CHAPTER - I

INTRODUCTION
# TABLE OF CONTENT

## CHAPTER: 1

<table>
<thead>
<tr>
<th>Chapter 1</th>
<th>INTRODUCTION</th>
<th>1-22</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>1.1</td>
<td>Herbal Drugs</td>
<td>1</td>
</tr>
<tr>
<td>1.2</td>
<td>Allergy</td>
<td>2-9</td>
</tr>
<tr>
<td>1.2.1</td>
<td>Types of allergy</td>
<td>3</td>
</tr>
<tr>
<td>1.2.2</td>
<td>Treatment of allergy</td>
<td>5</td>
</tr>
<tr>
<td>1.2.3</td>
<td>Ayurveda and allergy treatment</td>
<td>6</td>
</tr>
<tr>
<td>1.2.4</td>
<td>Traditionally used anti-allergic plant</td>
<td>6</td>
</tr>
<tr>
<td>1.2.5</td>
<td>Ayurvedic formulations used in treatment of allergy</td>
<td>6</td>
</tr>
<tr>
<td>1.2.6</td>
<td>Experimental models for screening of antiallergic agent</td>
<td>7</td>
</tr>
<tr>
<td>1.3</td>
<td>Antioxidants</td>
<td>10-12</td>
</tr>
<tr>
<td>1.3.1</td>
<td>Models for screening of antioxidant activity</td>
<td>11</td>
</tr>
<tr>
<td>1.4</td>
<td>References</td>
<td>13-22</td>
</tr>
</tbody>
</table>
1. INTRODUCTION

1.1 Herbal Drugs

Herbal medicines consist of herbs, herbal materials, herbal preparations and finished herbal products which contain as active ingredients, parts of plants, or other plant materials, or combinations defined by World Health Organization (WHO).\(^1\) Herbs used as medicinal purposes for many centuries. Now a day, herbal medicines are used globally as home remedies in treatment of various ailments. People rely mainly on herbal drugs to meet their primary health care needs in some developing countries. Herbal medicines are also gaining popularity as alternative and complementary therapies in many industrialized and developed countries. There has been an increase in scientific studies on herbal medicines, due to insufficient availability of reliable data still today.

Herbal medicines are an integral part of the development of modern civilization. Most of the medicinal uses of plants have been developed through observations by trial and error. Tribes added the medicinal herbs in their regimen of curing various diseases, on the bases of herbals knowledge observed in routine life. Collection of systematic information on herbs, ultimately lead to develop well-defined monographs and herbal pharmacopoeias in different countries.

The WHO estimates 80% of the world population (4 billion) presently consume herbal medicine for some aspect of primary health care. Herbal medicine is the major component in all indigenous traditional medicine and a common element in ayurvedic, homeopathic, naturopathic, traditional, and native. WHO notes that out of 119 plant-derived pharmaceutical medicines, about 74% are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by human beings. Currently, major pharmaceutical companies are conducting extensive research on plant materials for their potential medicinal value.

Substances derived from the plants remain the basis for a large proportion of the commercial medications used today for the treatment of various diseases.\(^2\) For example, ephedra is an herb used in traditional Chinese medicine for more than 2000 years to treat asthma and other respiratory problems. Ephedrine derived from it is used in the
commercial pharmaceutical preparations for the relief of asthma symptoms. Foxglove (digitalis) plant used since 1775; presently the powdered leaf of it or active constituents (digoxin) is known as the cardio tonic.

Traditional medicine is the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences which are indigenous to different cultures, whether explicable or not, used in the maintenance of health, as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnesses. Traditional medicine has been developed based on history and culture of the countries or regions. The term “traditional medicine” covers a wide variety of therapies and practices which vary greatly from country to country and from region to region. Traditional, complementary, or alternative medicine has many positive features. Traditional medicine and its practitioners play an important role in treating chronic illnesses, and improving the quality of life of those suffering from minor illness or from certain incurable diseases.³

Traditional medicine are facing many challenges and difficulties like; lack of legal recognition, appropriate law and regulations, knowledge of preservation and protection, appropriate control mechanism and approaches to control safety, efficacy and quality, adequate support to the research resources, training, education programmes and licensing practice for qualified practice of traditional medicine or medicinal plants, practice and practitioner.⁴

