Compositae)

In this ring A in over 90% of the cases is substituted by two phenolic hydroxyl groups at C- 5 and C- 7. These hydroxyl groups are either free or esterified, and one of them may be engaged in glycosidic linkage. Other substitutions are possible, as free or etherified hydroxyl groups at C-6 or C- 8 or both in a carbon – carbon bond with a saccharide. The ring B, substituted in the 4’-position in 80% of cases, may be 3’, 4’di-substituted or, less frequently, 3’, 4’, 5’, -trisubstituted, the substituents are OH or OCH\textsubscript{3} groups. The other positions (2’ and 6’) are substituted only exceptionally.

3. FLAVONOL:

(Generally in woody angiosperms)

Quercitol (Ruta graveolens, Fagopyrum esculentum, Sambucus nigra) Kaempferol (Sambucus nigra, Cassia senna, Equisetum arvense, Lamium album, Polygonum bistorta)
These flavonols and their glycosides are universally distributed, but some of the substitution patterns are restricted to some families like Lamiaceae, Rutaceae, and Asteraceae.

4. FLAVONONE:

These molecules are characterized by the absence of a 2,3-double bond, and by the presence of at least one asymmetric center. In flavanones C-2 is normally in the 2S configuration. These flavonoids are somewhat less common than their unsaturated homologs, and it is not worthy that some families tend to accumulate their C-alkylated derivatives (Asteraceae, Fabaceae).

5. ANTHOCYANINS:

The term anthocyanins initially coined to designate the substance responsible for the color of the corn flower, applies to a group of water soluble pigments responsible for the red, pink, mauve, purple, blue, or violet color of most flowers and fruits. These pigments occur as glycosides (the anthocyanins), and their aglycones (the anthocyanidins) are derived from 2-phenylbenzopyrylium cation. Anthocyanins are present in all of the aangiosperms, although they are generally characteristics of flower petals and of the fruits, anthocyanins can also be found in bromeliaceae. Anthocyanins whose vivid color attracts insects and birds play a major role in pollination and seed dispersal. A high coloring power and the absence of toxicity lend these natural coloring glycosides the potential to replace synthetic color in food technology. Therapeutical applications of anthocyanins are limited to treatment of vascular disorders; the drugs containing them are used for the extraction of anthocyanins and the preparation of galanicals designed to treat the symptoms linked to capillary and venous fragility.

6. ISOFLAVANOIDs:

All molecules in this group can be related to skeleton of 3-phenylchromane. They are present in Dicotyledons, They are in face almost specific to Fabaceae only. Nearly 700 isoflavonoids are known they are classified in to dozens of types, in all types we can note the high frequency of isoprenylated derivatives, and consequently of furandihydropuran, and pyran type structures. The most common compound is isoflavone, which occurs in the Free State, or more rarely as glycosides. Isoflavonoids have an additional ring such as in case of pterocarps and their derivatives and also in coumaranochromones. Other isoflavonoids have a coumarinic structure induced by
the oxidation of an isoflavone. Some polycyclic compounds have an additional carbon atom, for example rotenoids arising from the oxidative cyclization products of a 2'-methoxyisoflavone. Most of these (flavanones, flavones, flavonols, and anthocyanines) bear ring B in position 2 of the heterocyclic ring. In isoflavonoids, Ring B occupies position 3. The isoflavonoids and the Neoflavonoids can be regarded as ABNORMAL FLAVONOIDS. 293

PHENOLIC COMPOUNDS IN PLANTS

Antioxidants

Flavonoids

Flavonols
Flavanones
Catechins

Found in tea leaves

Anthocyanins
fruit and vegetable
coloring

Genistein
Diadzein

Found in soy
Converted to

Isoflavones

Powerful anti-cancer
And anti-heart disease
Properties

Dihydroflavonols
Chalcones
Quercetin

Found in onions
Red wine, green tea, onions

Strong antioxidants

Ellagic acid
Berries a good source

Tannic acid
red and green tea
Vanillin

Caffeic acid
fruits, vegetables
Chlorogenic acid

Ferulic acid
fruits, vegetables

Curcumin
turmeric and mugwort
Coumarins
citrus fruits

Phenolic Acids

Hydroxycinnamic acid derivatives

Lignans
become phytoestrogens

Flax seed & other grains

Phenolic Compounds

phytoestrogens

Found in:
Fruits
Vegetables
Grains
SOLUBILITIES AND EXTRACTION OF FLAVONOIDS:

