CHAPTER – 2

REVIEW OF LITERATURE

2.1 Introduction

People have now been turning towards the use of herbs as an alternative and complementary to modern medicine (Lucas, 2010). The use of indigenous plants to cure infectious diseases by the local people is very common. These indigenous plants or their products are termed as ethno-medicine. Although, there is few literatures found, on mechanism of action of these plant based phyto-medicines. But the traditional knowledge suggests that these plants are potent to cure infectious diseases. Nowadays, the ethno-medicines have been receiving attention by scientist and pharmaceutical research industries with the aim to find out more effective substitute (Dogruoz et. al., 2008; Samee et. al., 2009 and Karsha and Lakshmi, 2010).

Human beings are highly dependent on the use of plants for their medicine. The uses of plants in various applications differ among different cultures due to availability of different varieties of plants in different parts of the world (Simpson, 1995).

Since the incept of time humans have relied on nature for their basic needs like food, shelter, clothing, transportation, flavours, fragrances, fertilizers and medicines (Cragg and Newman, 2005). The use of flora for medicinal purpose is very old and this is why the herbs and shrubs are considered as the base of sophisticated traditional medicine systems, The traditional medicine systems is known to provide remedies to mankind for thousands of years and is still serving the mankind with new remedies. Although some of the therapeutic properties attributed to plants have proven to be erroneous, medicinal plant therapy is based on the empirical findings of hundreds and probably thousands of years of use (Tekwu et. al., 2013).

2.2 Medicinal Plants

Plant parts have been used as medicine from time pre-Vedic period. In several developing countries still the priority is given to the plants for the health care (Hoareau and Silva,
1999). The ancient literature of Unani, Ayurveda, Siddha and Chinese has described the use and the benefits of the herbal medicines. These literatures give detailed extensive knowledge of traditional medicine (Sen and Batra, 2012). Weiner and Weiner (1994) has beautifully described herbal medicine was human’s first line of defence before the dawn of history. We learnt the use of plants from instinct and from the observation of animals and birds. Through trial human learnt which plant and preparations served them best. The knowledge was transferred to helping others and eventually rescuing animals. Initially plants were collected from the wild. Later the plants were planted in herbal gardens and ultimately pharmaceutical companies isolated bioactive compounds from plants and then synthesized them chemically (Mokwala, 2007).

Hoareau and Silva (1999) has quoted in their study that the developed countries are very much towards the traditional medicine, approximately 1,400 herbal preparations were taken for the study. Synthetic chemistry and biotechnology are developing very fast but plants are still an essential source of preventative medicine as well as of curative medicine (Srivastava et. al., 1996).

The first written records were from Mesopotamia and date from about 2600 BC (Heinrich et. al., 2004). The records were written on clay tablets in cuneiform and the substances that were used are oils of Cedrus species (cedar) and Cupressus sempervirens (cypress), Glycyrrhiza glabra (licorice), Commiphora species (myrrh) and Papaver somniferum (poppy juice), all of which are still in use today for the treatment of ailments ranging from coughs and colds to parasitic infections and inflammation. In ancient Egypt, bishop’s weed (Ammi majus) was reported to be used to treat vitiligo, a skin condition characterized by a loss of pigmentation (Staniszewska et. al., 2003). More recently, a drug (β-methoxypsoralen) has been produced from this plant which helps in to treat psoriasis and other skin disorders and T-cell lymphoma as well (Beissert and Schwarz, 2002).

The curiosity in natural products as a source of potential chemotherapeutic agents continues. Approximately 50 percent of all the drugs in clinical use in the world today are related to the natural products and their derivatives. Higher plants contribute approximately 25 percent of the total (Farnsworth et. al., 1985 and Cragg and Newman, 2005).

It is reported by the World Conservation Monitoring Centre (1992) that nearly half of the flowering plant species of the world are found in the tropical forests. Tropical rain forests
play an important role and also support a vast reservoir of potential drug species. There is an urgent need to more compounds only about 1 percent of tropical species have been studied for their pharmaceutical potential (Cragg and Newman, 2005). This data is even lower for species confined to the unexplored tropical rain forests. To date about 47 drugs found in the world have come from tropical plants (Soejarto and Farnsworth, 1989). The probable undiscovered pharmaceuticals for modern medicine have often been cited as one of the most important reasons for protecting tropical forests (Abelson, 1990 and Oldfield, 1989). Therefore the high annual extinction rate is a matter for concern.

2.3 Traditional medicine

For thousands of years, plants have been utilized as medicines (Samuelsson, 2004). The traditional medicines were initially taken in the crude form of the drugs like tinctures, teas, poultices, powders, and other herbal formulations (Balick and Cox, 1997). The knowledge of the specific plants to be used in specific disease and the methods of application for particular ailments were passed down through oral tradition. Eventually information regarding medicinal plants was recorded in herbal pharmacopoeias (Balunas and Kinghorn, 2005).

The Greeks, in the ancient Western world, contributed appreciably to the realistic development of the use of herbal drugs. However, the European healing system is said to have originated with Hippocrates (460–377 BC) and Aristotle (384–322 BC), who had developed ideas from the ancient beliefs of India and Egypt. The philosopher and natural scientist, Theophrastus (~300 BC), in his history of plants, mainly wrote about the medicinal properties of herbs, and also studied the ability to change their characteristics through cultivation. Dioscorides, a Greek physician (100 AD), during his travels with Roman armies, also recorded about the collection, use of medicinal herbs. Similarly Galen (130–200 AD) practiced and taught pharmacy and medicine in Rome. He published approximately 30 books on medicines. He was also famous for his complex prescriptions and formulas used in formulating drugs, which even contains dozens of ingredients (galenicals) (Weiher et. al., 1999).

Medicine from Greek and Roman were basically based on the belief that the world is composed of four elements-earth, wind, fire and water. Each of these has its corresponding humours, which are linked to the four vital fluids within the body. The four humours
(blood, phlegm, black bile and low bile) influence both health and temperament (respectively sanguine, phlegmatic, melancholic and choleric) of an individual (Gurib-Fakim, 2006).

During that time severe measures were taken such as bloodletting (reducing excess blood) and purging (to remove excess black bile). The four humours were also linked with cold, heat, dampness and dryness and each of these would have a respective range of cold, hot, damp or dry herbs that were hypothetically able to re-establish imbalances. European tradition had also many regional influences that influenced local folk practices and traditions (Weiher et al., 1999). As early as 800 AD, medicinal plants were cultivated in monasteries in Central Europe. One of the famous healers of this era was Hildegard of Bingen (1098–1179). In later years a Swiss alchemist known as Paracelsus (1493–1541) emphasized on the importance of the correct dosage for medical treatments (Gurib-Fakim, 2006) in any disease.

Herbal medicine has created an important position in day to day life of the people in many countries in Europe. In European countries, the use of herbal tea is very popular. The ‘natural products’ taken in their crude form (unprocessed) as teas or decoctions, more sophisticated phyto-medicines (standardized and formulated extracts of plants, often subject to rigorous testing in humans) are a popular alternative to medicinal products which had been derived from pure synthetic chemicals (Vicker and Zollman, 1999).

Traditional herbal remedies of Europe have become widely known as a result of commercialization and a number of active compounds have been isolated from medicinal plants and are used today as single chemical units (Pieroni, 2000).

The oldest written information about the tradition from the Arab can be obtained from the Sumerians and Akkadians of Mesopotamia, and dates back to the same period as the archaeological records of Shanidar IV (Heinrich et al., 2004). The earliest found record, related to the medicinal plants, dates from 60000 before the common-era (BCE) were found in the grave of the Neanderthal man from Shanidar IV, an archaeological site in Iraq. The pollens from several species of plants were also found useful as medicines among which are: Centaurea solstitialis (Asteraceae), Ephedra altissima (Ephedraceae), Althea sp. (Malvaceae) amongst others. Some of these species may have been used by the Neanderthal people as well and may also be part of a tradition for which Shaidar IV
represents the first available record (Cragg and Newman, 2005). The Middle East is known as the base of civilisation and also many plants cultivated nowadays were domesticated from this area. The Babylonians, Assyrians and Sumerians recorded herbal remedies in cuneiform writing on numerous clay tablets. The Code of Hammurabi (ca. 1700 BC), a comprehensive set of civil laws carved in stone and commissioned by the King of Babylon also enlists several medicinal herbs (Spiegel and Springer, 1997).