It is estimated that at least 25% of all modern medicines are derived, either directly or indirectly, from medicinal plants, primarily through the application of modern technology to traditional knowledge. In the case of certain classes of pharmaceuticals, such as antitumor and antimicrobial medicines, this percentage may be 60%. In developing countries, 70%–95% of population relies on traditional medicines for primary care.⁵

1.2 Allergy

About 30-40% of world population is affected by allergic conditions.⁶ The first allergic history was reported in the year 2641 BC after wasp sting.⁷ In the middle age rose fever with hay fever like symptoms was found. The first clinical description of hay fever was given by John Bostock⁸. The term "allergy" was originally introduced by von Pirquet⁹ in
1906, meaning "changed reactivity." World allergy organization (WAO) define, allergy is a hypersensitivity reaction initiated by immunological mechanisms. Allergy can be antibody or cell mediated. In the majority of cases the antibody responsible for an allergic reaction belongs to the IgE isotype and these individuals may be referred to as suffering from an IgE-mediated allergy. In non-IgE-mediated allergy the antibody can belong to the IgG isotype, eg, anaphylaxis due to immune complexes containing dextran. Both IgE and IgG antibodies are found in allergic bronchial pulmonary aspergillosis (ABPA). Allergic contact dermatitis is mediated by lymphocytes.

Allergens are antigens which cause allergy. Most allergens reacting with IgE and IgG antibody are proteins, often with carbohydrate side chains, pure carbohydrates, low molecular weight chemicals, eg, isocyanates and anhydrides acting as haptens. In allergic contact dermatitis, the allergens are low molecular weight chemicals reacting with cells eg, chromium, nickel and formaldehyde.

1.2.1 Types of allergy

Allergy is a reaction between immune system and a foreign body or an allergen. The body’s immune system or the defense mechanism works overtime fighting these allergens hence, it is also called as hypersensitivity. Steps involve in allergy are i) appearance of antigen, ii) synthesis of antibodies that is specific to antigen and iii) antigen –antibody reaction. Allergy is humoral or cell mediated. It can be caused due to medicines, food, insect, pollen, insect stings, and mold spores. It causes symptoms like sneezing, runny nose, itching rashes, urticaria, swelling, asthma. Coomb and Gell described four types of hypersensitivity; e.g. type-I or anaphylaxis, type-II or Cytotoxic, type III or toxic complex reaction and type IV or cell mediated.

Type I Hypersensitivity (Atopic allergy): It is characterized by an allergic reaction that occurs immediately following contact with the antigen, referred to as the allergen. Atopic allergy is mediated by IgE, antibodies which are bound to the mast cells. When allergens cross-link the IgE molecules degranulation is induced and mediators such as histamine and other vasoactive amines are released and produce the allergic reactions. Total and allergen specific circulating IgE antibodies can be measured in serum. Common environmental allergens inducing IgE-mediated allergy are cat, dog and horse epithelium,
tree, grass, weed pollen, house dust mites and molds. Food allergens are also a common cause of allergy, even though food allergy is more frequently seen in children than in adults. In recent years "allergy" only has become synonymous with Type I.

The term atopy was coined in 1923 by Coca and Cooke\textsuperscript{17} to describe clinical allergy of an inherited nature. Nowadays an atopic constitution implies a hereditary tendency to produce IgE to common environmental allergens and an increased risk of developing asthma, rhino-conjunctivitis (hay fever), urticaria and atopic eczema. Atopic patients also have an increased tendency to be allergic to food substances, which can result in various symptoms, including described above and others of the gastrointestinal tract.

**Type II Cytotoxic type:** In these type of immune reactions the antibodies are free in the serum while antigens are bound to the surface of certain cells or a component of the cell membrane. Often the substances concerned are small molecules, haptens that are fixed to the cell surface, thus resulting in the formation of the antigen. When the antibodies (of IgG or IgM type) react with the antigen, complement is activated, the cell is damaged and lysis may occur. A complement induced accumulation of granulocytes, together with release of anaphylatoxins and histamine, can lead to further damage. Drug reactions, transfusion reactions to erythrocytes, hemolytic disease of the newborn and autoimmune hemolytic anaemia are some of the examples of type II hypersensitivity. The pathology depends on the molecules and tissues targeted by the antibodies.