Although as a general rule, glycosides are water-soluble and soluble in alcohol
a fair number are sparingly soluble in a polar organic solvents. When they have at
least one free phenolic group, they dissolve in alkaline hydroxide solutions. Solvents
of medium polarity directly extract lipophilic Flavonoids of leaf, tissues. Glycosides
can be extracted, at high temperature, by acetone or alcohol (Ethanol, Methanol)
mixed with water (20 to 50% depending on whether the drug is fresh or dried).
Solvent evaporation under vacuum can be next followed, when only the aqueous
phase is left, by a series of liquid – liquid extraction by non miscible solvents;
petroleum ether which eliminates chlorophyll and lipids; diethyl ether which extracts
free aglycones and ethyl acetate which dissolves the majority of glycosides. The free
saccharides remain in the aqueous phase with the most polar glycosides when these
are present.

The separation and purification of the different Flavonoids is based on the
usual chromatographic techniques (on polyamide, cellulose or sephadex gel) as in
case of the most of the secondary metabolites, I the last few years HPLC has taken a
place of choice in the battery of isolation techniques for glycosyl flavonoids.

CHARACTERIZATION OF FLAVONOIDS:

Although several color reactions allow the characterization of aglycone and
glycosides in crude extracts, preliminary work on these extracts is classically
dominated by TLC analysis (but paper chromatography has not yet been abandoned)
The chromatogram can be studied:

➢ Directly, since chalcones and aurones are usually visible, and turn orange and
red, respectively in the presence of ammonia vapors.
➢ By examination under UV light before and after spraying with aluminum trichloride, and before and after exposure to ammonia vapors.

➢ After spraying with a 1% solution of the ester of 2-amino ethanol and diphenyl-boric acid, in other worlds the “Naturstoff Reagenza”, by examination under UV light them under visible light.

➢ After spraying with ferric chloride, anisaldehyde diazotized, sulfanilic acid and other general reagents for phenols

➢ By utilizing more or less specific reactions or properties such as reaction with magnesium powder for flavanones and dihydroflavanones, or with zinc for flavanoids, both in presence of hydrochloric acid, reaction of dihydrochalcones, first with sodium borohydrides, then with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.

➢ Structural elucidation, Mass spectrometry and NMR techniques are generally emphasized; UV also can provide very useful information. The usefulness of UV data extends to the use, in routine HPLC analysis, of diode array detectors.

QUANTIFICATION OF FLAVONOIDS:

The classic quantification methods are colorimetric or spectrophotometric. HPLC now makes possible a rapid and precise estimate of all flavonoids present in a drug therefore it is widely used.

ANTIOXIDANT FLAVONOIDS:

Flavonoids or bioflavonoids, are biquitous group of polyphenolic substances which are present in most plants, concentrating in seeds, fruits, skin or peels, bark and flowers. A great number of plant medicine contain flavonoids, which have been reported by as having antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, antithrombic and vasodialator actions, flavonols, flavanones,
flavones, flavan-3-ols (catechins), anthocyanins and isoflavones, flavonoids have been shown in no. of studies to be potent antioxidants, capable of scavenging hydroxyl radicals, superoxide anions, and lipid peroxyl radicals.

**Rutin** SYN; Rutoside, quercetin-3-rutinoside and sophorin

Rutin is a bioflavonoid. Rutin is a flavonol glycoside comprised of the quercetin and the disaccharide rutinose (rhamnose and glucose). Rutin has strong antioxidant properties. Rutin has also the property to chelate metal ions, such as iron, hereby reducing the Fenton reaction (production damaging oxygen radicals).

**Lycopene**

Lycopene belongs to the family of carotenoids. It has a structure that consists of a long chain of conjugated double bonds, with two open-end rings. Lycopene is very efficient antioxidant, which can neutralize oxygen-derived free radicals. The oxidative damage caused by these free radicals has been linked to many degenerative diseases such as cardiovascular diseases, premature aging, cancer and cataracts.

**Asaxanthin syn:** 3,3’- Dihydroxy-b,b-carotene-4,4’ -dione

Astaxanthin is a red carotenoid pigment. Astaxanthin is similar in structure than beta-carotene. Microscopic small plants produce Astaxanthin: the micro-alga Haematococcus pluvialis. Astaxanthin is a powerful antioxidant.