The manner of documentation of Egyptians was quite different and unique as they kept their knowledge regarding their medical as well as their pharmaceutical in paintings on the walls of the tombs dated back from the Old Kingdom and on papyrus which is made from *Cyperus aquaticus*. The writings were known to be very useful and important and among these writings, the Ebers Papyrus is known to be the most important, which was known to be originated from around 1500 BC and to contain, ancient medicinal knowledge from before 3000 BC (Oubre et. al., 1997). The famous 20 m papyrus scroll reputedly found in a tomb is inscribed in Egyptian hieroglyphics and named after Prof. Ebers Georges at Thebes in 1872. It is deposited at the University of Leipzig 1873 and two years later G. Ebers published a facsimile edition (Ghalioungui, 1987). The medical handbook, Ebers Papyrus, contains the treatment almost all types of illnesses and includes empirical as well as symbolic forms of treatment and the diagnostic knowledge given is accurate and very impressive. During 5th to 12th centuries (AD) which is also known as the dark and middle ages, the countries like Germany, Ireland, and England were accountable for taking care of the remains of Western knowledge, whereas the Arabs not only preserving the knowledge from Greco-Roman expertise, but also they have expanding and developing these preserving to include the use of their own resources, together with the Chinese and Indian herbs, till then unknown to the Greco-Roman world. The Arabs were known to be the first one who has established privately owned drug stores in the world in the 8th century. Canon medicinae, known as the final codification of all Greco-Roman medicine, is as a great contribution of Avicenna, a Persian pharmacist, physician, philosopher and also a great poet. Canon medicinae included elements of other cultures healing system and forms the basis for a distinct Islamic healing system known today as Unani-Tibb (Sheehan and Hussain, 2002).

Some of the famous medicinal plants of the Middle East and Egypt are: *Allium cepa*
Review of Literature


Ancient medicine provides a base for the modern allopathic medicine. (The new remedies were discovered and commercialized as it has been known till now). While European traditions also influences modern western pharmacognosy. All the societies have developed their own herbal traditions, but all the herbal tradition are still not been studied. The study of such traditions will provide an insight into how the field has developed and also it is an interesting example of our ability to develop a diversity of cultural practices (Gurib-Fakim, 2011).

Ancient traditional medicine from Africa is known to be the vast and most diverse of all medicinal systems. Africa is generally considered to be the base of humankind, with a rich biological and cultural diversity and marked regional differences in healing practices. The literature of medicinal uses of African plants is urgently required because of the rapid loss of the natural habitats of these plants due to human activities. The African continent is suffering with a very serious problem of deforestation, which is known to be at the highest rates of deforestation in the world and the worst part of that is there are still some plants which are not yet reported. This is one of the greatest losses for the continent as it also has a high rate of endemism, Madagascar is listed on the top with 82 percent (Green and Sussman, 1990).

African traditional medicine has diverse forms. It is considered to be holistic, as it is related to both the body and the mind. The local practitioner generally diagnose and treat the psychological part of an illness before prescribing medicines to treat the symptoms. Well known African medicinal plants include *Acacia senegal* (gum arabic), *Agathosma betulina* (buchu), *Aloe ferox* (Cape aloes), *Aloe vera* (north African origin), *Artemisia afra* (African wormwood), *Aspalanthus linearis* (rooibos tea), *Boswellia sacra* (frankincense), *Catha edulis* (khat), *Commiphora myrrha* (myrrh), *Harpagophytum*
Review of Literature

procumbens (devil’s claw), Hibiscus sabdariffa (hibiscus, roselle), Hypoxis hemerocallidea (African potato), Prunus africana (African cherry). Madagascar has contributed Catharanthus roseus (rosy periwinkle) and has the potential of contributing more in view of the diversity of the flora and fauna (Newman et. al., 2000 and Neuwinger, 2000).

The indigenous healer or Shaman of the United States of America treated illnesses by addressing both the physical and spiritual dimension of diseases. These Shamanistic ceremonies involve chanting, dancing and other rituals aimed at expelling evil forces so that the patient or the community as a whole can be healed (Fabricant and Farnsworth, 2001). Early incomers learnt from local practices and they eventually adopted many of the herbal remedies, which later formed the basis of the early United States Pharmacopoeia. Among the well known medicinal plants of the United States are Echinacea purpurea commonly known as Echinacea and Hydrastis canadensis with common name Goldenseal. Many studies have shown that still herbs and botanicals commonly used in the USA and Canada are still considered as nutritional supplements and not as medicines in their own rights (Pieroni, 2000; Heinrich et. al., 2004 and Gurib-Fakim, 2006).

Apart from other countries Chinese herbal medicinal is being used by most of the countries. The ancient Chinese system of medicine is believed to be more than 5000 years old. This system is based on two separate theories related to the natural laws that govern good health and longevity, namely yin and yang, and the five elements (Kapoor, 1990 and Patwardhan et. al., 2005). The traditional Chinese medicine has been written in a systematized manner between 100 and 200 BC. The most complete reference to Chinese herbal prescription is the Modern Day Encyclopedia of Chinese Materia Medica published in 1977. It lists nearly 6000 medicines out of which 4800 are of plant origin (Magner, 1992). The medicinal products originating from China are Croton tiglium (purging croton), Duboisia hopwoodii (pituri), Eucalyptus globules (bluegum), Melaleuca alternifolia (tea tree), Myristica fragrans (nutmeg and mace), Piper methysticum (kava kava), Strychnos nux-vomica (strychnine), Styrax benzoin (benzoin) and Syzygium aromaticum (coves) (Maher, 1999; Kapoor, 1990; Newman et. al., 2000 and Gurib-Fakim, 2006) are well known. Similarly Angelica polymorpha var. Sinensis (dang gu), Artemisia annua (qing hao), Ephedra sinica (ma huang), Paeonia lactiflora (bai shao yao), Panax ginseng (ren shen) and Rheum palmatum (da huang) (Magner, 1992; Padua de et. al., 1999 and
Gurib-Fakim, 2006) are other well known Chinese medicinal plants.

Among all medicinal traditions the most ancient tradition is known to be Ayurveda. It is probably older than traditional Chinese medicine and is considered to be the origin of systemized medicine. It is actually a practical and holistic set of guidelines to maintain balance and harmony in the system. Dioscorides (who influenced Hippocrates) is thought to have taken many of his ideas from India. Ancient Hindu writings on medicine contain no references to foreign medicines whereas Greek and Middle Eastern texts refer to concepts and drugs of Indian origin (Magner, 1992 and Chopra, 2000).

2.4 Indian System of Medicine

The word Ayurveda is derived from the Indian words Ayar means life and Veda means knowledge or science and hence means the science of life (Ayyanar, 2012 and Patwardhan, 2000).

Ayurveda was supposed to ensure a long life, which was supposed to be the instrument for achieving righteousness (dharma), wealth (artha) and happiness (sukha). In India, wisdom and knowledge have been passed on from one generation to another through songs and poems, which were learnt by hearts by the scholars and physicians. The Veda is an ancient text in four parts (Rig Veda, Sama Veda, Yajur Veda and Atharva Veda), the earliest of which date back to 2000 years BC. The principles of Ayurvedic medicine and the medicinal uses of plants are contained in Rig Veda in the form of thousands of poetic hymns. The first school to teach Ayurvedic medicine was at the University of Banaras in 500 BC where the great Samhita, the encyclopaedia of medicine, was written. After 700 years later of Samhita, another encyclopaedia was written, and these two together forms the basis of the Ayurveda (Chopra, 2000). Ayurveda is similar to Galenical medicine in that it is based on body humours (dosas) and the inner life force (prana) that is believed to maintain digestion and mental activity. The living and the non living environment, including humans, are considered to be elements such as earth (prithvi), water (jada), fire (tejac), air (vaju) and space (akasa) (Ayyanar, 2012).

The concept of impurity and cleansing are also essential to understand these traditions. The imbalance between these elements is known as illness and it is the goal of the treatment to restore this balance (Magner, 1992). Treatment of any disease is based on the symptoms
and on pattern of imbalances, often detected by checking the pulse or observing the patient’s tongue. Warm or hot herbs, such as ginger and cinnamon, are used to treat the ailments associated with cold symptoms such as cold hands, abdominal pains and indigestion (Kapoor, 1990 and Padua et al., 1999).