**Type III Toxic complex reaction:** Precipitins, the free circulating antibodies belong to the IgG class, cause these reactions.\textsuperscript{18} The complement system is activated when these antibodies react with locally introduced antigens to produce an antigen-antibody complex. This activation of complement, together with complement induced granulocyte accumulation and release of histamine via anaphylatoxins, results in tissue damage, an Arthur’s reaction. Allergic alveolitis is an example of such an immune complex reaction: inhalation of antigens such as molds, plant or animal antigens results in tissue changes, giving rise to symptoms such as coughing, dyspnoea, fever and cyanosis. The tissue damage may be irreversible in persistent allergic condition. Persistent infections and autoimmunity could cause other categories of immune-complex diseases. Another example of this type reaction is serum sickness, antigen introduction, in the form of a
foreign protein, results in the formation of IgG antibodies. The antigen-antibody complex formed from circulating antigen and a precipitated antibody is deposited in different tissues (e.g. lung), resulting in tissue damage. It has been shown that the immune complex which is formed with an excess of antigen is the most damaging. The symptoms of serum sickness can partly be of a general nature such as fever, pain in the joints, skin changes or swelling of the lymph glands, but they can also be localized to organs such as the heart or the kidneys.

Type IV cell mediated type: This type of hypersensitivity differs from the other types of reactions in that it is not caused by antibodies, but by lymphocytes cells which are immunologically specific with receptors for the antigens, tissue antigens or small molecular substances, fixed to the cell membrane. These reactions called delayed hypersensitivity reactions, which occurs 12-48 hours after exposure to the antigen. It leads to inflammatory tissue damage and infiltration of cells, which are principally mononuclear (lymphocytes and macrophages). The inflammatory reaction leads to irreversible damage with deterioration of the tissue. The classical examples of type IV hypersensitivity are the positive tuberculin reaction, contact dermatitis (e.g. caused by nickel or chrome) and rejection of tissues transplanted from other individuals. Histamine is one of the important mediators of allergy, inflammation and bronchoconstriction, which were released after degranulation of mast cell by antigen exposure. On activation, mast cells released immediately the preformed and the \textit{de novo} synthesized other mediators proteases, leukotrienes, prostaglandins and cytokines. As a consequence, the acute reactions such as vasodilation, increased vascular permeability, and bronchoconstriction will be induced. In addition, allergic responses also trigger the influx and activation of a variety of inflammatory cells including eosinophils and lymphocytes. Rapidly released mediators and numerous cytokines produced by mast cells are strongly believed to induce and sustain these responses, which may contribute to chronic inflammation. Targeting histamine, either preventing its release from mast cell or histaminergic receptor antagonist becomes part of therapy in allergic diseases.

1.2.2 Treatment of allergy \textsuperscript{20}

The avoidance of allergen is first step to controlling allergies. The skin allergy can be reduced by using soothing creams and wet wrapping. Drugs used to treat allergies;
i. Drug blocks the activity of mediators which are released during allergic reactions. e.g. Antihistamines and leukotriene antagonists.

ii. Drugs relax the constricted muscle around the airways of the lungs, or shrink congested tissue, or reverse the effects of the chemicals released during allergic reactions. e.g. bronchodilators, decongestants and epinephrine.

iii. Drugs which reduce inflammation. e.g. corticosteroids.

iv. Drug modifies the immune response. e.g. allergen immunotherapy. antihistamines and leukotriene antagonists

1.2.3 Ayurveda and allergy treatment

In Ayurveda the main causative factor of allergy is from improperly digested food called *Ama*. Allergic reaction illnesses (ARIs), drug allergies, or hypersensitivities are considered *Kapha* dominated diseases in Ayurveda, e.g. asthma and eczema. In Allopathic medicine, the treatment of these diseases clusters around the use of steroids, antihistamines and bronchodilators (beta-adrenergic blockers). The treatment of allergic reactions in Ayurveda essentially consists of identifying the allergens, avoiding the exposure to them, using drugs to relieve acute symptoms, improving digestion and cleaning the intestine of toxic materials in the gut (*ama*). Emesis is recommended to treat allergy and asthma because ARI are considered *kaphaja* disease.