**Quercetin**

Quercetin is the most abundant of the flavonoids. Quercetin belongs to the flavonoids family and consists of 3 rings and 5 hydroxyl groups. Quercetin is also a building block for other flavonoids.

Quercetin has many health promoting effects, including improvement of cardiovascular health, reducing risk for cancer. Quercetin has anti-inflammatory and
anti-allergic effects. All these activities are caused by the strong antioxidant action of quercetin.

**Lutein**

Lutein is an antioxidant, which belongs to the carotenoid. Lutein can lower the risk of age-related vision loss or age-related macular degeneration (AMD) is caused by steady damage of the retina. Lutein probably acts by preventing oxidative damage of the retina cells. Lutein can also reduce the risk for artery diseases. 294

**MATERIALS AND METHODS:**

Antioxidant activity can be measured by using in vitro and in vivo methods and animal studies. The Chemistry responsible for these effects is ready donation of electrons to reactive oxygen species (ROS) by antioxidants, which then quenches the ROS and produces more stable and thus less damaging species.

**In vitro and in vivo Measurement**

The interpretation of results obtained from in vitro and in vivo measurements of antioxidant activity of a compound or a crude plant extract, must be dealt with caution as the antioxidant effect of a tested compound may vary considerably with the method and conditions used, thus selection of the appropriate assay to be used should be based on the intended application of the antioxidant. 295

**Conjugated diene assay**

This method allows dynamic quantification of conjugated dienes as a result of initial PUFA (Poly unsaturated fatty acids) oxidation by measuring UV absorbance at 234 nm. The principle of this assay is that during linoleic acid oxidation, the double bonds are converted into conjugated double bonds, which are characterized by a strong UV absorption at 234 nm. The activity is expressed in terms of Inhibitory concentration (IC50).
Super oxide radical scavenging activity

In-vitro and in vivo super oxide radical scavenging activity is measured by riboflavin/light/NBT (Nitro blue tetrazolium) reduction. Reduction of NBT is the most popular method. The method is based on generation of super oxide radical by auto oxidation of riboflavin in presence of light. The super oxide radical reduces NBT to a blue colored formation that can be ensured at 560nm. The capacity of extracts to inhibit the colour to % is measured.

Reducing Power Method

This method is based on the principle of increase in the absorbance of the reaction mixture. Increase in the absorbance indicates increase in the antioxidant activity. In this method antioxidant compound forms a colored complex with potassium ferricyanide, trichloro acetic acid and ferric chloride, which is measured at 700 nm. Increase in absorbance of the reaction mixture indicates the reducing power of the samples.

Peroxynitrite radical scavenging activity:

Researchers now recognize Peroxynitrite, as the culprit in many toxic reactions that were previously ascribed to is chemical precursors, superoxide and nitric oxide. Hence, an in vitro and in vivo method for scavenging of peroxy radical has been developed to measure antioxidant activity. The scavenging activity is measured by monitoring the oxidation of dihydrorhodamine on a microplate fluorescence spectrophotometer at 485nm.

ABGS (2, 2-azinobis (3-ethyl benzothiazoline-6-sulfonicacid) diamonium salt) method:

This is a measure of antioxidant activity as opposed to antioxidant concentration, which might include a proportion of biologically inactive antioxidants.
It also permits the measurements of antioxidant activity of mixtures of substances and hence helps to distinguish between additive and synergistic effects. The antioxidant activity of wines was measured by using this method. The assay is based on interaction between antioxidant and ABTS+ radical cat ion, which has a characteristic color showing maxima at 645, 734 and 815 nm.

**DMPD (N, N-dimethyl-p-phenylene diamine dihydrochloride) Method:**

This assay is based on the reduction of buffered solution of colored DMPD in acetate buffer and ferric chloride. The procedure involves measurement of decrease in absorbance of DMPD in presence of scavengers at its absorption maxima of 505 nm. The antioxidant activity of wines was measured by using this method. The activity was expressed as percentage reduction of DMPD.