Prominent Ayurvedic medicinal plants include *Azadirachta indica* (neem), *Centella asiatica* (gotu kola), *Cinnamomum camphora* (camphor), *Elettaria cardamomum* (elaoor cardamomum), *Rauwolfia serpentina* (Indian snake root), *Santalum album* (sandalwood), *Terminalia* species (myrobolan), *Withania somnifera* (aswargandha) (Kapoor, 1990; Magner, 1992; Padua de et al., 1999; Gurib-Fakim, 2006 and Ayyanan, 2012) and many other as well.

### 2.5 Plants

Plants not only provide shelter, food etc but also produce substances that help protect humans from microorganisms, herbivores, competing plants, and aide in reproduction. They also produce chemicals for offensive chemical warfare targeting cell proliferation of pathogens. Such chemicals may have general or specific activity against key target sites in bacteria, fungi, viruses, or neoplastic diseases (Wedge and Camper, 1999). It has been estimated that more than 250,000 species of higher plants exist and that only a fraction have been investigated to characterize the chemical constituents for biological effects (Clark and Walker, 1999).

A successful drug discovery program requires several key components for the evaluation of plants. It is essential to have a continuing collection of new and preferably unique plants at a rate sufficient to generate several good leads per year. There must be a selective and effective set of bioassays to detect which plant extracts contain bioactive constituents. An effective mechanism for screening out compounds that have previously been tested or otherwise uninteresting will allow drug companies to concentrate their work on compounds that have a high potential for becoming a marketable drug. An efficient fractionation and structure determination program will facilitate the isolation and identification of bioactive products (Kingston et al., 1999).

The samples collected should have maximum chemo diversity and therapeutically useful biological activity within a minimum number of collected samples. Samples may be
collected randomly or based on the traditional information on medicinal plants. Since many of the samples collected are found in remote areas of the world (that is rain forests), it is beneficial to collaborate with the endogenous people for collection. These folks possess a vast knowledge of the plants in their part of the world. In particular, shamans or witch doctors use these plants in tribal medicine and have an understanding of how plants and other substances may be used for treatment of illnesses (Kingston et. al., 1999).

Some of the very useful verdicts in sampling of the plants include quantity collected, storage and process samples (Clark and Walker, 1999). Any part of the plant may be taken as samples like roots, bark, twigs, flower, fruits or leaves, whole plant or one or many parts of the plant can also be taken for the particular study. They are then dried and sent to the laboratory for extraction. If collaboration with different people is possible, the plants may be collected from gardens of tribal shamans or healers. Detailed information should be collected about each plant, which should includes various medicinal uses, area where found, local name, part of plant used, habitat, soil, visibility, abundance, process of preparation of medicine by shaman, dosage, method of application and side effects (Kingston et. al., 1999).

After conducting the testing process of samples, plants having bioactivity are recollected to confirm the initial results. The extract from the plant is then fractionated in an effort to isolate and characterize the active entity. The samples are fractionated by drying then further reconstituted in solvents. Solvents like ethyl acetate and methanol may be used for this extraction process (Kingston et. al., 1999). Initial fractionation of crude extracts can be done by counter current chromatography. This separation method is known to be one of the least destructive to the compound of interest. The fractions are isolated and put in separate tubes and tested for their biological activity. The fractions may be considerably pure and isolated in reasonable quantities after a single chromatographic step (Alvi, 1999).

### 2.5.1 Plants as a source of drug targets

Drug discovery from medicinal plants include numerous fields of inquiry and various methods of analysis. The process typically begins with a botanist, ethnobotanist, ethno-pharmacologist, or plant ecologist who collects and identifies the plants of interest. The collection may involve species with known biological activity for which active compounds have not been isolated (that is traditionally
used herbal remedies) or may involve taxa collected randomly for a large screening program. It is essential to respect the intellectual property rights of a given country where plants of concern are collected (Baker et al., 1995). Phyto-chemists (natural product chemists) prepare extracts from the plant material, subject these extracts to biological screening in pharmacologically relevant assays, and commence the process of isolation and characterization of the active compounds through bioassay-guided fractionation. Molecular biology has become essential to medicinal plant drug discovery through the determination and implementation of appropriate screening assays directed towards physiologically relevant molecular targets. Pharmacognosy encapsulates all of these fields into a distinct interdisciplinary science (Sarker, 2012).

The number of methods used to obtain compounds for drug discovery includes isolation from plants and other natural sources, synthetic chemistry, combinatorial chemistry, and molecular modelling (Ley and Baxendale, 2002; Geysen et al., 2003 and Lombardino and Lowe, 2004). Despite the recent interest in molecular modelling, combinatorial chemistry, and other synthetic chemistry techniques by pharmaceutical companies and funding organizations, the natural products, and particularly that of medicinal plants, remain an important source of new drugs, drug leads, and chemical entities (Newman et al., 2000 and Butler, 2004). In both 2001 and 2002, approximately one quarter of the best-selling drugs worldwide were natural products or were derived from natural products (Butler, 2004). An example is Arteether, a potent anti-malaria drug. It is derived from artemisinin, a sesquiterpene lactone isolated from Artemisia annua (Asteraceae) (Graul, 2000). There are number of evidence of drug discovery from medicinal plants and future endeavours face many challenges. The Pharmacognosists, phyto-chemists and other natural product scientists needs continuously to improve the quality and quantity of compounds that enter the drug development phase to keep pace with other drug discovery efforts (Butler, 2004). The process of drug discovery has been estimated to take an average of 10 years upwards (Reichert, 2003) and cost more than 800 million US dollars (Dickson and Gagnon, 2004). It has been found that only one in 5000 lead compounds have successfully been advance through clinical trials and have been approved for use, whereas lead identification is only the first step in a
lengthy drug development process. There is also lead optimization (involving medicinal and combinatorial chemistry), development (including toxicology, pharmacology, pharmacokinetics, ADME [absorption, distribution, metabolism, and excretion], and drug delivery), and clinical trials which takes considerably longer time.

In comparison of other drug discovery methods, drug discovery from medicinal plants has traditionally been lengthier and more complicated. Therefore, many pharmaceutical companies have eliminated or scaled down their natural product research (Butler, 2004; Koehn and Carter, 2005). Recently, there has been an interest generated for rediscovering the natural products due to its no side effects.

2.6 Phyto-constituents of Medicinal Plants

The metabolic processes occurring in plants result in production of many different kinds and types of organic compounds or metabolites. These metabolites are further divided into primary and secondary metabolites. The primary metabolites are like chlorophyll, amino acids, nucleotides, simple carbohydrates or membrane lipids and more importantly play roles in photosynthesis, respiration, solute transport, translocation, nutrient assimilation and differentiation. While the secondary metabolites different from primary metabolites and are unevenly distributed throughout in the plant kingdom. It is found that sometimes particular secondary metabolites are often found in only one plant species or a taxonomically related group of species, whereas the basic primary metabolites are found throughout the plant kingdom (Taiz and Zeiger, 2006). During the recent past it has been found that many secondary metabolites do indeed have specific functions that are vital for the fitness of a plant producing them. The main roles are as follows:

- They defence the plant against herbivores (insects, vertebrates)
- They protect the plant against fungi and bacteria
- They defence the plant against viruses
- They defence the plant against other plants competing for light, water and nutrients
- They acts as signal compounds to attract pollinating and seed dispersing animals
• Gives protection against UV-light or other physical stress

• Signals for communication between plants and symbiotic micro-organisms (e.g. N-fixing Rhizobia or mycorrhizal fungi).

They have also provided an invaluable resource that has been used to find new drug molecules (Gurib-Fakim, 2006). Till date approximately 12,000 secondary metabolites have been isolated and this is much less than 10 percent of the total (Schultes, 1978 and Rawal et al., 2015). Useful phyto-chemicals are divided into several categories, as given in Table 2.1.

2.7 Free Radicals

The free radicals are identified as molecules having an unpaired electron in the outer orbit. (Gilbert 2000 and Agarwal, 2006). They are usually unstable and very reactive. Reactive oxygen species (ROS) include free radicals are superoxide anion (O₂⁻), hydroxyl (OH’), peroxyl (RO₂⁻), alkoxyl (RO’), and hydroperoxyl (HO₂⁻) radicals. Nitric oxide (NO) and nitrogen dioxide (‘NO₂) are two highly reactive nitrogen free radicals (Sikka, 2001 and Kothari, 2010).

Denham Harman known as the pioneer of free radical research, for the first time made the connection between free radical chemistry and ageing in the early 1950's. He reported that the 2-MEA (2 - mercaptoethylamine), a radiation protection compound synthesised by the AEC (Atomic Energy Commission), could extend life span by decreasing the level of free radical reactions.