1.2.4 Traditionally used anti-allergic plant

*Adhatoda vasica, Albezia lebbeck, Allium cepa, Allium Sativa, Alpinia galangal, Benincasa hispida, Boerhavia diffusa, Calotropis procera, Cedrus deodara, Curcuma longa, Cyperus rotundus, Datura stramonium, Dolichos biflorus, Elettaria cardamomum, Emblica officinalis, Fagonia cretica, Galphimia glauca, Glycyrrhiza glabra, Inula racemosa, Mentha spicata, Mucuna pruriens, Ocimum sanctum, Phyllanthus surinaria, Picrorhiza kurora, Piper longum, Rhus succedanea, Solanum xanthocarpum, Terminalia bellerica, Tylophora indica, Vitis vinifera, Withania somnifera and Zingiber officinale.*

1.2.5 Ayurvedic formulations used in treatment of allergy

Kantaryadikvath, Srmgyadikvath, Vasadikvath, Agastyaharitikiavaleha, Chavanprashavaleha, Chitrakaharitikiavaleha, Vasaavaleha, Vyaghriharitikiavaleha, Draksharishta,
1.2.6 Experimental models for screening of antiallergic agent

A. Acetyl choline or histamine-induced bronchospasm in guinea pig\textsuperscript{24,25}: Asthma can be experimentally induced in guinea pig by exposing to acetylcholine or histamine in an aerosol chamber. A guinea pig shows progressive signs of breathing difficulty leading to convulsions, asphyxia and death. The time until the appearance of convulsion is called pre-convulsion time (PCD). The end point for PCD is determined from the time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsions. Similarly, PCD is determined with selected extracts. The protection offered by the extracts can be calculated by the following formula.

\[
\text{Percentage protection} = (1 - \frac{T1}{T2}) \times 100
\]

Where;
\(T1\) is time for PCD onset (Without treatment) and
\(T2\) is the time for PCD onset. (Treated with extracts)

B. \textit{In vitro} studies on isolated guinea pig ileum\textsuperscript{26}: Guinea pig ileum is rich with histamine receptor. Histamine causes contraction of ileum. The contractile response of ileum to histamine is determined in presence and absence of selected extract.

C. Milk-induced leucocytosis in mice\textsuperscript{27}: The boiled and cooled milk injected in mice subcutaneously induces leukocytosis, one of the symptoms of allergic condition. Difference in total leukocyte count before plant extract administration and after milk injection indicates the antiallergic potential of plant extracts.

D. Isolated goat tracheal chain\textsuperscript{28}: Isolated adult goat tracheal tissue produce contraction in presence of histamine, acetyl choline, 5-hydroxytryptamine or bradykinin. The contractile response of goat tracheal chain to histamine is determined in presence and absence of selected extract. Percent of maximum contractile responses is determined in the absence and presence of plant extract.

E. Mast cell stabilization activity\textsuperscript{29,30}: Peritoneal fluid of rats contains mast cell. The mast cell can be collected from peritoneal fluid. The compound 48/80 causes
degranulation of mast cell and liberates histamine. The selective dye like toluidine blue stains disrupted mast cells while undisrupted mast cells remain as such. Percentage protection from degranulation of mast cells can be determined in presence and absence of plant extracts.

F. Passive paw anaphylaxis in rats\textsuperscript{31,32}: Passive paw anaphylaxis is induced in Wistar rats egg albumin (s.c.) adsorbed on aluminum hydroxide gel. After sensitization, blood serum injected in the left hind paw of animals. The contra lateral paw received unequal volume of saline. Drug treatment is given after sensitization. Then animals are again challenged in the left hind paw with egg albumin. The difference in the reading prior to, and after antigen challenge represented the edema volume and the percent inhibition of volume is calculated by using the following formula.

\[
\text{Percentage inhibition} = 1 - \left( \frac{V_t}{V_c} \right) \times 100
\]

Where:

\( V_t = \) Mean relative change in paw volume in test group

\( V_c = \) Mean relative change in paw volume in control group.

G. Antipruritic activity\textsuperscript{33}: Scratching is induced by administering compound 48/80 subcutaneously in to dorsal surface of the neck of mice. The incidence of scratching behaviour on the whole body and the site injected with compound 48/80 can be determined with and without treatment of selected plant extracts.