**Oxygen Radical Absorbance Capcity (ORAC):**

ORAC is an exciting and revolutionary new rest tube analysis that can be utilized to test "Antioxidant Power" of foods and other chemical substances. It calculates the ability of a product or chemical to protect against potentially damaging free radicals. This analytical procedure measures the ability of a food, vitamin, nutritional supplement, or other chemicals to protect against the attack by free radicals, or to act as an Antioxidant. The test is performed using Trolox (a watersoluble analog of Vitamin E) as a standard to determine the Trolox Equivalent (TE). The ORAC value is then calculated from the Trolox Equivalent and expressed as ORAC units or value. The higher the ORAC value, the greater the "Antioxidant Power". This assay is based on generation of free radical using AAPH (2,2-azobis 2-amido propane dihydrochloride) and measurement of decrease in fluorescence in presence of free radical scavengers. Hong et al. (1996) have reported automated ORAC assay. In this assay b-phyceroerythin (b-PE) was used as target free radical
damage, AAPH as peroxo radical generator and Trolox as a standard control. After addition of AAPH to the test solution, the fluorescence is recorded and the antioxidant activity is expressed as trolox equivalent.

b-Carotene Linoleate model:

This is one of the rapid method to screen antioxidants, which is mainly based on the principle that Linoleic acid, which is an unsaturated fatty acid, gets oxidized by “Reactive Oxygen Species” (ROS) produced by oxygenated water. The products formed will initiate the b-carotene oxidation, which will lead to discoloration. Antioxidants decrease the extent of discoloration, which is measured at 434nm and the activity is measured.

Xanthine oxidase Method:

This is one of the recent methods for evaluation of antioxidant activity. The percentage inhibition in the xanthine oxidase activity in presence of anti oxidants is measured. Xanthine oxidase enzyme produces uric acid together with super oxide radicals from xanthine and the amount of uric acid is measured at 292nm.

FRAP Method:

FRAP (Ferric Reducing Ability of Plasma) is one of the most rapid test and very useful for routine analysis. The antioxidative activity is estimated by measuring the increase in absorbance caused by the formation of ferrous ions from FRAP reagent containing TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) and FeCl₃·6H₂O. The absorbance is measured spectrophotometrically at 595nm.

TRAP Method:

This method is defined as total radical trapping antioxidant parameter. The fluorescence of R-Phycoerythrin is quenched by ABAP (2,2’-azo-bis (2-amidino-propane) hydrochloride) as a radical generator. This quenching reaction is measured
in presence of antioxidants. The antioxidative potential is evaluated by measuring the delay in decoloration.

**Cytochrome C test:**

Superoxide anions were assayed spectrophotometrically by a cytochrome reduction method described by Mc-Cord and Fridovich (1969). Xanthine oxidase converts xanthine to uric acid and yields superoxide anions and these radicals directly reduce ferri-cytochrome C to ferro-cytochrome C, having an absorbance change at 550 nm. When test compounds showed superoxide scavenger activity, there was a decrease in the reduction of ferri-cytochrome C.

**Erythrocyte ghost system:**

This method involves isolation of erythrocytes ghost cells and the induction of lipid peroxidation using erythrocyte ghosts and the induction of tetra-butyl hydroxy peroxide (t-BHP). TBARS (thio barbituric acid reactive substance) produced during the reaction is measured at 535 nm.²⁹⁶

**Microsomal lipid peroxidation or Thibarbituric acid (TBA) assay:**

TBA test is one of the most frequently used tests for measuring the peroxidation of lipids. Method involves isolation of microsomes from rat liver and induction of lipid proxides with ferric ions leading to the production of small amount of Malonaldehyde (MDA). TBA reacts with MDA to form a pink chromagen, which can be detected spectrophotometrically at 532 nm.²⁹⁷

**MODEL: I**

**Reaction with 1-1-Diphenyl-2Picryl-Hydrazyl (DPPH):**

DPPH is a molecule containing a stable free radical. In the presence of an antioxidant which can donate an electron to DPPH, the purple color which is typical to free DPPH radical decays and the change in absorbency at 517 nm is followed
either spectrophotometrically. This simple test can provide information on the ability
of a compound to donate a hydrogen atom, on the number of electrons a given
molecule can donate, and on the mechanism of antioxidant action. In cases where the
structure of the electron donor is not known (e.g., a plant extract), this method can
afford data on the reduction potential of the sample, and hence can be helpful in
comparing the reduction potential of unknown materials. A similar method, using a
different stable radical (galvinoxyl) was introduced.

FREE RADICAL SCAVENGING CAPACITY ON DPPH RADICAL

Mechanism:

Free radical scavenging potentials of the extracts were tested against a
methanolic solution of 1, 1-diphenyl-2-picryl hydrazyl (DPPH). Antioxidants react
with DPPH and convert it to 1, 1-diphenyl-2-picryl hydrazine. The degree of
discoloration indicates the scavenging potential of the antioxidant extract. The
change in the absorbance produced at 517 nm has been used as a measure of
antioxidant activity.

