CHAPTER – 1

Introduction
CHAPTER - 1

Introduction

1.1 General Introduction:

Medicinal plants are an important part of our natural wealth. In many developing countries, traditional medicines are still the mainstay of health care and drugs. Even in developed countries, the raw materials for manufacturing essential drugs are extracted from medicinal plants. The popularity of herbal medicines is connected with their easy access, therapeutic efficiency, relatively low cost and the assumption for absence of toxic side effects.

About 80% of the world’s inhabitants rely mainly on traditional medicines for their primary health care (Owolabi et al., 2007). Modern pharmacopoeia still contains at least 25% drugs derived from plants and many others, which are synthetic analogues, built on prototype compounds isolated from plants.

India has a rich heritage of traditional medicines and the traditional health care system. It has several traditional medical systems such as Ayurveda and Unani, which has survived through more than 3000 years, mainly using plant-based drugs. Over the centuries, the use of medicinal herbs has become an important part of daily life despite the progress in modern medical and pharmaceuticals research. Approximately 3000 plants species are known to have medicinal properties in India (Prakasha et al., 2010). The Botanical Survey of India records over 15,000 plant species occurring in the country, of which at least 7,500 species have been used for medicinal purposes (Attisso M.A., 1983)

In recent years, the increasing demand for herbal medicines becomes as an alternative conventional medicine even in the industrialized countries and the adoption of crude extracts of plants for self-medication has gained more importance. Each medicinal plant species has its own nutrient composition besides having pharmacologically important phytochemicals. These nutrients are essential for the physiological functions of human body. The phytotherapy acts as a bridge between traditional and modern medicines. The
developments of plant derived drugs have always been a multi-step procedure starting with a crude extract followed by the standardized extract and ending up with isolated constituents.

The present thesis deals with the analysis of medicinal plants for their proximate composition, antioxidant activity and elemental content.

1.2 Proximate composition:
1.2.1 Extractable matter:
   Extractable matter is the amount of active constituents extracted with different solvents from the medicinally important plant material. These solvents are normally water, alcohol, ether and chloroform. The extractive values provide information of the extent of polar, medium polar and non-polar components present in medicinal plant material.

1.2.2 Moisture content:
   Moisture content is the quantity of water present in a material. The presence of excess water in medicinal plants will encourage microbial growth. Limits for water content should therefore be set for every given plant material. The accurate method for determining the amount of water is the Karl Fischer titration method.

1.2.3 Ash content:
   It is the nonvolatile inorganic matter of a compound which remains after subjecting it to a high decomposition temperature. Ash represents the mineral salt or inorganic matter content of the drug. The total ash includes both physiological ash, which is derived from the plant tissue itself and non-physiological ash which is the residue of the extraneous matter adhering to the plant. Low amount of total ash indicates that the inorganic matter and non-physiological matter such as silica is less in medicinal plant material.
1.2.4 Crude fibers:

Fibers include cellulose, hemicelluloses, pectin and lignin. It represents only 60% to 80% of the cellulose and 4% to 6% of the lignin. Most of them are polysaccharides. Within the past decade food composition databases have reflected technological advances by listing specific values for total, soluble and insoluble dietary fibers. Wheat, rye, rice and most other grains are primarily composed of insoluble fiber (Englyst et al., 1982).

1.2.5 Fats and Waxes (Lipids):

Lipids are of great importance to the body as they are main storage form of energy. All Lipids are hydrophobic in nature. This group of molecules includes fats and oils, waxes, phospholipids and steroids. Waxes are esters of fatty acids with long chain monohydric alcohols. Natural waxes are often mixtures of such esters and may also contain hydrocarbons (Gidez L. I., 1984).

1.3 Phytochemicals:

Phytochemicals are defined as non-nutritive bioactive plant chemicals in fruits, vegetables, grains and other plants having protective or disease preventive properties and are considered to reduce the risk of chronic diseases. Some well-known phytochemicals include carotenoids, phenolics, alkaloids, nitrogen-including compounds and organo-sulfur compounds. It was proven that phytochemicals working together with nutrients found in fruits, vegetables and nuts, helps in slowing down the aging process and reduce the risk of many diseases including cancer, heart disease, stroke, high blood pressure, cataracts, osteoporosis and urinary tract infections.

1.3.1 Phenolic compounds:

Phenolic compounds are natural antioxidants having an aromatic ring with one or more hydroxyl groups. Numerous types of phenolics are found in nature, including simple phenol, phenylproponoids, benzoic acid derivatives, flavonoids, stilbenes, tannins, lignans and lignins. Phenolic compounds in fruits and vegetables are the secondary metabolites in plants that are derived from the metabolism phenylalanine and tyrosine (Van Sumere C. F., 1989). Phenolic compounds play a crucial role in the growth and
reproduction of plants, and also act as antifeedants and antipathogens (Butler L. G., 1992). The presence of phenols is considered to be potentially toxic to the growth and development of pathogens. Phenolic function as antibiotics, natural pesticides, signaling substances, protective substance against ultraviolet light, insulating materials to make cell walls impermeable to gas and water, and give structural stability to plants. Many properties of plant products, such as the astringency of foods or their potential antinutritional properties are associated with the presence and content of phenolics (Butler L.G., 1992). Among the variety of polyphenolic compounds, phenolic acids have attracted considerable interest in the past few years because they exhibit many potential health benefits such as antiallergenic, antiatherogenic, antiinflammatory, antimicrobial, antioxidant, antithrombotic cardioprotective and vasodilatory effects (Manach and Mazur, 2005).