Harman has also proposed that a single common process, the production of free radicals, was responsible for ageing and death of all living things (Harman, 1956 and 1992). This theory was based on the chemical nature of free radical reactions and their ubiquitous prominent presence in living systems (Harman, 1956).

The free radical theory of ageing has shown that the free radicals especially the oxygen-derived free radicals are very much responsible for ageing and age-related problems at cellular and tissue levels. It has concluded that cellular ageing is associated with oxidative stress. In a study conducted by Sies (1986), stated that the pro-oxidant molecules when accumulated in a sufficient quantity, suppress the anti-oxidant compound.
Hitherto had some doubt related to the free radical theory, but this theory gained much importance and interest after the discovery of superoxide dismutase (SOD) by Mc Cord and Fridovich (1969). Superoxide dismutase (SOD) is a natural enzyme and possesses an ability of scavenging the superoxide free radicals produced in the body.

Many studies have been done to know the concept of aging. But the study on the free radicals and the free radical theory has gained much interest than any other concept in ageing, such studies proved as an evidence to support Harman's original conception, which was that free radical damage is a major factor contributing to the ageing process and probably too many other age-related diseases such as atherosclerosis, cancer, arthritis and neurodegenerative diseases (Table 2.2) (Beckman and Ames, 1998 and Willcox et. al., 2004).

A review (The free radical theory of ageing matures) study conducted by Beckman and

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<th>S.No.</th>
<th>Class</th>
<th>Subclass</th>
<th>Example</th>
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<tbody>
<tr>
<td>1</td>
<td>Phenols</td>
<td>Simple phenols</td>
<td>Catechol and Epicatechin</td>
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<td></td>
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<td>Phenolic Acids</td>
<td>Cinnamic Acid</td>
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<td>Quinones</td>
<td>Hypericin</td>
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<td>Flavonoids</td>
<td>Chrysin</td>
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<td>Flavones</td>
<td>Abyssinone</td>
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<td>Flavonols</td>
<td>Totarol</td>
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<td>Tannins</td>
<td>Ellagitannin</td>
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<td>Coumarins</td>
<td>Warfarin</td>
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<td>2</td>
<td>Terpenoids, Essential oils</td>
<td></td>
<td>Capsaicin</td>
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<td>3</td>
<td>Alkaloids</td>
<td></td>
<td>Berberine &amp; Piperine</td>
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<td>4</td>
<td>Lectins and Polypeptids</td>
<td></td>
<td>Fabatin</td>
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<td>5</td>
<td>Polyacetylenes</td>
<td></td>
<td>8S-Hetadeca-2(Z),9(Z)-diene-4,6-diyne-1,8-diol</td>
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Table No. 2.1: Major Classes of Anti-oxidant Compounds in Plants (Source-Cowan, 1999)
Ames (1998) have stated in their study that the status of the free radical theory by categorizing the literature in terms of the different types of experiments performed. They have also reported that a large amount of data is present which showed that the oxidants play a very important role in ageing and it is also found to be responsible for the aetiology of many degenerative diseases. Another review study carried out by Diplock et. al., (1998) have also published an extensive review, in which he had analysed critically the scientific basis of oxidative damage and its significant causative factor in the development of human disease and he has found that the anti-oxidants are competent of preventing or stopping these disease processes. Diplock et. al., (1998) also concluded that there was sufficient evidence that mechanisms which involve free radicals are implicated at some stage of the growth of human diseases, and that maintenance of well-being and health depends on the supply through the diet of anti-oxidants which modulate free radical processes in vivo.

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<th>S.No.</th>
<th>Category</th>
<th>Example</th>
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<tbody>
<tr>
<td>1.</td>
<td>Visual disorders</td>
<td>Cataract and age-related macular degeneration (Diplock et. al.,1998)</td>
</tr>
<tr>
<td>2.</td>
<td>Neuronal diseases</td>
<td>Parkinson's disease (Jenner et. al., 1992), schizophrenia</td>
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<td></td>
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<td>(Smythies, 1998; Reddy and Yao, 1999)</td>
</tr>
<tr>
<td>3.</td>
<td>Shock</td>
<td>Septic, haemorrhagic and burnshock are all associated with severe oxidative stress and depletion of anti-oxidant defences (Goode and Webster, 1993)</td>
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<tr>
<td>4.</td>
<td>Respiratory diseases</td>
<td>Asthma, lung cancer, cystic fibrosis, especially during exacerbations, exposure to environmental pollutants (O₁, NO₂,SO₂, auto exhaust), emphysema</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Van der Vliet and Cross, 2000)</td>
</tr>
<tr>
<td>5.</td>
<td>Digestive system</td>
<td>Inflammatory bowel disease, ulcerative colitis (Grisham, 1993) diseases.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Muscular Skeletal Rheumatoid arthritis (Halliwell et. al., 1992)</td>
</tr>
<tr>
<td>6.</td>
<td>Infectious diseases</td>
<td>AIDS, malaria (Halliwell and Gutteridge, 1999; Mollace et. al., 2001)</td>
</tr>
<tr>
<td>7.</td>
<td>Endocrine disease</td>
<td>Diabetes (Paolisso et. al., 1995)</td>
</tr>
</tbody>
</table>

Table No. 2.2: Range of human diseases in which oxidative damage have been claimed to play a role in the pathogenesis
Azzi et al., (2004a) proposed that anti-oxidants may only be useful if the molecular mechanism of the oxidative stress situation or imbalance is known especially since anti-oxidants can protect, or increase injury, depending on the situation. The free radicals are not only by-products, but also play an important role in cell signal transduction, apoptosis and infection control (Bonnefoy et al., 2002 and Halliwell, 2005).

2.7.1 Free radicals and Oxidants

Study of free radicals and reactive oxygen species (ROS) is now days, gaining a lot of interest and considered as medical revolution that promises a new age of health. The reactive oxygen species (ROS) and the reactive nitrogen species (RNS), both are commonly called as free radicals. The other non-radical reactive derivatives called oxidants. Radicals are highly reactive as they are unstable than the non-radical species. The non-radical species are produced from molecules as by-products during the breakage of a chemical bond in such manner that each fragment gets one electron, this cleavage of a radical gives another radical and so on, and also by the redox reactions (Bahorun et al., 2004 and Halliwell and Gutteridge, 2007). Reactive oxygen species (ROS) are not only free radicals but acts as oxidants which easily initiates free radical reactions in living organisms. The exogenous sources of ROS include auto-exhaust, tobacco smoke, ozone, certain pollutants, exposure to sunlight, organic solvents, X-rays, and pesticides. ROS causes oxidative damage to bio-molecules like DNA, RNA, lipids, proteins and carbohydrates, resulting into mutations, wrong repair mechanism, surface receptor altering enzymes and other essential functions. ROS have been associated with more than 100 diseases. The diseases in which ROS involved are ageing, AIDS (acquired immunodeficiency syndrome), malaria, cardiovascular diseases, cancer, diabetes, etc. (Bendich, 1996 and Bingham et al., 2003).

2.7.2 Sources of Free Radicals

The free radicals originate not only from the environment but also from other free radicals in chain reactions and from many normal biological processes in vivo. Free radical reactions take place continuously in cells and tissues in the body from both enzymatic and non-enzymatic reactions. Enzymatic reactions serving as sources of free radical reactions include those involved in phagocytosis, prostaglandin
biosynthesis and in the Cytochrome system. The free radicals also come up in the non-enzymatic reactions of oxygen with organic compounds as well as those initiated by ionising radiation (Beckman and Ames, 1998 and Diplock et al., 1998).

The sources of free radicals from the environment comprise tobacco smoke, ozone derived from air pollution, automobile exhaust emissions, excessive radiation, pesticides, deep fried foods, hydrogenated oils and toxic metals which we inhale or digest (Pryor et al., 1995; Diplock et al., 1998). The destructive free radical nitrogen dioxide (NO₂) is one of the examples, which is the result of a reaction between nitric oxide (NO·) and oxygen (O₂), is produced in cigarette smoke and vehicle exhaust. It has been associated with the respiratory illnesses and irreversible lung damage (Pryor and Stone, 1993; Halliwell, 1994 and Van de Vliet and Cross, 2000).