Procedure:

For the present study the samples were prepared in different concentrations i.e.
10-100 μg/ml in AR grade methanol. The samples of above concentrations were
mixed with 3 ml of 100 μM of DPPH prepared in AR grade methanol and make up the
final volume up to 4 ml with AR grade methanol. The absorbance of the resulting
solutions and the negative (with same chemicals except sample) were recorded after
20 mins. At room temperature, against BHT and ascorbic acid. The disappearance of
DPPH was read spectrophotometrically at 517 nm using a JASCO V 530 UV-Visible
Spectrophotometer. Radical Scavenging Capacity (RSC) in percent was calculated by
following equation.
RSC (%) = 100 x \frac{A_{\text{negative}} - A_{\text{sample}}}{A_{\text{negative}}}

⇒ RSC = Radical Scavenging Capacity

⇒ A_{\text{negative}} = Absorbance of negative

⇒ A_{\text{sample}} = Absorbance of sample.

From the obtained RSC values the % inhibition were calculated, which represents the concentration of the scavenging compound that caused % neutralization.298

MODEL: II

NITRIC OXIDE INHIBITION ACTIVITY:

Principle: Nitric oxide, because of its unpaired electron, is classified as a free radical and displays important reactivity's with certain types of proteins and other free radicals. In vitro inhibition of nitric oxide radical is also a measure of antioxidant activity. Griess reagent is used to detect nitrite photo metrically. The reagent contains two chemicals, sulfanilic acid and N-(1-naphthaldenediamine) under acidic condition sulfanilic acid is converted by nitrite to a diazonium salt, which readily couples with N-(1-naphthaldenediamine) to form a highly colored azo dye that can be detected at 548nm.299

Procedure:

Nitric oxide radical scavenging activity was determined according to the method reported by Garrat (28). Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess Illosvoy reaction. 2ml of 10mM sodium nitroprusside in 0.5 ml phosphate buffer saline(pH 7.4) was mixed with 0.5 ml of extract at various concentrations and the mixture incubated at 25°C for 50 min. From the incubated mixture 0.5 ml was taken out and added into 1.0 ml sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room
temperature for 5 min. Finally, 1.0 ml naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min. The absorbance at 540 nm was measured with a spectrophotometer. The nitric oxide radicals scavenging activity was calculated.

According to the following equation:

\[
\% \text{ inhibition} = 100 \times \frac{A_{\text{negative}} - A_{\text{sample}}}{A_{\text{negative}}}
\]

\[
\text{RSC} \% = 100 \times \frac{A_{\text{negative}} - A_{\text{sample}}}{A_{\text{negative}}}
\]

⇒ RSC = Radical Scavenging Capacity
⇒ A_{\text{negative}} = Absorbance of negative
⇒ A_{\text{sample}} = Absorbance of sample.
⇒ BHT was used as a positive control.

From the obtained RSC values, the % values were calculated, which represent, the concentration of the scavenging compound that caused % neutralization.  

MODEL: III

REAGENTS FOR ESTIMATION OF LIPID PEROXIDATION\textsuperscript{301}

a) HCl (0.25 N): 2.125 ml of concentrated HCl
b) TCA (15%): Take 15 g of TCA.
c) TBA (0.375%): Take 375 mg of TBA
d) TCA-TBA-HCl reagent: Dissolve the above chemicals and make up the volume to 100 ml.

Procedure:

Lipid peroxidation was estimated in terms of thiobarbituric acid reactive species (TBARS), using malondialdehyde (MDA) as standard by the method of Buege and Aust. 1.0 ml of the sample extract was added with 2.0 ml of the TCA-TBA-HCl reagent (15% w/v TCA, 0.375% w/v TBA and 0.25 N HCl). The contents
was boiled for 15 minutes, cooled and centrifuged at 10000 rpm to remove the precipitate. The absorbance was read at 535 nm and malodialdehyde concentration of the sample was calculated using extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$.