1.3.2 Alkaloids:

Alkaloids are the natural compounds found in all plants that include nitrogen. The alkaloids are the plant bases. They are essentially basic nitrogenous compounds of vegetable origin, possessing physiological actions. They are found in all parts of plants, but mostly in fruits, stem, bark, roots, leaves and seeds. Alkaloids are generally insoluble in water and soluble in ether or chloroform and other non polar solvents. Along with that alkaloids have significant therapeutic value and form the ingredients of many important medicines. It has been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity (Nobori et al., 1994). Many alkaloids derived from plants have anticancer properties.

1.3.3 Flavonoids:

Flavonoids are polyphenolic compounds that are ubiquitous in nature and are categorized, according to chemical structure into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones. Over 4,000 flavonoids have been identified many of which occur in fruits, vegetables and beverages. The flavonoids have been reported to have antiviral, antiallergic, antiplatelet, antiinflammatory, antitumor and antioxidant activities. Besides this flavonoids have also been reported to act as anticancer
agents via regulation of signal transduction pathways of cell growth and proliferation, suppression of oncogenes and tumor formation, induction of apoptosis, modulation of enzyme activity related to detoxification, oxidation and reduction, stimulation of the immune system and DNA repair and regulation of hormone metabolism (Aron et al., 2008).

1.3.4 Amino acids:

Amino acids are basic unit of protein containing an amino group and a carboxylic group and plays major role in regulating multiple processes related to gene expression, including modulation of the function of the proteins that mediate messenger RNA (mRNA) translation (Scot and Leonard, 2006). They also help in tissue protein formation. Few amino acids are involved in enzyme formation. Hormones like insulin, growth hormone and glucagon are made up of amino acids. Adrenaline, nor-adrenaline and thyroxin are made up of single amino acid. Glutathione, a physiologically active peptide is also made up of amino acids. Amino acids are involved in synthesis of melanin. It has been reported that amino-acid balance in cancer patients often differs from that in healthy individuals, because of metabolic changes (Jun et al., 2010). Amino acids are absorbed through stomas in plants. It has been observed that amino acids influence the physiological activities of the plant. The basic classification of amino acid is shown in Table 1.1.
Table 1.1: Amino acids classification

<table>
<thead>
<tr>
<th>Essential Amino acid</th>
<th>Non- essential Amino acid</th>
<th>Special amino acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>Cysteine</td>
<td>GABA</td>
</tr>
<tr>
<td>Methionine</td>
<td>Tyrosine</td>
<td>DOPA</td>
</tr>
<tr>
<td>Valine</td>
<td>Serine</td>
<td>Citrulline</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>Alanine</td>
<td>Ornithine</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>Asparagines</td>
<td>Taurine</td>
</tr>
<tr>
<td>Histidine</td>
<td>Aspartic acid</td>
<td></td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>Glutamic acid</td>
<td></td>
</tr>
<tr>
<td>Threonine</td>
<td>Glycine</td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>Hydroxylysine</td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>Proline</td>
<td></td>
</tr>
</tbody>
</table>

1.3.5 Oxidative stress:

Oxidative stress is a condition resulting from an imbalance between the production of free radicals and antioxidant defense systems in which oxidation predominates (Halliwell and Whiteman, 2004). It plays a role in cellular processes, such as aging and apoptosis. In a balanced cell state, ROS are produced as a byproduct of metabolic processes and the level of ROS can be controlled with antioxidants, such as vitamin E and vitamin C (Spitz et al., 2004). In a state of cellular imbalance damage is caused to nuclear and mitochondrial DNA, proteins and lipids. If this damage is irreparable then mutagenesis, carcinogenesis and cell death can occur. Oxidative stress has been linked to diseases, including some allergic and inflammatory skin diseases (Okayama Y., 2005).

1.3.6 Free radicals:

Free radicals are reactive species which contain one or more unpaired electrons and are capable of independent existence. There are many types of radicals, but those which concerned in biological systems are derived from oxygen, and known as reactive oxygen species (ROS). Free radicals and other reactive species are continuously produced in the
body and have important functions for the immune system. Free radicals can also be generated in non-enzymatic reactions of oxygen with organic compounds as well as those initiated by ionizing radiations (Fig. 1.1). ROS are potentially very toxic to cells. Oxidative stress might cause damage to the biomolecules such as lipids, protein and DNA, which may increase the risk of development of several diseases.

Free radicals and other reactive oxygen species are derived either from normal essential metabolic processes in the human body or from external sources. Some internally generated sources are mitochondria (Balaban et al., 2005), phagocytic cells, reactions involving iron and other transition metals, peroxisomes, exercise and inflammation while the externally generated sources of free radicals are cigarette smoke, environmental pollutants, radiation, ultraviolet light, certain drugs, pesticides, Ozone, alcohol consumption, viral infections (Halliwell B., 1996).