2.8 Formation of Reactive Oxygen Species and Actions In Vivo

Large numbers of reactive oxygen species are responsible for oxidative damage in the human body. Some of the physiologically relevant free radicals include hydroxyl (OH·), nitric oxide (NO·), superoxide anion (O₂·−), peroxyl (RO₂·) and nitrogen dioxide (NO₂·), and the non radicals, hydrogen peroxide(H₂O₂), hypochlorous acid (HOCl), singlet oxygen (¹O₂), ozone (O₃) and peroxynitrite (ONOO⁻) which, although not technically oxygen free radicals, are included in this family due to their radical-forming capacity.

The one electron reduction product of oxygen, Superoxide anion radical (O₂·−), (Halliwell, 1994), is the most important source of initiating radicals in vivo formed from the reduction of molecular oxygen by many physiological processes either intentionally or accidentally. Phagocytic cells like macrophages consciously produce superoxide to help inactivate viruses and bacteria (Babior et al., 1973 and Halliwell, 1994). Usually within the cell during electron chain transport in the mitochondria sometimes there occurs chemical leakage whereas another source which serves as an important source of reactive species. Another source is autoxidation (a spontaneous oxidation reaction in which a molecule reacts with oxygen via a free radical, self catalysed route). Superoxide radicals can damage red blood cells, cause lungs damage and degrade synovial fluid, possibly leading to arthritis (Lin, 1993 and Halliwell, 1994). Superoxide can also produce other reactive
species by a variety of reactions in biological systems (Beckman and Ames, 1998).

Hydrogen peroxide (H$_2$O$_2$) is a non-radical derivative of oxygen (Aruoma, 1993). It is continuously produced in vivo as a result of many physiological processes including phagocyte activity, peroxisomal fatty acid metabolism and normal aerobic respiration where mitochondria consume oxygen, reducing it by sequential steps to produce water (H$_2$O), hydrogen peroxide (H$_2$O$_2$) and superoxide (O$_2^-$) (Ames et al., 1993). When superoxide (O$_2^-$) radicals is allowed to react with hydrogen peroxide (H$_2$O$_2$) in the process of a metal which act as a catalyst as in Fenton reaction iron catalysed Haber Weiss reaction, then the highly reactive oxygen centred hydroxyl radical (OH) is generated (Beckman and Ames, 1998). These free radicals generated attack all molecules in the human body and leads to cell membrane damage and genetic mutation (Lin, 1993, Snowdon et al., 1996). These radicals account for a large part of the damage done by ionising radiation (Halliwell, 1994). The hydroxyl radical produced reacts in vivo at the site of formation or close to it. The degree of the damage depends on where they are produced (Halliwell, 1984).

The superoxide (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$) cannot attack DNA alone or initiate lipid peroxidation (Halliwell, 1994). Therefore, further Interest was mainly focused on the ability of superoxide (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$) to generate the dangerous species. Yet another highly reactive non-radical species generated from energy transfer, singlet molecular oxygen (¹O$_2$), is thought to be formed in vivo for instance, from exposure to sunlight or ozone (O$_3$) (Beckman and Ames, 1998 and Diplock et al., 1998). The singlet oxygen can damage lipids, DNA and RNA (Pearson and Shaw, 1982).

Another recently recognised free radical is Nitric oxide (NO), which is of the only free radical with biological significance. It is known as messenger molecule, generated from the amino acid L-arginine that participates in a broad range of physiologic processes such as vasodilation, bronchodilation, neurotransmission and microbial-host defence (Moncanda and Higgs, 1993 and Van de Vliet and Cross, 2000). The nitric oxide (NO) also contributes to the tissue injury mechanism but when generated in excess then becomes highly cytotoxic (Beckman and Ames, 1998). The peroxynitrite (ONOO-) is proved as a powerful oxidant which can cause lipid peroxidation and leads to the formation of Nitric oxide (NO) which reacts with superoxide ((O$_2^-$), (Beckman and Ames, 1998 and Van de Vliet and Cross, 2000).
2.9 Formation of ROS and RNS in the Living Cells

Formation of ROS and RNS occurs in the cells by enzymatic and non-enzymatic reactions. The free radicals produced by enzymatic reactions, gets involved in the respiratory chain, phagocytosis, prostaglandin synthesis and cytochrome system P450 (Pacher et al., 2007; Genestra, 2007 and Halliwell, 2007).

2.9.1 DNA Damage

Reactive oxygen species causes oxidative damages to DNA, on nuclear and mitochondrial level. The damages which were mainly done to DNA is through base modification, oxidation of deoxyribose, strand breakage and DNA—protein cross-links. The ROS-induced DNA damages cause various mutagenic alterations too. When the ROS interferes with normal cell signaling, the gene expression gets altered. ROS have also been known for the activation of mutations in human gene. The oxidative damage by ROS in mitochondrial DNA leads to the formation of unusual components of the electron transport chain resulting in the formation of more ROS by increased leakage of electrons, and therefore causes further cell damage. The oxidative damage to mitochondrial DNA ultimately supports cancer and aging (Bandopadhyay et al., 2000).

2.9.2 Carcinogenesis

Many studies have been done till the date to study the involvement of free radicals in carcinogenesis, mutation and transformation. But no definite results were found for the involvement of free radicals in these processes, it has been found that the reactive species found in bio system causes mutation, transformation and ultimately cancer. The known biological effect of radiation is the induction of mutagenesis which occurs mainly through the free radicals especially by the hydroxyl radical, produced by radiolysis of water, by damaging DNA. These biological effect causes cell mutagenesis and carcinogenesis. Some studies done on the lipid peroxides like benzo (a) pyrene, have shown that these compounds have a tendency to activate carcinogens, and such compounds are also found to be capable of activating some types of promoters of carcinogens (Hertog et al., 1992, 1993a and 1993b).
2.10 Anti-oxidants

Anti-oxidants are defined as molecules which, in small concentrations protect, and prevent or reduce the extent of oxidative destruction of bio-molecules (Halliwell, 1990). In recent years there has been an increased interest in the appliance of anti-oxidants to medical treatment as it have found to be linking the development of human diseases to oxidative stress. The free radicals play many important physiological roles. The anti-oxidants were stored in the body and they neutralized the free radicals produced during various body functions.

2.10.1 Anti-oxidant Defence Enzyme Network

Epidemiological and animal studies suggest that the regular consumption of fruits, vegetables and whole grains, reduces the risk of chronic diseases associated with oxidative damages. Carotenoids, tocopherols, ascorbates, lipoic acids and polyphenols are strong natural anti-oxidants with free radical scavenging activity (Prakash and Sharma, 2014). The free radicals cause damage to the cells and organ systems of the body, to protect cells and organ systems of the body by the adverse effect of the reactive oxygen species, the body of the human beings have developed an anti-oxidant protection system which is highly complex yet sophisticated. It involves a variety of components, both endogenous and exogenous in origin, that function interactively and synergistically to neutralize free radicals (Table 2.3). Anti-oxidant defense system against oxidative stress is composed of several lines, and the anti-oxidants are classified into four categories based on function (Table 2.4).

2.10.2 Mechanisms of Action of Anti-oxidants

The principle of mechanisms of action of anti-oxidants has been proposed by Vaya and Aviram (2001). One of the first mechanisms is a chain-breaking mechanism, by which the primary anti-oxidant donates an electron to the free radical present in the system (e.g., lipid radical) forming a new radical, more stable than the initial one. Such primary anti-oxidants consist of compounds such as flavonoids, tocopherol and ascorbic acid. Another mechanism involves removal of ROS initiators (secondary anti-oxidants) by quenching chain-initiating catalysts. In this
mechanism the deactivation of high-energy species like $\text{O}_2^-$, absorption of ultra violet light, chelations of metal catalysing free radical reactions, or by inhibition of peroxidases, such as xanthine oxidase or lipoxygenases (Vaya and Avrim, 2001) takes place. Compounds that can react with the initiating radical, inhibit the initiating enzyme, or reduce the $\text{O}_2$ level without generating ROS, can be considered as a secondary anti-oxidant (Aazza et. al., 2011).