**MODEL: IV**

**REAGENTS FOR ESTIMATION OF SUPEROXIDE DISMUTASE**

1. Sodium Pyrophosphate buffer (0.052 M, pH 8.3): 2.31 gm in 100 ml of distilled water adjusted to pH 8.3
2. 186µM Phenazine metha sulphate (PMS): 5.7 mg in 100 ml of distilled water.
3. 300µM nitro blue tetrazolium (NBT): 2.45 mg in 1 ml of distilled water.
4. 780µM NADH: 5.53 mg in 1 ml of distilled water.
5. N-butanol.

**Procedure:**

The estimation will be done as per Kakkar et al. The liver tissue was homogenized by using a Potte-Ellvehjem glass homogenizer for 30 sec either in ice cold 0.9% saline (5%). Briefly, to 0.4ml of the homogenate was added 1.2 ml of sodium pyrophosphate buffer (pH 8.3, 0.052 M), 0.1 ml of 186 µM of phenazine metha sulphate (PMS), 0.3 ml of 300 µM nitro blue tetrazolium (NBT) and 0.8 ml of distilled water was added to make up the volume up to 3 ml. The reaction was started by addition of 0.2ml of NADH (780 µM). It was incubated at 30°C for 60 sec. The reaction was stopped by addition of glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4ml of n-butanol. The mixture was allowed to stand for 10 min, centrifuged and butanol layer will be taken out. Colour intensity of the chromogen was measured against butanol at 560 nm using Spectrophotometer. A system devoid of enzyme activity was defined as enzyme concentration required
decreasing the rate of reaction by 50% in one min under the assay conditions. The results are expressed as units (U) of SOD activity / g wet tissue.

MODEL: V

REAGENTS FOR ESTIMATION OF CATALASE:

a) 0.2 M phosphate buffer (pH 8.0): 50 ml of potassium di-hydrogen orthophosphate (0.2M) + 46.1 ml NaOH (0.2M), volume made up to 200 ml and pH adjusted to 8.

b) 2 M Hydrogen peroxide: 22.45 ml of H2O2, volume made up to 100 ml.

c) 5% potassium dichromate: 5 gm in 100 ml of distilled water.

d) Dichromate-acetic acid reagent – 5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratios.

Procedure:

The liver tissue was weighed and 10% homogenate was prepared with 0.2 M phosphate buffer pH 8.0. After centrifugation, the clear supernatant was used for assay of enzyme activity. The reaction mixture (1.5ml, vol.) contained 1.0 ml of 0.01 M pH 7.0 phosphate buffer, 0.1 ml of tissue homogenate (supernatant) and 0.4 ml of 2 M H2O2. The reaction was stopped by the addition of 2.0 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio). Colour intensity was measured colorimetrically at 620 nm and expressed as % inhibition of H2O2 consumed /min/mg protein as described by Sinha.
RESULTS:

Table-7.3: EFFECT OF SCOPARIA DULCIS ON DPPH FREE RADICAL SCAVENGING PROPERTY AFTER 5 MINUTES

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ±SEM</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>2.056±0.05</td>
<td>--</td>
</tr>
<tr>
<td>Alcoholic Extract</td>
<td>0.83±0.02</td>
<td>59.57</td>
</tr>
<tr>
<td>Ethyl Acetate Extract</td>
<td>0.71±0.03</td>
<td>65.47</td>
</tr>
<tr>
<td>Aqueous Extract</td>
<td>0.94±0.04</td>
<td>54.29</td>
</tr>
<tr>
<td>Pet. Ether Extract</td>
<td>0.9835±0.03</td>
<td>52.17</td>
</tr>
<tr>
<td>Benzene Extract</td>
<td>1.444±0.06</td>
<td>29.7</td>
</tr>
<tr>
<td>Chloroform Extract</td>
<td>1.288±0.02</td>
<td>37.37</td>
</tr>
</tbody>
</table>

Above results were compared with negative control at $\lambda = 517$ nm.

Fig No.: 7.1 Histogram showing RSC of DPPH
Table-7.4: Free Radical Scavenging Activity of Nitric Oxide by different Extracts

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ±SEM</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>1.792±0.05</td>
<td>--</td>
</tr>
<tr>
<td>Alcoholic Extract</td>
<td>0.38±0.02</td>
<td>78.8</td>
</tr>
<tr>
<td>Ethyl Acetate Extract</td>
<td>0.6061±0.03</td>
<td>66.18</td>
</tr>
<tr>
<td>Aqueous Extract</td>
<td>1.0693±0.04</td>
<td>38.82</td>
</tr>
<tr>
<td>Pet. Ether Extract</td>
<td>1.387±0.04</td>
<td>22.62</td>
</tr>
<tr>
<td>Benzene Extract</td>
<td>1.725±0.06</td>
<td>3.76</td>
</tr>
<tr>
<td>Chloroform Extract</td>
<td>1.6201±0.05</td>
<td>9.61</td>
</tr>
</tbody>
</table>

Above results were compared with negative control at \( \lambda = 540 \text{ nm} \).