![Figure 1.1: Different ways for formation of free radicals](image)

### 1.3.6.1 Types of free radicals:

There are numerous types of free radicals that can be formed within the body. The most common ROS which are biologically significant are superoxide radical (O$_2^-$), hydroxyl radical (·OH), peroxyl radical (ROO’), hydrogen peroxide (H$_2$O$_2$), singlet oxygen (‘O$_2$), nitric oxide (NO), peroxynitrite (ONOO$^-$) and hypochlorous acid (HOCl).
In the human body $\text{O}_2^-$ is continuously formed during metabolism. The rate of formation depends on the amount of oxygen flowing through the mitochondria at any given time. Hydroxyl radicals are short-lived but the most damaging radicals within the body. This type of free radical can be formed from $\text{O}_2^-$ and $\text{H}_2\text{O}_2$ via the Harber-Weiss reaction. The interaction of copper or iron and $\text{H}_2\text{O}_2$ also produces $\text{OH}^-$. Hydrogen peroxide is produced in vivo by many reactions. Transition metal ions are important in the production of ROS. The ability of metal ions to donate and accept single electrons is the basis for the formation and propagation of many ROS. Both copper and iron gain or lose electrons during redox reactions.

1.4 Antioxidants:

Antioxidants in the broad sense are all substances that can protect materials against auto-oxidation, irrespective of the mechanism of action. They have the ability to repair damage done by free radicals and this is thought to reduce cancer risk and aging. They vary widely in chemical structure and have diverse mechanisms of action. Antioxidants can inhibit or retard oxidation in two ways: either by scavenging free radicals, where the compound is described as a primary antioxidant, or by a mechanism that does not involve direct scavenging of free radicals, in which case the compound is a secondary antioxidant. The components of primary antioxidants are consumed during the induction period. Secondary antioxidants operate by a variety of mechanisms including binding mental ion catalysts, scavenging oxygen, absorbing UV radiation and converting hydroperoxides to non-radical species or intercepting single oxygen. Different mechanisms of antioxidant activity are shown in Table 1.2.
Table 1.2 – Different mechanisms of antioxidant activity (Hall C., 2001)

<table>
<thead>
<tr>
<th>Antioxidant class</th>
<th>Mechanism of antioxidant activity</th>
<th>Examples of antioxidants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proper antioxidants</td>
<td>Inactivating lipid free radicals</td>
<td>Phenolic compounds</td>
</tr>
<tr>
<td>Hydroperoxide stabilizers</td>
<td>Preventing decomposition of hydroperoxides into free radicals</td>
<td>Phenolic compounds</td>
</tr>
<tr>
<td>Synergists</td>
<td>Promoting activity of proper antioxidants</td>
<td>Citric acid, ascorbic acid</td>
</tr>
<tr>
<td>Metal chelators</td>
<td>Binding heavy metals into inactive compounds</td>
<td>Phosphoric acid, Maillard reaction compounds, citric acid</td>
</tr>
<tr>
<td>Singlet oxygen quenchers</td>
<td>Transforming singlet oxygen into triplet oxygen</td>
<td>Carotenes</td>
</tr>
<tr>
<td>Substances reducing Hydroperoxides</td>
<td>Reducing hydroperoxides in a non-radical way</td>
<td>Proteins, amino acids</td>
</tr>
</tbody>
</table>

1.4.1 Types of Antioxidants:

According to their origin antioxidants are divided in two groups namely, natural antioxidants and synthetic antioxidants. Whereas, based on the nature of antioxidants, the human antioxidant system can be divided into two main groups, enzymatic antioxidants and non-enzymatic antioxidants.

1.4.1.1 Enzymatic antioxidants:

The major primary intracellular endogenous antioxidant defenses are called as enzymatic system. This enzymatic antioxidant system includes superoxide dismutase (SODs), catalase (CAT) and glutathione peroxidase (GSHPx).
1.4.1.2. Non-enzymatic antioxidants:

Non-enzymatic antioxidants are classified into two groups: endogenous antioxidants and exogenous antioxidants.

**Endogenous antioxidants:**

The major endogenous antioxidants found in human plasma are the transition metal binding proteins. This includes ceruloplasmin, transferring, hepatoglobin and albumin.

**Exogenous antioxidants**

Antioxidants from our diet play an important role in helping endogenous antioxidants for the neutralization of oxidative stress (Lien Ai et al., 2008). The best known examples are vitamins such as ascorbic acid, vitamin E, carotenoids, quinines, and polyphenols.

1.4.2 Types of antioxidant assays:

On the basis of the chemical reactions involved, major antioxidant activity assays can be divided roughly into two categories (Huang et al., 2005):

1) Hydrogen atom transfer (HAT) and
2) Single-electron transfer (SET) reaction–based assays

These two mechanisms yield identical results, but they differ in terms of kinetics and the potential for side reactions to occur.

1.4.2.1 Hydrogen atom transfer (HAT) assay:

HAT-based procedures measure the classical ability of an antioxidant to quench free radicals by hydrogen donation:

\[ X^* + AH \rightarrow XH + A^* \] ..........................1.1

Where, (AH = any H donor). Antioxidant activity measurements of HAT assays are based on competition kinetics. HAT reactions are solvent and pH independent and
usually are quite rapid—typically they are completed in seconds to minutes. A disadvantage of the procedure, however, is that the presence of reducing agents, including metals, is a complication that can lead to high apparent reactivity. Total phenolic and total flavonoids assays fall in this category because they donate hydrogen ions while maintaining a stable structure (Chance B., 1979).