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Enzymatic and non-enzymatic anti-oxidants</th>
<th>Location</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Superoxide dismutase (SOD)</td>
<td>Mitochondria and cytosol</td>
<td>Dismutase superoxide radicals</td>
</tr>
<tr>
<td>2.</td>
<td>Glutathione peroxidase (GSH)</td>
<td>Mitochondria and cytosol</td>
<td>Removes hydrogen peroxide and organic hydroperoxide</td>
</tr>
<tr>
<td>3.</td>
<td>Catalase (CAT)</td>
<td>Mitochondria and cytosol</td>
<td>Removes hydrogen peroxide</td>
</tr>
<tr>
<td>4.</td>
<td>Vitamin E</td>
<td>Cell membrane</td>
<td>Major chain-break anti-oxidant in cell membrane</td>
</tr>
<tr>
<td>5.</td>
<td>Uric acid</td>
<td>Product of purine metabolism</td>
<td>Scavenger of OH radicals</td>
</tr>
<tr>
<td>6.</td>
<td>Glutathione</td>
<td>Nonprotein thiol in cell</td>
<td>Serves multiple roles in the cellular anti-oxidant defense</td>
</tr>
<tr>
<td>7.</td>
<td>$\alpha$-lipoic acid</td>
<td>Endogenous thiol</td>
<td>Effective in recycling vitamin C, may also be an effective glutathione substitute</td>
</tr>
<tr>
<td>8.</td>
<td>Carotinoids</td>
<td>Lipid soluble anti-oxidants, located in membrane tissue</td>
<td>Scavengers of reactive oxygen species, singlet oxygen quencher</td>
</tr>
<tr>
<td>9.</td>
<td>Metals ions sequestration: transferrin, ferritin, lactoferrin,</td>
<td></td>
<td>Chelating of metals ions, responsible for Fenton reactions</td>
</tr>
<tr>
<td>10.</td>
<td>Nitric oxide</td>
<td></td>
<td>Free radical scavenger, inhibitor of LP</td>
</tr>
</tbody>
</table>

**Table No. 2.3:** Important Enzymatic and Non-enzymatic Physiological Anti-oxidants
(Source-Singh, et. al., 2012)
2.10.3 Anti-oxidants and Lifespan

A study conducted by Cutler (1991) on the lifespan of mammals and their level of anti-oxidants (anti-oxidants and ageing) showed in their study that the longevity is very much related to the factors of ageing in group of mammals including human beings also. It was found that the level of anti-oxidants present in different mammals is different and is directly related to the species expected life spans. It

Table No. 2.4: Various Types of Anti-oxidants based on their Functions (Source-Singh, et al., 2012).
was also found that the human beings possess the highest level of anti-oxidants and so they were found to have the longest life expectancies as compared to others mammals.

The experimental studies have also done by Short shown that the life span of laboratory animals can be prolonged with anti-oxidants (Short et. al., 1997). Yet another studies have demonstrated that the mice fed with 0.5 – 1 percent anti-oxidants increased their life span to 30-40 percent. (Harman, 1968 and Pearson and Shaw, 1982). Further these anti-oxidants are used as food such as synthetic anti-oxidants such as Sevitoquin (commonly used as a stabiliser in chicken food) and butylated hydroxy-toluene (BHT) an anti-oxidant used as food additive. The taste and smell of synthetic anti-oxidants reduces the food intake, which is known to retarded ageing in mammals (Scott, 1995).

It was found in some research studies that anti-oxidant capable of inhibiting essential free radical reactions would become toxic if taken more than required e.g. Butylated hydroxy-toluene (BHT) is toxic in the mouse when added to the diet at levels more than 0.5 percent by weight (Horrum et. al., 1987).

2.10.4 Epidemiological Studies

Most of the researches which has been done have centred on diet-derived anti-oxidants such as vitamins C and E and carotenes. In a review by Diplock et. al., (1998) it was concluded that though epidemiological studies support the hypothesis that these vitamins may play a beneficial role in reducing the risk of several chronic age-related diseases, but still findings for human intervention studies are inconsistent. Many synthetic anti-oxidants have been proved although more efficient in vitro than biological anti-oxidants of the body defence system, produce unwanted side reactions unrelated to their biological functions (Rekka and Kourounakis, 1991). The β carotene for example can either inhibit or exacerbate the growth of cancer cells, depending upon the dosage (Lowe et. al., 1999 and Wang and Russell, 1999). A large human intervention trial carried out on smokers to investigate the efficacy of β carotene and alpha-tocopherol supplementation in reducing the incidence of lung and other cancers gave unexpected results. None of the smokers showed benefit, and also an 18 percent increase in the incidence of
lung cancer was also observed among those who only received β carotene (Ommen et al., 1994; 1996 and Collins, 1999).

The unoxidized form of β carotene appears to be an anti-carcinogen, but its oxidised products appear to facilitate carcinogenesis. The carcinogenic response in lung tissue to high dose β carotene observed in the trial is thought to be caused by the instability of β carotene in the ROS rich environment of the lungs, particularly in cigarette smokers (Crayhon, 2001 and Wang and Russell, 1999). This is especially possible because smoke decreases tissue levels of other anti-oxidants such as ascorbic acid and alpha-tocopherol, which in general have a stabilising effect on the unoxidised form of β carotene (Crayhon, 2001).

Dr. Jeff Evans an oncologist, Cancer Research Campaign, University of Glasgow quoted that even in successful human trials using β carotene supplementation, protection lasts only as long as the supplement continues and ends on its discontinuation (Evans, 2001). The anti-oxidant vitamins used alone especially in doses considerably higher than normal dietary intake can produce beneficial, detrimental or insignificant results depending on the circumstances (Boik, 2001).

The epidemiological studies shows that increased intake of fruits and vegetables confirm their protective function against various diseases such as cardiovascular disease and cancer (Eastwood, 1999). This protective effect may be due to a variety of constituents, including not only a combination of vitamins, minerals and fibre, but a number of phyto-chemicals, including flavonoids (Aruoma, 1993 and Eastwood, 1999).

The studies for electron transfer carried out by Anderson et al., (2000) support the possibility that dietary flavonoids can repair wide range of oxidative radical damage sustained by DNA by a mechanism of hydrogen atom transfer from the flavonoids to the free radical which is sited on DNA, results in the potent and fast chemical repair of some of the oxidative damage resulting from hydroxyl radical attack.
2.11 Dietary Supplementation with Anti-oxidant Enzymes

The therapeutic benefits of oral supplementation with isolated anti-oxidant enzymes are mixed. For example, superoxide dismutase (SOD) taken as a nutritional supplement is ineffective due to the destruction of the enzyme during the digestive process (Dev-Marderoscan, 2001). The results for Coenzyme Q10 [2, 3 dimethoxy-5 methylbenzoquinone (CoQ 10)] a naturally occurring fat soluble quinine taken as a food supplement, are more potential (Lamson and Brignall, 1999 and Dev-Marderoscan, 2001). Morton et. al., (1957) introduced the name ubiquinone, meaning the ubiquitous quinone because it is ubiquitous in eukaryotic cells. Mellors and Tappel (1966) proved that in its reduced form, in vitro it was an effective anti-oxidant against lipid peroxidation in membranes. Ubiquinone is found in foods, Ames and co-workers proposed that optimal levels of dietary ubiquinone/ubiquinol could be important in many of the degenerative diseases of ageing (Ames et. al., 1993 and Burke et. al., 2001).

2.12 Natural Anti-oxidants and Chemoprevention

It has been found that the plants possess numerous anti-oxidants as their defense system against active oxygen species, which are very potent. Numbers of microbes, seaweeds, animal products such as fermented products, protein hydrolysis, were found to be potent anti-oxidants. Daily foods like fruits, vegetables, tea etc have a wide range of molecules capable of scavenging free radicals. These molecules are known as natural anti-oxidants. Among various anti-oxidants, the secondary products from plants are of great interest (Bendich, 1996).

The important secondary products from plants are plant phenolics (flavonoids, phenylpropanoids, coumarines), polyphenolic (proanthocyanidins, tannins), nitrogen containing compounds (alkaloids, isocyanate, indoles, non-proteins amino acids), carotenoids, chlorophyll derivatives, phytosterols.