![RSC of Nitric Oxide by Different Extracts](image)
Table-7.5: Free Radical Scavenging Activity of Lipid Peroxidation by different Extracts

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ±SEM</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>0.386±0.06</td>
<td>--</td>
</tr>
<tr>
<td>Alcoholic Extract</td>
<td>0.113±0.04</td>
<td>71.16</td>
</tr>
<tr>
<td>Ethyl Acetate Extract</td>
<td>0.139±0.02</td>
<td>65.82</td>
</tr>
<tr>
<td>Aqueous Extract</td>
<td>0.1663±0.05</td>
<td>56.91</td>
</tr>
<tr>
<td>Pet. Ether Extract</td>
<td>0.2128±0.04</td>
<td>45.07</td>
</tr>
<tr>
<td>Benzene Extract</td>
<td>0.2591±0.02</td>
<td>32.87</td>
</tr>
<tr>
<td>Chloroform Extract</td>
<td>0.2421±0.03</td>
<td>37.27</td>
</tr>
</tbody>
</table>

Above results were compared with negative control at $\lambda = 535$ nm.
Table: 7.6 Histogram showing RSC of Lipid Peroxidase Free Radical Scavenging Activity of Superoxide Dismutase by different Extracts

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ±SEM</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>1.099±0.04</td>
<td>--</td>
</tr>
<tr>
<td>Alcoholic Extract</td>
<td>0.2508±0.05</td>
<td>77.17</td>
</tr>
<tr>
<td>Ethyl Acetate Extract</td>
<td>0.2995±0.05</td>
<td>74.74</td>
</tr>
<tr>
<td>Aqueous Extract</td>
<td>0.3456±0.04</td>
<td>68.55</td>
</tr>
<tr>
<td>Pet. Ether Extract</td>
<td>0.4146±0.03</td>
<td>62.27</td>
</tr>
<tr>
<td>Benzene Extract</td>
<td>0.4576±0.02</td>
<td>58.36</td>
</tr>
<tr>
<td>Chloroform Extract</td>
<td>0.367±0.04</td>
<td>66.60</td>
</tr>
</tbody>
</table>

Above results were compared with negative control at $\lambda = 560$ nm.

Fig No.: 7.4 Histogram showing RSC of Superoxide Dismutase
Table: 7.7 Histogram showing RSC of Lipid Peroxidase Free Radical Scavenging Activity of Catalase by different Extracts

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean± SEM</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>2.389±0.07</td>
<td>--</td>
</tr>
<tr>
<td>Alcoholic Extract</td>
<td>0.3345±0.02</td>
<td>85.99</td>
</tr>
<tr>
<td>Ethyl Acetate Extract</td>
<td>0.4141±0.03</td>
<td>93.96</td>
</tr>
<tr>
<td>Aqueous Extract</td>
<td>0.0641±0.02</td>
<td>97.31</td>
</tr>
<tr>
<td>Pet. Ether Extract</td>
<td>0.5088±0.04</td>
<td>78.7</td>
</tr>
<tr>
<td>Benzene Extract</td>
<td>0.361±0.02</td>
<td>84.88</td>
</tr>
<tr>
<td>Chloroform Extract</td>
<td>0.5071±0.04</td>
<td>77.7</td>
</tr>
</tbody>
</table>

Above results were compared with negative control at $\lambda = 620$ nm.

Fig No.: 7.5 Histogram showing RSC of Catalase
Effect of extracts of *Scoparia dulcis* Linn. on oxidative status