1.4.2.2 Single-electron transfer (SET) assay:

SET-based methods detect the ability of a potential antioxidant to transfer one electron to reduce a species, including metals, carbonyls and radicals:

\[
X^- + AH \rightarrow X^- + AH^+ \quad \text{................... 1.2}
\]
\[
X^- + H_2O^+ \rightarrow XH + H_2O \quad \text{................... 1.3}
\]
\[
M(III) + AH \rightarrow AH^+ + M(II) \quad \text{................. 1.4}
\]

The relative reactivity in SET methods is based primarily on deprotonation and ionization potential. SET reactions are usually slow and can require long time to reach completion, so antioxidant activity calculations are based on percent decrease in product rather than on kinetics. When H\(^+\) has a sufficient lifetime, secondary reactions become a significant interference in assays and can even lead to toxicity or mutagenicity in vivo. SET assays are very sensitive to ascorbic and uric acid, which are important in maintaining plasma redox tone. Unfortunately, trace compounds and metals interfere with SET methods and can account for high variability and poor reproducibility of results. The antioxidant assays such as Ferric reducing antioxidant power (FRAP) used for measuring the antioxidant power. In case of DPPH free radical scavenging assay and ABTS radical assay reactions operates by both HAT and SET mechanisms (Prior et al., 2005). The summary of antioxidant assays is shown in Table 1.3.
<table>
<thead>
<tr>
<th>Antioxidant assay</th>
<th>Simplicity</th>
<th>Instrumentation required</th>
<th>Biological relevance</th>
<th>Mechanism</th>
<th>Time required</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORAC</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>HAT</td>
<td>++</td>
</tr>
<tr>
<td>TRAP</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>HAT</td>
<td>+++</td>
</tr>
<tr>
<td>FRAP</td>
<td>+ ++</td>
<td>+</td>
<td>-</td>
<td>SET</td>
<td>- -</td>
</tr>
<tr>
<td>TEAC</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>SET</td>
<td>-</td>
</tr>
<tr>
<td>FC</td>
<td>+ ++</td>
<td>-</td>
<td>-</td>
<td>SET</td>
<td>+</td>
</tr>
<tr>
<td>ABTS</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>HAT</td>
<td>+</td>
</tr>
</tbody>
</table>

+, ++, +++ = desirable to highly desirable characteristic.
—,——, — — — = less desirable to highly undesirable characteristic.

1.4.3 Antioxidant assays:
1.4.3.1 Phenolic content:
Singleton and Rossi (Singleton and Rossi, 1965) adapted this assay first time for analysis of food products. Since then, the total phenols assay has been used in many studies and is now commonly known as the total phenols (or phenolics) assay. Total phenolics methodology consists of the addition of Folin-Ciocalteau reagent to a sample held in the dark for two hours, followed by measurement of the absorption. Intensity of the light absorption is directly proportional to the concentration of phenols. Results are expressed as ferulic acid equivalents of dry weight of the sample using the standard curve. The total phenols assay by FCR method is convenient, simple and reproducible.

1.4.3.2 Total flavonoids:
Flavonoids are most common and widely distributed group of plant phenolic compounds that are characterized by a benzo-y-pyrone structure, which is ubiquitous in fruits and vegetables. The AlCl₃ method is used for quantification of the total flavonoids content of the plant extracts. Total flavonoids can be determined in the sample extract by
reaction with sodium nitrite, followed by the development of colored flavonoid aluminum complex formation using aluminum chloride which can be monitored spectrophotometrically at 510 nm. Flavonoids are important for human health because of their high pharmacological activities as radical scavenger.

1.4.3.3 DPPH radical scavenging assay:
A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical 2, 2-Diphenyl-1-picrylhydrazyl (DPPH). It is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity.

The DPPH radical is long-lived organic nitrogen radical and has a deep purple color. It is commercially available and does not have to be generated before assay. The molecule of 1, 1-diphenyl-2-picrylhydrazyl is characterized as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole, so that the molecules do not dimerise. The delocalization also gives rise to the deep violet colour, characterized by an absorption band in ethanol solution centered at about 520 nm. When a solution of DPPH is mixed with that of a substance it donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet colour.
1.4.3.4 ABTS radical scavenging assay:

The ABTS assay was first reported by Miller et al. (Miller et al., 1993). ABTS$^-$ is generated by mixing ABTS solution with potassium persulfate under darkness at room temperature (23°C) for 16 h. The solution can then be diluted with 50% ethanol and the absorbance can be measured at 734 nm. The mechanism by which the ABTS$^{+}$ radical cation reacts with the antioxidant extract is shown below.

ABTS$^{-}$ ($\lambda_{\text{max}} = 734$ nm)

ABTS$^{2-}$ (colorless)

ABTS assay has been used in many research laboratories for studying antioxidant capacity. The advantage of using this method is that it is rapid and can be used over a wide range of pH values (Arnao et al., 1999) in both aqueous and organic solvent systems.