H. In vitro studies on isolated Rat ileum\textsuperscript{34}: Acetyl choline cause bronchial contraction, so it exaggerates the allergic condition. Rat’s ileum is rich with the acetyl choline receptor. The contractile response of ileum to acetyl choline is determined in presence and absence of selected extract.

Herbs are used in treatments of allergic ailments like hay fever and asthma. These herbs work to decrease the production of chemicals responsible for allergy. Herbs with natural antihistamine activity act by reducing formation of histamine.\textsuperscript{35} The list of some plants reported antiallergic activity is given in Table1.1.
### Table 1.1 List of Plants having antiallergic activity

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<thead>
<tr>
<th>Plant</th>
<th>Part</th>
<th>Plant</th>
<th>Part</th>
</tr>
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<tbody>
<tr>
<td>Abrus precatorius³⁶</td>
<td>Leaves</td>
<td>Gleditsia sinensis³⁷</td>
<td>Fruits</td>
</tr>
<tr>
<td>Acanthopanax senticosus³⁸</td>
<td>Root</td>
<td>Hemides musindicus³⁹</td>
<td>Root</td>
</tr>
<tr>
<td>Ailanthus excelsa⁴⁰</td>
<td>stem bark</td>
<td>Inula japonica⁴¹</td>
<td>Flowers</td>
</tr>
<tr>
<td>Albizia lebeck⁴²</td>
<td>Bark, flower</td>
<td>Lagenaria siceraria⁴³</td>
<td>Leaf</td>
</tr>
<tr>
<td>Amaranthus spinosus⁴⁴</td>
<td>Leaves</td>
<td>Lycopus lucidus⁴⁵</td>
<td>--</td>
</tr>
<tr>
<td>Aristolochia Indica⁴⁶</td>
<td>Root</td>
<td>Magnolia officinalis⁴⁷</td>
<td>Bark</td>
</tr>
<tr>
<td>Aristolochia atagala⁴⁸</td>
<td>Root</td>
<td>Mirabilis jalapa⁴⁹</td>
<td>Root</td>
</tr>
<tr>
<td>Artemisia iwayomogi⁵⁰</td>
<td>--</td>
<td>Momordica dioica⁵¹</td>
<td>Fruit</td>
</tr>
<tr>
<td>Asystasia gangetica⁵²</td>
<td>Leaf</td>
<td>Morus bombycis⁵³</td>
<td>--</td>
</tr>
<tr>
<td>Bauhinia variegata⁵⁴</td>
<td>Bark</td>
<td>Myrica esculenta⁵⁵</td>
<td>stem bark</td>
</tr>
<tr>
<td>Bidens pilosa⁵⁶</td>
<td>--</td>
<td>Myrica sapida⁵⁷</td>
<td>--</td>
</tr>
<tr>
<td>Bunium persicum⁵⁸</td>
<td>--</td>
<td>Passiflora incarnata⁵⁹</td>
<td>Leaves</td>
</tr>
<tr>
<td>Camellia japonica⁶⁰</td>
<td>Leaf</td>
<td>Perilla frutescens</td>
<td></td>
</tr>
<tr>
<td>Carum coticum⁶¹</td>
<td>--</td>
<td>Prunella vulgaris⁶², ⁶³</td>
<td>--</td>
</tr>
<tr>
<td>Cassia alata⁶⁴</td>
<td>Leaves</td>
<td>Pterocarpus marsupium⁶⁵</td>
<td>Bark</td>
</tr>
<tr>
<td>Cassia sophera⁶⁶</td>
<td>Leaves</td>
<td>Punica granatum⁶⁷</td>
<td>Flower bud</td>
</tr>
<tr>
<td>Cecropia glaziov⁶⁸</td>
<td>Leaves</td>
<td>Rehmannia glutinosa⁶⁹</td>
<td>--</td>
</tr>
<tr>
<td>Centella asiatica⁷⁰</td>
<td>--</td>
<td>Rumex gmelini</td>
<td>Herb</td>
</tr>
<tr>
<td>Cichorium intybus⁷¹</td>
<td>--</td>
<td>Salvia plebeia⁷²</td>
<td>--</td>
</tr>
<tr>
<td>Clerodendron trichotomum⁷³</td>
<td>Leaves</td>
<td>Sanseveiria trifasciata⁷⁴</td>
<td>Leaves</td>
</tr>
<tr>
<td>Clitoria ternatea⁷⁵</td>
<td>Roots</td>
<td>Schisandra chinensis⁷⁶</td>
<td>Fruit</td>
</tr>
<tr>
<td>Crocus sativus⁷⁷</td>
<td>--</td>
<td>Solanum nigrum⁷⁸</td>
<td></td>
</tr>
<tr>
<td>Curculigo orchoides⁷⁹</td>
<td>Rhizome</td>
<td>Sphaeranthus indicus⁸⁰</td>
<td>Whole plant</td>
</tr>
<tr>
<td>Cyperus rotundus⁸¹</td>
<td>Rhizome</td>
<td>Striga orobanchioides⁸²</td>
<td>Whole plant</td>
</tr>
<tr>
<td>Dolichos biflorus⁸³</td>
<td>Seed</td>
<td>Strychnos potatorum⁸⁴</td>
<td>Seed</td>
</tr>
<tr>
<td>Eclipta alba⁸⁵</td>
<td>Whole herb</td>
<td>Syzygium cumini⁸⁶</td>
<td>Leaf</td>
</tr>
<tr>
<td>Elaeagnus pungens⁸⁷</td>
<td>Leaf</td>
<td>Tephrosia purpurea⁸⁸</td>
<td>Root</td>
</tr>
<tr>
<td>Elephantopus scaber⁸⁹</td>
<td>Leaves</td>
<td>Teucrium japonicum⁹⁰</td>
<td>--</td>
</tr>
<tr>
<td>Ficus bengalensis⁹¹</td>
<td>Bark</td>
<td>Vitis amurensis⁹²</td>
<td>Fruits</td>
</tr>
<tr>
<td>Ficus religiosa⁹³</td>
<td>Leaves</td>
<td>Xylopia aethiopica⁹⁴</td>
<td>Fruit</td>
</tr>
<tr>
<td>Gastrodia elata⁹⁵</td>
<td>Roots</td>
<td>Zataria multiflora⁹⁶</td>
<td>--</td>
</tr>
<tr>
<td>German chamomile⁹⁷</td>
<td>Rhizomes</td>
<td></td>
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</table>
1.3 Antioxidants