<table>
<thead>
<tr>
<th>Group</th>
<th>DPPH (%Inhibition)</th>
<th>Nitric Oxide (%Inhibition)</th>
<th>Lipid Peroxidation (%Inhibition)</th>
<th>Superoxide Dismutase (%Inhibition)</th>
<th>Catalase (%Inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>2.056±0.05</td>
<td>1.792±0.05</td>
<td>0.386±0.06</td>
<td>1.099±0.04</td>
<td>2.389±0.07</td>
</tr>
<tr>
<td>Alcoholic Extract</td>
<td>0.83±0.02</td>
<td>0.38±0.02</td>
<td>0.113±0.04</td>
<td>0.2508±0.05</td>
<td>0.3345±0.02</td>
</tr>
<tr>
<td></td>
<td>(59.57)*</td>
<td>(78.8)*</td>
<td>(71.16)*</td>
<td>(77.17)*</td>
<td>(85.99)*</td>
</tr>
<tr>
<td>Ethyl Acetate</td>
<td>0.71±0.03</td>
<td>0.6061±0.03</td>
<td>0.139±0.02</td>
<td>0.2995±0.05</td>
<td>0.4141±0.03</td>
</tr>
<tr>
<td>Extract</td>
<td>(65.47)*</td>
<td>(66.18)*</td>
<td>(65.82)*</td>
<td>(74.74)*</td>
<td>(93.96)*</td>
</tr>
<tr>
<td>Aqueous Extract</td>
<td>0.94±0.04</td>
<td>1.0693±0.04</td>
<td>0.1663±0.05</td>
<td>0.3456±0.04</td>
<td>0.0641±0.02</td>
</tr>
<tr>
<td></td>
<td>(54.29)*</td>
<td>(38.82)*</td>
<td>(56.91)*</td>
<td>(68.55)*</td>
<td>(97.31)*</td>
</tr>
<tr>
<td>Pet. Ether Extract</td>
<td>0.9835±0.03</td>
<td>1.387±0.04</td>
<td>0.2128±0.04</td>
<td>0.4146±0.03</td>
<td>0.5088±0.04</td>
</tr>
<tr>
<td></td>
<td>(52.17)</td>
<td>(22.62)</td>
<td>(45.07)</td>
<td>(62.27)</td>
<td>(78.7)</td>
</tr>
<tr>
<td>Benzene Extract</td>
<td>1.444±0.06</td>
<td>1.725±0.06</td>
<td>0.2591±0.02</td>
<td>0.4576±0.02</td>
<td>0.361±0.02</td>
</tr>
<tr>
<td></td>
<td>(29.7)</td>
<td>(3.76)</td>
<td>(32.87)</td>
<td>(58.36)</td>
<td>(84.88)</td>
</tr>
<tr>
<td>Chloroform Extract</td>
<td>1.288±0.02</td>
<td>1.6201±0.05</td>
<td>0.2421±0.03</td>
<td>0.367±0.04</td>
<td>0.5071±0.04</td>
</tr>
<tr>
<td></td>
<td>(37.37)</td>
<td>(9.61)</td>
<td>(37.27)</td>
<td>(66.60)</td>
<td>(77.7)</td>
</tr>
</tbody>
</table>

Values are in Mean ± SEM; n = 6, ns = not significant

* significant
DISCUSSION

Present studies include the systematic phytochemical and antioxidant activity of Scoparia dulcis Linn. Antioxidant activity of pet ether, benzene, chloroform, ethyl acetate, alcohol and aqueous extract of Scoparia dulcis Linn. Were evaluated using different in vitro and in vivo models.

- Five models used for the in vitro and in vivo studies includes
- Free radical scavenging activity of superoxide dismutase
- Free radical scavenging activity of catalase
- Free radical scavenging activity of lipid peroxidation
- Free radical scavenging capacity (RSC) on DPPH radical
- Free radical scavenging capacity on Nitric oxide radical

The results obtained were comparing to with negative control. In free radical scavenging against DPPH. Alcoholic extract showed (59.5%), in nitric oxide (78.8%), in lipid Peroxidation (71.16%), in Superoxide Dismutase (77.17%) and in Catalase (85.99%) of inhibition of free radicals. So we can conclude that flavonoids containing extracts have shown significant anti oxidant activity.

Antioxidant Activity:

Antioxidant activity of different extracts such as, petroleum ether, benzene, chloroform, ethyl acetate, alcohol and aqueous extracts of Scoparia dulcis Linn. were evaluated using different in vitro and vivo models. Five models were used for in vitro and vivo studies.

- Free radical scavenging on DPPH radical
- Free radical scavenging capacity on nitric oxide radical
- Free radical scavenging activity of Lipid Peroxidase
Free radical scavenging activity of Catalase
Free radical scavenging activity of Superoxide

The results obtained were compared with negative control, ethyl acetate, alcoholic and aqueous extracts have shown significant percentage inhibition.