1.4.3.5 Ferrous reducing antioxidant power assay:

The method is based on the reduction of Fe$^{3+}$-TPTZ complex (colorless complex) to Fe$^{2+}$-tripyrldyltriazine (blue colored complex) formed by the action of electron donating antioxidants at low pH. This reaction is monitored by measuring the change in absorbance at 593 nm. The mechanism is as shown below.
The method is simple, rapid, inexpensive and does not require sophisticated instrumentation. TPTZ is deficient as the ideal reaction stoichiometry between Fe (III) and TPTZ is 1 to 2. The oxidant is not just Fe(III)(TPTZ)$_2^2+$, it also contains other Fe (III) species which can lead to potential problems as many metal chelators in food extract could bind Fe(III) and form complexes that are also capable of reacting with antioxidants.

**1.4.3.6 Reducing power assay:**

The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity. In this assay, the yellow color of the test solution changes to green depending on the reducing power of test specimen. The antioxidant ability of certain compounds is associated with their reducing power. Reducing power assay is based on the principle that substances, which have reduction potential, react with potassium ferricyanide (Fe$^{3+}$) to form potassium ferrocyanide (Fe$^{2+}$), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. Increased absorbance of the reaction mixture indicates increase in reducing power.

**1.5 Trace elements:**

A trace element is defined as an element in a sample that has an average concentration in the range of 1 part per billion to 100 parts per million (Skoog et al., 2003). Trace elements can be classified as essential and non-essential elements. Essential trace elements are also known as micronutrients and are vitally important for proper maintenance of various metabolic body functions. They are required by human beings in
the range of 50 µg/day to 18 mg/day. The insufficient intake of these elements by human beings and other living bodies over a long period of time results in the impairment of normal body functions. Supplementation of the required elements helps in preventing or curing the corresponding deficiency (Mertz W., 1981). The safe and acceptable intake of some of the essential trace elements by human beings as recommended by WHO (WHO, 1996) are given in Table 1.4.

Table 1.4 WHO recommended safe and adequate dietary intake of some elements for adults (WHO, 1996)

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Recommended daily allowance</th>
<th>Tolerated upper limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>1200 mg</td>
<td>2500 mg</td>
</tr>
<tr>
<td>Magnesium</td>
<td>320-420 mg</td>
<td>750 mg</td>
</tr>
<tr>
<td>Iron</td>
<td>15-10 mg</td>
<td>45 mg</td>
</tr>
<tr>
<td>Zinc</td>
<td>12-15 mg</td>
<td>40 mg</td>
</tr>
<tr>
<td>Selenium</td>
<td>55-70 µg</td>
<td>400 µg</td>
</tr>
<tr>
<td>Copper</td>
<td>0.9 mg</td>
<td>10 mg</td>
</tr>
<tr>
<td>Manganese</td>
<td>1.8-2.3 mg</td>
<td>11 mg</td>
</tr>
<tr>
<td>Nickel</td>
<td>400-700 µg</td>
<td>1000 µg</td>
</tr>
<tr>
<td>Cobalt</td>
<td>10-20 µg</td>
<td>250 µg</td>
</tr>
<tr>
<td>Sodium</td>
<td>1500 mg</td>
<td>2300 mg</td>
</tr>
<tr>
<td>Potassium</td>
<td>4700 mg</td>
<td>N/A</td>
</tr>
</tbody>
</table>

The importance of trace elements in human being is discussed below:

1.5.1 Calcium:

It is one of the most abundant elements in the body, 99 % being found in the skeleton. It is primarily present in bone tissue as the hydroxyapatite form of calcium phosphate. The basic function of calcium is to provide a strong framework supporting and protecting delicate organs. On the cellular level, calcium is used to regulate the permeability and electrical properties of biological membranes, which in turn control
muscle and nerve functions, glandular secretions, and blood vessel dilation and contraction.

1.5.2 Magnesium:

It is the fourth most abundant mineral in the body. Numerous biochemical and physiological processes require magnesium, including energy production, protein synthesis, muscle contractions and vascular tone. It is a component of several enzymes implicated in the metabolism of carbohydrates, lipids and proteins. Hence the deficiency may lead to a range of serious biochemical and functional problems. There is an increased interest in the role of magnesium in preventing and managing disorders such as hypertension, cardiovascular disease and diabetes.

1.5.3 Iron:

It plays a key role in many biochemical reactions. Haemoglobin iron represents approximately 60% of total body iron, whereas myoglobin represents only about 3-7% of total iron (McDowell L.R., 1992). In humans, iron is an essential component of proteins involved in oxygen transport. A deficiency of iron limits oxygen delivery to cells, resulting in fatigue, poor work performance, and decreased immunity. On the other hand, excess amounts of iron can result in toxicity and even death (Corbett J.V., 1995).

1.5.4 Selenium:

It is an important component of antioxidant enzymes such as glutathione peroxidase (GPx), thioredoxin reductase (TrxR) and iodothyronine deiodinases (IDD). It may also protect the animal organism from detrimental effects of heavy metals including cadmium, mercury and silver (McDowell L. R., 1992). It is toxic if taken in large quantities and may result in hair loss, tooth decay, brittle nails, white spots, poor appetite, sour taste in the mouth and change in skin pigmentation.

1.5.5 Zinc:

It activates several enzymes and is a component of many important metalloenzymes, DNA and RNA. The element is critically involved in cell replication
and in the development of cartilage and bone. The deficiency of zinc leads to an underperforming immune system open to infections, allergies, night blindness, loss of smell, falling hair, white spots under finger nails, skin problems, sleep disturbances.