The most commonly distributed group of phyto-chemicals is polyphenols, ranging from simple phenols to highly polymerized tannins. The intake of polyphenols is very beneficial to human beings as they block specific enzymes that cause inflammation. The polyphenols also takes part in the prostaglandin pathways by protecting platelets from clumping.
The flavonoids act as anti-oxidant, anti-hepatotoxic, anti-allergic, anti-inflammatory, hypoglycemic, immuno-stimulant and also stabilizing capillary permeability. Specifically phenols help in lowering the risk of cardiovascular diseases, cancer, reduce inflammation, Parkinson’s disease, Alzheimer’s disease, age related vision disorders and asthma (Escarpa and Gonzalez, 2001 and Nichenametia et. al., 2006). Therefore, the studies of phyto-chemicals have shown strong potential with free radical scavenging capacity and their mode of action in human health care system as nutraceuticals and functional foods (Rowan-Robinson et. al., 1997). Some important known phyto-chemical constituents are given below-

### 2.12.1 Polyphenols

The polyphenols are benzene ringed phyto-chemicals present in almost all the parts of the plants, functioning in various important and protective roles. Some natural sources that contain polyphenols are shown in Table 2.5. A recent study revealed that polyphenolics also showed indirect anti-oxidant effects by the induction of endogenous protective enzymes. Many studies have also proved the benefits of polyphenolic mediated regulation in cellular processes such as inflammation and cancer. Phenolic acids and flavonoids also have anti-oxidant and anti-carcinogenic activity. Some epidemiological studies have shown the inverse relationships between the intake of flavonoids (flavonols and flavones) and stomach cancer and the risk of coronary heart disease. Many secondary products also act as strong bio anti-mutagens. The anti-mutagen activity of green tea extract has been studied, for which epigallocatecin gallate present in the green tea seems to be most responsible (Wang et. al., 1989 and 1994).

Phenolic acids and flavonoids are the most occurring polyphenols in plants. The total dietary phenols contain 60 percent of flavonoids and 30 percent of phenolic acids. (Escarpa and Gonzalez, 2001 and Nichenametia et. al., 2006). Polyphenols from apples, bellies, chamomile, citrus, dandelion, fruits, green tea, grapes, ginkgo, hawthorn, licorice, onions, red wine, rosemary, thyme, vegetables and beverages have been studied for their anti-oxidant activity and biological properties which showed the presence of phenols which enhances the efficacy of vitamin C, act against allergies, tumors, ulcers, platelet aggregation, reduce the risk of cancer and are also effective in controlling hypertension (Kondratyuk and Pezzuto, 2004).
Flavonoids and their derivatives were found from the Shikimate pathway has been classified into anthocyanidins, flavones, flavanones, flavanols, isoflavones, and proanthocyanidins. Flavonoids possess ideal structure for free radicals scavenging activity and have been found to be more effective anti-oxidants in vitro than tocopherols and ascorbates (Amie et al., 2003). They are efficient reducing agents that can stabilize the polyphenols derived radicals and delocalize the unpaired electrons. Flavonoids can also generate H₂O₂ by donating a hydrogen atom from their pyrogallol or catechol structure to oxygen, through a superoxide anion radical (Andjelkovid et al., 2006). The pyrogallol-type compounds generate more H₂O₂, than that of catechol. H₂O₂ has been reported to raise levels of intracellular Ca²⁺, activate transcription factors, repress expression of certain genes, promote or inhibit cell proliferation, cytotoxic, activate or suppress certain signal transduction pathways, promote or suppress apoptosis (Rietjens et al., 2002).

2.12.2 Carotenoids

Carotenoids are the plant pigments, present in the human diet as micro-components of fruits and vegetables. There are approximately 700 naturally occurring carotenoids which are known as biological anti-oxidants and protect tissues and cells from the harmful effects of free radicals. Carotenoids are found in many human foodstuffs, of both plant and animal origin, but are widely present in fruits and vegetables. The major carotenoids are α-carotene, β-carotene, β-cryptoxanthin, lutein and lycopene. Few carotenes like alpha, beta and epsilon carotenes possess vitamin A activity and out of them β-carotene is the most active. Natural β-carotene is the precursor of vitamin A and has preventive action against eye diseases and cancer. Lycopene gives tomatoes their red color and is particularly effective at quenching the destructive singlet oxygen. Along with carotene and lutein, it provides protection against lung, breast, uterus and prostate cancers. Limonoids, the second major subclass of terpenoids, are the biologically active phyto-chemicals present in citrus which act as anti-oxidant and protect lung tissues from free oxygen radicals. In vitro studies show that limonin, nomilin and lirnonoid glycosides have significant ability to inhibit proliferation of human breast cancer (Ortuno et al., 2006 and Sun et al., 2005).

The epidemiologic studies proposed that dietary intake of carotenoids influences
the risk for certain types of cancer, cardiovascular disease and other chronic
diseases. Although, it would be ideal to use humans directly to answer critical
questions regarding carotenoid absorption, metabolism and effects on disease
progression, appropriate animal models offer many advantages. Each potential
model has strengths and weaknesses (Willcox et al., 2004; Kris-Etherton et al.,
2002 and Papas, 1999). However, ferrets, mice and rats can be used to study
cancer, whereas primates and gerbils are probably more appropriate for studies on
biomarkers of heart disease (Papas, 1999).

Epidemiologic studies also indicated that an increased intake of vegetables and
fruits that contain carotenoids is linked with a decreased risk of many types of
cancer including breast, lung, and those affecting the gastrointestinal tract
(Kornsteiner et al., 2006) and also a decreased risk of cardiovascular disease (Park
and Pezzuto, 2002). Consumption of specific vegetables and fruits has also been
associated with a decreased risk of prostate cancer and β-carotene supplementation
has been shown to enhance natural killer cell activity in elderly men (Lee et al.,
2001). In contrast of which it has been reported that supplementation of β-carotene
may increase the risk of lung cancer (Lutsenko et al., 2002). Many clinical studies
suggested it a protective effect.

Inhibitory effects were reported in two of the studies using aberrant crypt foci, an
intermediate lesion leading to colon cancer, as an end point and in two mammary
tumor studies, one using the dimethylbenz (a) anthracene model, and the other the
spontaneous mouse model. Inhibitory effects were also reported in mouse lung and
rat hepato-carcinoma and bladder cancer models. In one of the report from the
researcher suggested no effect in the N-nitroso-methylurea-induced mammary
tumor model when crystalline lycopene or a lycopene-rich tomato carotenoid
oleoresin was administered in the diet. Several retrospective and prospective
epidemiological studies indicate that tomato consumption (Lee et al., 2001),
lycopene intake (Izzo et al., 2002), and serum lycopene levels are associated with
decreased risk of cancers, most notably prostate and lung cancer.

2.12.3 Vitamin E (Tocopherols and Tocotrienols)

Vitamin E belongs to a family of eight molecules having a chromanol ring
(chroman ring with an alcoholic hydroxyl group) and a 12-carbon aliphatic side chain containing two methyl groups in the middle and two more methyl groups at the end. The tocopherols and tocotrienols are non-polar constituents of biological membranes that exist in nature in lipid phase. The vitamin E is known as a natural anti-oxidant which fights with the damaging natural substances which are known as free radicals. Mode of action of the Vitamin E is by working in lipids (fats and oils), making it complementary to vitamin C, which finally fights free radicals dissolved in water. As an anti-oxidant, vitamin E has been widely accepted for preventing heart disease and cancer. α-tocopherol is the most abundant form, with high vitamin E activity and singlet oxygen scavenging ability than other forms of tocopherols (Rietjens et. al., 2002). The anti-carcinogenic activity of vitamin E is contributes largely to its potent anti-oxidant activity where the major hydrophobic chain-breaking anti-oxidant protects membrane lipids from oxidation.

Vitamin E has also been reported to interfere with hormone signaling, which is particularly relevant to prostate carcinogenesis. Data from mechanistic studies showed that vitamin E acts synergistically with other anti-oxidant nutrients in vitro. For example, vitamin C and various flavonoids have been known to regenerate α- tocopherol from α-tocopheroxyl radicals, which are formed when the parent molecule reduces free radicals (May, 1999; Pedrielli and Skibsted, 2002 and Zhu et. al., 1999). In addition to which the α-tocopherol has also been shown to regulate the expression of several genes involved in growth, apoptosis, and inflammation, as well as several anti-oxidant defense genes (Azzi et. al., 2004b).

Synergy between anti-oxidants has also been observed in human prostate cancer cell lines, although the resulting effects may not be attributable to their anti-oxidant activity. A study conducted on people consuming vitamin E showed that the people with highest amounts of vitamin E intake had the greatest benefit in lung cancer as compared to the people with lowest amounts of vitamin E intake. It was found that there was a 61 percent reduction in lung cancer risk with the intake of vitamin E high dose (Mahabir et. al., 2008). The role of vitamin E in cancer prevention is ambiguous and data of observed study done also gave positive result. The supplementation with vitamins E, C, and beta carotene did not prevent cancer incidence in randomized clinical trials (Bjelakovic et. al., 2004) nor did it affect
cancer mortality (Lin et. al., 2009).