It is widely accepted that antioxidants have protective effect against some pathological events. There is a worldwide trend toward the use of natural antioxidant.

Reactive oxygen species (ROS) such as singlet oxygen, superoxide anion (O$_2^-$) and hydroxyl (.OH) radical and hydrogen peroxide (H$_2$O$_2$) are often generated as byproducts of biological reactions or from exogenous factors. These reactive species exert oxidative damaging effects by reacting with nearly every molecule found in living cells. Such species are important causative factors in the development of diseases such as diabetes, stroke, arteriosclerosis, cancer, cardiovascular diseases and the aging process. Human body has multiple mechanisms especially enzymatic and non-enzymatic antioxidant systems to protect the cellular molecules against reactive oxygen species (ROS) induced damage.\(^9\) However, the innate defense may not be enough for severe or continued oxidative stress. Hence, certain amounts of exogenous antioxidants are constantly required to maintain an adequate level of antioxidants in order to balance the ROS in human body. When free radicals or oxidants are produced in abundance, cells suffer from oxidative stress. Fortunately, compounds called antioxidants quickly balance the free radicals, inhibiting oxidative stress. Antioxidants reduce the effect of dangerous oxidants by binding together with these harmful molecules, decreasing their destructive power. Antioxidants can also help to repair damage cells.