1.5.6 Nickel:

It is found in the enzyme superoxide dismutases, which is an important antioxidant. It influences absorption and metabolism of iron. Acute nickel exposure is associated with a variety of clinical symptoms and signs which include gastrointestinal disturbances (nausea, vomiting, abdominal discomfort and diarrhoea), visual disturbance (temporary left homonymous hemianopia), headache, giddiness, wheezing and cough. Excess nickel in the body is also associated with a high incidence of heart disease, thyroid disease and cancer.

1.5.7 Sodium:

It is an element that is vital to human life. Together with potassium and chlorine, it forms a very important part of blood plasma. It also allows our body to maintain the right blood chemistry and the correct amount of water in our blood. Normal functioning of our nervous system also depends on this important element. Too much sodium can damage kidney and increases the chances of high blood pressure.

1.5.8 Manganese:

It is actually an extremely important element that the body uses for a variety of things. It supports the immune system, regulates blood sugar levels and is involved in the production of energy and cell reproduction. Additionally, manganese works with vitamin K to support blood clotting and with B-complex vitamins it helps to control the effects of stress.

1.5.9 Copper:

It is an element that is very important for our good health. It along with vitamin C is important for keeping blood vessels and skin elastic and flexible. This important
element is also required by the brain to form chemicals that keep us awake and alert. The deficiencies of copper include anemia and arthritis.

1.5.10 Potassium:

It is an important electrolyte in the body which is intimately associated with sodium metabolism. It is essential for the transport of nutrients into each cell and waste products out of each cell and helps normalize the heartbeat. It also plays an important role of a catalyst for many types of enzymes inside the human body. Deficiency of potassium may lead to nervous disorders, constipation, slow irregular heartbeat and muscle damage.

1.5.11 Cobalt:

It forms the core of vitamin B-12 and required for normal functioning of the pancreas. It helps in repair of myelin sheath, increase the effectiveness of glucose transport and building of red blood cells. Cobalt has metabolic links with iron and copper which can be depressed at high levels of cobalt intake leading to anemia, nerve disorders and abnormalities in cell formation.

1.6 Bioaccessibility:

Bioaccessibility is a term used to describe the proportion of a nutrient in food that can be utilized for normal body function (Judprasong et al., 2005). In general, bioaccessibility is affected by the type and/or composition of food and also by the simulated gastrointestinal conditions which may affect the distribution of initial species. Many factors affect the bioaccessibility of a compound; these may be divided into exogenous factors such as the complexity of the food matrix, the chemical form of the compound of interest, structure and amount of co-ingested compounds (Scholz and Williamson., 2007) as well as endogenous factors including mucosal mass, intestinal transit time, rate of gastric emptying, metabolism and extent of conjugation and protein-binding in blood and tissues. There are two approaches to estimate the bioaccessibility of minerals for the animal, in -vivo and in -vitro techniques.
1.6.1 In-vivo techniques:

In-vivo studies are both expensive and laborious, and the possibility of measuring certain parameters during the experiments are often limited (Danielsson et al., 1995). In this technique, bioavailable amount of an element of interest is estimated as the difference in the concentration of the element in ingest and excreta, using radiotracers. Basic disadvantage of the method is the exposure of ionizing radiations (Welch and House, 1984). Most of the in vivo studies were carried out on Fe and Zn (McCance and Widdowson, 1942).

1.6.2 In-vitro techniques:

In-vitro methods are rapid and inexpensive (Miller, et al. 1981). It involves the simulation of the gastric and intestinal digestive conditions in the laboratory. As the experiments are carried out under ‘simulated’ digestive conditions, the results may not be as accurate as those obtained by in-vivo studies. The results obtained by in vitro methods are based on the formation of digestive products that are soluble or dialyzable. However, these methods are efficient to identify potential food products as nutrient supplements (Van Campen and Glahn, 1999). In-vitro method is routinely used to estimate the bioaccessible concentrations of essential elements in the diet. It is shown that the bioaccessible values obtained by these methods can be well co-related with that of human subjects (Menson and Cook, 1979) and many animal models (Forbs et al., 1989).

1.7 Inductively coupled plasma atomic emission spectrometry (ICP-AES):

It is an analytical technique used for the detection of trace metals. It is a type of emission spectroscopy that uses the inductively coupled plasma to produce excited atoms that emit electromagnetic radiation at wavelengths characteristic of a particular element. The intensity of this emission is indicative of the concentration of the element within the sample. A schematic diagram of ICP-AES is shown in Fig. 1.2.
Figure 1.2: Schematic diagram of Inductively coupled plasma atomic emission spectrometry (ICP-AES)

The ICP-AES is composed of two parts: the ICP torch and the optical spectrometer. The ICP torch consists of 3 concentric quartz glass tubes. Argon gas is typically used to create the plasma. When the torch is turned on, an intense electromagnetic field is created within the coil by the high power radio frequency signal flowing in the coil. This RF signal is created by the RF generator. The argon gas flowing through the torch is ignited with a Tesla unit that creates a brief discharge arc through the argon flow to initiate the ionization process. Once the plasma is ignited, the Tesla unit is turned off.