The body’s antioxidant supply can be classified into two groups: Primary antioxidants are made by the body, thus internally provided. This internal antioxidant defence system includes superoxide dismutase (SOD), catalase and glutathione peroxidase, which are the first and most powerful line of defence against oxidative stress. Secondary antioxidants are externally provided from dietary sources, such as vitamins (vitamin A, C and E), minerals (selenium, zinc, copper and manganese) and other substances, including polyphenols found in grapes and green tea. These dietary antioxidants contribute to the antioxidant reserve, yet play a secondary role to the body’s own antioxidants. Choosing raw fruits and vegetables rather than cooked, provide the highest concentration and best absorption of antioxidants. Dietary supplements are also available for those that do not consume enough antioxidant producing foods. Natural antioxidants are structurally
A phenolic or polyphenolic compound occurs in plants. Dietary supplements such as vitamins A, C and E and flavonoid prevent oxidative damage. Antioxidants are major five types.

i. **Primary antioxidants**: They terminate the free radical chain, acts as electron donors. e.g. BHT, BHA, Tocopherols.

ii. **Oxygen scavengers**: They are reacting with oxygen. e.g. Vitamin C

iii. **Secondary antioxidants**: They decompose lipid hydroperoxidase into stable end products.

iv. **Enzymic antioxidants**: It removes oxygen or highly oxidative species. e.g. glucose oxidase or super oxide dismutase.

v. **Chelating agents**: They are synergistic substance which enhances the activity of phenolic antioxidants. e.g. Citric acid and amino acid.

### 1.3.1 Models for screening of antioxidant activity

Antioxidant activity cannot be measured directly but rather, by effects of the antioxidant, in controlling the extent of oxidation strategies have been developed for measuring the antioxidant activity, as the ability to scavenge free radicals generated in aqueous and lipophilic phase. The ability to scavenge specific radicals may be targeted as, for example, hydroxyl radical, super oxide radical or nitric oxide radical.

A. **DPPH free radical scavenging activity**: DPPH (1, 1-diphenyl-2-picryl-hydrazyl, Deep violet coloured) is a free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule yellow coloured diphenyl picryl hydrazine. The antioxidants reduce and decolorize DPPH by their hydrogen donating ability. The reduction capability of DPPH radical by antioxidants is determined by the decrease in absorbance at 516nm.

B. **Reducing power by FeCl₃**: Assay of reducing activity is based on the reduction of Fe³⁺/ferricyanide complex to the ferrous form in presence of antioxidants. The Fe²⁺ is then measured by the formation of Perl's Prussian blue at 700nm using spectrophotometer. Higher absorbance of the reaction mixture indicates greater reducing power.
C. **Metal chelating activity**\(^{106}\): Ferrous ion form red coloured chelate with ferrozn. The extent of formation of complex is inhibited in presence of other chelating agent. Resulting, the decreasing intensity of red colour is due to inhibition of formation of ferrozine-Fe\(^{2+}\)complexes. Determination of the colour reduction measures the chelating activity of drug.

D. **Nitric oxide scavenging activity**\(^{107}\): Nitric oxide synthases generates NO- radical in biological tissue. The compound sodium nitroprusside is able to decompose in aqueous solution at physiological pH (7.2) to produce producing NO\(^-\) radical. It reacts with oxygen to produce stable nitrate and nitrite. The amount of nitrite ions can be estimated by use of Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to inhibit formation of nitric oxide.

E. **Phosphomolybdenum assay**\(^{108}\): The antioxidant capacity is determine by phosphomolybdenum assay based on the reduction of Mo (VI) to Mo (V) by the antioxidants and subsequent formation of a green phosphate/Mo (V) complex at acidic pH. The higher absorbance value indicates higher antioxidant activity.

F. **β-carotene linoleic acid method**\(^{109,110}\): It based on the principle that linoleic acid, unsaturated fatty acid, gets oxidized by in presence of oxygen and produced oxygenated water. It causes oxidation of β-carotene resulting discoloration of β-carotene. Antioxidant decreases the extent of decolouration of β-carotene. The extent of antioxidant potential can be measured by inhibition of decolouration of β-carotene by using spectrophotometer.

G. **Hydrogen peroxide scavenging (H\(_2\)O\(_2\)) assay**\(^{111,112}\): H\(_2\)O\(_2\) rapidly decomposed into oxygen and water and this may produce hydroxyl radicals cause DNA damage in biological tissue. The concentration of H\(_2\)O\(_2\) is determined in presence and absence of antioxidant. As per Haliiwell et al. interaction of iron with free radical leads to formation of highly active tissue damaging species. The 2-deoxy ribose reacts with hydroxyl radical in presence of thiobarbituric acid to form pink chromogen. The concentration of chromogen is measure using spectrophotometer.
1.4 References


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CHAPTER: 1

INTRODUCTION


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