A peristaltic pump delivers sample into a nebulizer where it is changed into mist and introduced directly inside the plasma flame. The sample immediately collides with the electrons and charged ions in the plasma and is itself broken down into charged ions. The various molecules break up into their respective atoms which then lose electrons and recombine repeatedly in the plasma, giving off radiation at the characteristic wavelengths of the elements involved. Within the optical chamber, after the light is separated into its different wavelengths the light intensity is measured with a photomultiplier tube. Using
these detector arrays, the intensities of all wavelengths can be measured simultaneously or sequentially.

1.8 Atomic absorption spectroscopy (AAS):

Atomic absorption spectrophotometry analyzes the concentration of elements in a liquid sample based on energy absorbed from certain wavelengths of light (usually 190 to 900 nm). It typically include a flame burner to atomize the sample, hollow cathode lamp, a monochromator and a photon detector (Fig. 1.3)

![Figure 1.3: A schematic diagram of atomic absorption spectrometer (AAS)](image)

In atomic absorption spectrometry, light of a specific wavelength is passed through the atomic vapor of an element of interest and measurement is made of the attenuation of the intensity of the light as a result of absorption.

Radiation from the source passes through a flame into which sample is aspirated. The metallic compounds are decomposed in the flame forming cloud of atoms. Atomic absorption measures the amount of light at the resonant wavelength which is absorbed as it passes through a cloud of atoms. By measuring the amount of light absorbed, a quantitative determination of the amount of element present can be made. The use of hollow cathode lamp and careful selection of wavelength allow the specific quantitative
determination of individual elements in the presence of others. The ease and speed at which precise and accurate determinations can be made with this technique have made atomic absorption one of the most popular methods for determination of metals.

1.9 **High performance liquid chromatography:**

Chromatographic process can be defined as separation technique involving mass-transfer between stationary and mobile phase. In HPLC a liquid mobile phase is used to separate the components of a mixture. Stationary phase can be a liquid or a solid phase. The components to be separated are first dissolved in a solvent, and then forced to flow through a chromatographic column under a high pressure. In the column, the mixture separates into its components. The amount of resolution is important, and is dependent upon the extent of interaction between the solute components and the stationary phase. The interaction of the solute with mobile and stationary phases can be manipulated through different choices of both solvents and stationary phases. As a result, HPLC acquires a high degree of versatility and it has the ability to easily separate a wide variety of chemical mixtures. A schematic diagram of HPLC instrument is shown in Fig. 1.4.

![Figure 1.4: A schematic diagram of High performance liquid Chromatography (HPLC)](image)
HPLC instrumentation includes a pump, injector, column, detector and data system. The heart of the system is the column where separation occurs. Since the stationary phase is composed of micrometer size porous particles, a high pressure pump is required to move the mobile phase through the column. Detection of the components depends upon the detector used. The response of the detector to each component is displayed on a chart recorder or computer screen and is known as a chromatogram. To collect, store and analyze the chromatographic data, computer, integrator, and other data processing equipment are frequently used.

1.10 High performance thin layer chromatography:

High performance thin layer chromatography (HPTLC) is a sophisticated instrumental technique based on the full capabilities of thin layer chromatography. The advantages of automation, scanning, full optimization, selective detection principle, minimum sample preparation, hyphenation enable it to be a powerful analytical tool for chromatographic information of complex mixtures of inorganic, organic and biomolecules. The modern HPTLC technique is sensitive and suitable for use in qualitative and quantitative analysis. HPTLC is a valuable tool for identification because it can provide chromatographic fingerprints that can be visualized and stored as electronic images. A schematic diagram of HPTLC analysis is shown in Fig. 1.5.
1.11 Review of literature:

In India, medicinal plants are widely used by all sections of the population, either directly in different indigenous systems of medicine or indirectly in the pharmaceutical preparations of modern medicines. The first step towards pharmaceutical preparations is phytochemical screening of plant extracts and/or extracts from traditional preparations used in popular medicine (Alonso Paz et al., 1995). There are many reports in the literature on proximate composition and phytochemical contents of various medicinal plants of different origins. Pal et al. (Pal et al., 2005) and Donya et al. (Donya et al., 2007) reported the proximate composition of whole bitter melon.

Survey of the literature shows that plants are endowed with free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids, lignins, stilbenes,
tannins, flavonoids, quinones, coumarin, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity (Zheng and Wang, 2001; Cai et al., 2003). Netzel et al. (Netzel et al., 2007) have investigated seven native fruits of Australia in terms of their antioxidant activities and phytochemical components and the results showed that some fruits displayed high level of total phenolics as well as antioxidant properties. Rajurkar and Hande (Rajurkar and Hande, 2011) analyzed 11 Indian medicinal plants for their phytochemical content and antioxidant activity. They have observed that all medicinal plants under study are good source of phytochemicals.

Prior et al. (Prior et al., 2005) observed that there are several factors that may impact the antioxidant activity of foods, this include genetics, harvest season, geographic and environmental conditions. Twenty four Indian medicinal herbs were studied by Ali et al. (Ali et al., 2008) they observed that all studied plants have great antioxidant potential. Ljubuncic et al. (Ljubuncic et al., 2006) observed that the aqueous extract of *T. polium* can effectively inhibit oxidative processes and has substantial antioxidant activity in vitro. Carini et al. (Carini et al., 2001) reported that the polar fraction isolated from the flowering tops of *H. stoechas* displays radical scavenging properties with potency comparable to that of Trolox.

Horváthová et al. (Horváthová et al., 2007) and Suhaj et al. (Suhaj et al., 2006) reported that irradiation at certain doses can facilitate the antioxidant activities of some dietary plants. Bergers W.W.A. (Bergers W.W.A., 1981), Patil et al. (Patil et al., 1999) and Penner and Fromm (Penner and Fromm, 1972) reported a time-dependent change with irradiation and storage in the antioxidant-rich phenolics, chlorogenic acid, scopoletin and quercetin. Pendharker and Nair (Pendharker and Nair, 1975) also reported an increase in PAL activity with irradiation. Jo et al. (Jo et al., 2003) reported an increase in DPPH scavenging activity in irradiated raw and cooked pork patties with added freeze-dried green tea leaf extract powder. Adamo et al. (Adamo et al., 2004) observed a degradation of polyphenolic compounds upon irradiation at 1.0–1.5 kGy range.

Trace elements play a very important role in the formation of the active chemical constituents present in medicinal plants and are responsible for their medicinal as well as toxic proprieties. Garg et al. (Garg et al., 2007) studied 15 Indian medicinal herbs for their elemental concentrations and found wide variation in elemental concentration.
Liang et al. (1998) measured various metallic elements in commercial Chinese medicines using AAS.

Rajurkar and Vinchurkar (Rajurkar and Vinchurkar, 1992) have analyzed some Ayurvedic preparations, incorporating different medicinal plants, by INAA technique using $^{252}$Cf source. Using the same technique Rajurkar and Pardeshi (Rajurkar and Pardeshi, 1997) have also analyzed several medicinal plants used in the treatment of diabetes mellitus and heart diseases.

Tokalioglu S. (Tokalioglu S., 2012) analyzed ten elements in thirty medicinal herb samples from Kayseri, Turkey by using ICP-MS after microwave digestion and observed the decreasing sequence of the mean metal levels in medicinal herbs as follows: Fe > Sr > Mn > Zn > Rb > Cu > - Ni > Cr > Co > Pb. Desideri et al. (Desideri et al., 2010) analyzed 23 elements by polarised X ray fluorescence spectrometer (EDPXRF) in 35 medicinal plants used in Italy. Saiki et al. (Saiki et al., 1990) have determined 15 elements in the extract of Brazilian medicinal plants and discussed the therapeutic action of the corresponding elements from the plants. Whereas Faknkun et al. (Faknkun et al., 1993) have determined several elements in Nigerian medicinal plants.

The mean Cr level in four types of Chinese medicinal plants by Fei et al. (Fei et al., 2010) was found to be 2.00 µg/gm. Hemalatha et al. (Hemalatha et al., 2007), Carbonaro et al. (Carbonaro et al., 2001), Garcia et al. (Garcia et al., 2009) and Camara et al. (Camara et al., 2005) have observed that even though the total iron content in the vegetables and fruit is relatively high, only a small fraction is bioaccessible. Kulkarni et al. (Kulkarni et al., 2006) have studied bioaccessibility of elements in wheatgrass by in-vitro gastrointestinal digestion method combined with neutron activation analysis (NAA).

Amino acids are necessary for protein synthesis and have various functions in the body. The role of neurotransmitter amino acids in the function of the nervous system has been the focus of increasingly intense research over the past several years. Hur et al. (Hur et al., 1989) observed the high concentration of glutamic acid in medicinal mushrooms.

Phenolic compounds are secondary plant metabolites, diverse in structure and with a wide phylogenetic distribution. Van Sumere et al. (Van Sumere et al., 1993) developed a method for separation of nearly 50 phenolic compounds from the rose flower pedals whereas, Escarpa and Gonzalez (Escarpa and Gonzalez, 1998) separated multiple
groups of the most prominent phenolics with a relatively short analysis time. Hakkinen et al. (Hakkinen et al., 1999) reported that the ferulic acid is most abundant phenolic acid in cranberry and blueberry fruit. Kahkonen et al. (Kahkonen et al., 1999) studied wheat bran extracts and observed that it contain several phenolic acids, including vanillic, p-coumaric and largely ferulic acid.

1.12 Scope of the work:

Medicinal plants were the unique source of medicines in past. After first half of 20th century therapeutics was based mostly on synthetic medicines. However, there is recently a trend again to return to natural sources of medicine and there is an increasing tendency to use ayurvedic medicines mainly of herbal origin. Hence a systematic study on phytochemical screening, elemental content and their antioxidant activity will help to create a base line data of the medicinal plants and in the preparation of ayurvedic drugs. The present study was undertaken with following objectives:

- Phytochemical screening of different medicinal plants.
- Determination of antioxidant activity of plant extracts and effect of gamma irradiation on it.
- Estimation of elemental concentration in medicinal plants and their bioaccessibility.
- Speciation of selenium in different medicinal plants.
- Identification and quantification of essential amino acids from medicinal plants under study by using High Performance Thin Layer Chromatography (HPTLC).
- Identification and quantification of phenolic acids from medicinal plant under study by using High Performance Liquid Chromatography (HPLC).