2.12.4 Ascorbic Acid

Ascorbic acid (vitamin C) is known as a powerful natural anti-oxidant which neutralizes damaging natural substances called free radicals. Studies have shown that it scavenges reactive oxygen species and also possess anti-carcinogenic effects (Kim and Lee, 2004 and Lee et. al., 2001). Nobel loreate Dr. Linus Pauling in 1960s stated that vitamin C could effectively treat both cancer and the common cold. The anti-oxidant mechanism of ascorbic acid is based on hydrogen atom donation to lipid radicals, quenching of singlet oxygen and removal of molecular oxygen (Singh et. al., 2012).

Ascorbic acid can be easily oxidized by factors like heat, light, water, pH, oxygen concentration and metal ions like Cu$^{+2}$ and Fe$^{+3}$. Ascorbic acid is found to be effective in heart diseases and some forms of cancer. Ascorbic acid along with tocopherol can reduce oxidative damage. Some studies conducted on vitamin C, showed that vitamin C is found to be toxic to tumor cells. Vitamin C kills the tumor cells without affecting the normal cells. It is found that vitamin C when given in low doses only acts as an anti-oxidant but with the increase in the dosages, the role of vitamin C changes and it becomes a pro-oxidant, inducing peroxide production (Singh et. al., 2012).

2.12.5 Lipoic Acid

Some sulphur containing compounds like glutathione (GSH), lipoic acid (1, 2-dithilane-3-pentanoic acid) and dihydrolipoic acid (DHLA) present in meat, liver and heart show anti-oxidant activities. They prevent oxidative damage of proteins; regenerate GSH in liver, kidney and lung tissues. Lipoic acid occurs in three different forms: R-Lipoic acid is the pure form of lipoic acid and is found in each and every cells of the body from the simplest organisms up to humans. S-Lipoic acid is a by-product from chemical synthesis and interferes with some of the beneficial properties of the R-form, especially, in interactions with proteins and enzymes. Some group of researchers believe that lipoic acid also found to inhibit the action of some genes that activate cancer cells to grow and it contain a
component which is known to be anti-cancerous or it acts as a complementary therapy to prevent some side effects of conventional cancer treatments.

The anti-oxidant α-lipoic acid (ALA) has been shown to affect a variety of biological processes associated with oxidative stress including cancer. In recent years, the studies in the cancer field, related to lipoic acid as an anti-cancerous agent has been increased. The results obtained from the anti-proliferation studies on cancerous cell-based models have showed that the tumor-suppressive effect of lipoic acid corresponds with apoptosis induction, a critical parameter impaired in cancer cells, and this induction is selectively exerted in cancer and transformed cell lines, while it has been less active on the normal non-transformed cells (Pack et. al., 2002 and Wenzel et. al., 2005). A recent study conducted showed that α-lipoic acid and DHLA can effectively induce apoptosis in human colon cancer cells by a pro-oxidant mechanism that is initiated by an increased uptake of oxidizable substrates into mitochondria (Wenzel et. al., 2005).

In one of the study, conducted on some patients with advanced cancers have shown that the α-lipoic acid had beneficial effects on the patients by increasing the glutathione peroxidase activity and by reducing oxidative stress (Mantovani et. al., 2003). A study conducted by some researchers on lipoic acid found that the lipoic acid showed its anti-tumor effects by inducing cell cycle arrest and cell death in human promyelocytic HL-60 cells which was achieved by inhibition of both cell growth and viability in a time- and dose-dependent manner. Lipoic acid has been reported to be effective in human leukemic T cells, lipoic acid potentiate Fas-mediated apoptosis through redox regulation without affecting peripheral blood monocytes from healthy humans (Sen et. al., 1999). In some other studies conducted on lipoic acid for their anti-oxidant property using anti-oxidant response element (ARE) reporter assay, it has also been shown to induce phase II protective genes. These genes are known to be helpful in the prevention of carcinogenesis, in non-cancerous animal and cell-based studies (Flier et. al., 2002 and Cao et. al., 2003).

2.12.6 Selenium (Se)

Selenium (Se) is a trace mineral and is found in water, soil, vegetables (garlic,
onion, grains, nuts and soybean), meat, sea food, yeast, liver (Willcox et al., 2004). Even at a very low dose, this mineral has many benefits regarding health like that of anti-oxidant, anti-carcinogenic and immuno-modulator (Pham-Huy et al., 2001). Studies have shown significant anti-cancer effect of selenium on breast, liver, lung and small intestinal tumor cells. The best role of selenium in mammalian cells is being a component of the seleno-enzyme, glutathione peroxidase which is localized in the cytosol and mitochondrial matrix, and it removes organic peroxides from the cell. Some reports are present which showed that selenium alters the metabolism of carcinogens or the interaction of chemical carcinogens with DNA (Medina and Morrison, 1988). Some other mechanistic studies have also shown that selenium alters cell proliferation and immunologic responses (Medina and Morrison, 1988 and El-Bayoumy, 1991).

Many researchers have stated that application or intakes of selenium at higher levels than required for normal metabolism inhibited carcino-genesis-tumorigenesis (El-Bayoumy, 1991 and Combs and Gray, 1998). A study conducted on 34,000 men showed that men with low baseline selenium levels were three times more susceptible to develop advanced prostate cancer than men with high selenium levels (Yoshizawa et al., 1998). Another study conducted on women proved that women born with mutations of the BRCAI gene have a very high risk of breast and ovarian cancer and selenium supplementation for 1-3 months reduced chromosome breaks in those women to normal levels (Kowalska et al., 2005).

Number of studies showed that the cancer risk in men has more profoundly influenced by selenium status than in the women. Factors which are responsible for the apparent difference in the effects of selenium on cancer incidence in men and women may include sex-based differences in the metabolism and/or tissue distribution of selenium, and also sex-related factors that influence tumor biology (Waters et al., 2004).

Whanger (1998) has shown that selenium addition to salt reduced cancer incidence. Similarly, selenium supplementation gives a significant decrease in DNA synthesis in breast cancers cells (MCF-7 and MUIR-3) and also increase in apoptosis. The selenium benefit was just as impressive in cancers of the lung (RH2), small intestine (HCF8), colon (Caco-2), and liver (Hep G2). Prostate cancers (PC-3 and LNCaP) as
well as colon cancer (T-84), although initially less affected by supplementation, became responsive when selenium was coadministered with Adriamycin or Taxol (Vadgama et. al., 2000).

<table>
<thead>
<tr>
<th>Class and Sub-class</th>
<th>Food or Beverages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td><strong>Fruits</strong>: blackberries, black current blackberries, black grapes, elderberries, strawberries, cherries, pomegranate juice, raspberry.</td>
</tr>
<tr>
<td></td>
<td>Others- red wine</td>
</tr>
<tr>
<td>Anthocyanidins</td>
<td><strong>Vegetables</strong>: cherry tomatoes, chives, capers, celery, onions, red onions, celery, broccoli dock leaves, fennel, hot peppers, spinach, lettuce,</td>
</tr>
<tr>
<td>Anthoxanthins</td>
<td><strong>Cereal</strong>: beans (green/yellow), buckwheat.</td>
</tr>
<tr>
<td>Flavonols</td>
<td><strong>Fruits</strong>: apples, apricots, grapes, plums, bilberries, blackberries, cherries, black currant juice, apple juice, <em>ginkgo biloba</em></td>
</tr>
<tr>
<td></td>
<td><strong>Others</strong>: red wine, tea (green, black), tea (black beverage), cocoa powder, turnip (green), endive, leek</td>
</tr>
<tr>
<td>Flavanones</td>
<td><strong>Citrus fruits and juices</strong>: lemon, lemon juice, lime juice, orange, tangerine juice</td>
</tr>
<tr>
<td>Flavones</td>
<td><strong>Fruits</strong>: celery, olives</td>
</tr>
<tr>
<td>Phenolic acids</td>
<td><strong>Vegetables</strong>: hot peppers, celery hearts, fresh parsley</td>
</tr>
<tr>
<td>Hydroxycinnamic acids</td>
<td><strong>Fruits</strong>: blueberry, cranberry, pear, cherry (sweet), apple, orange, grapefruit, cherry juice, apple juice, lemon, peach</td>
</tr>
<tr>
<td>Trihydroxystilbenes</td>
<td><strong>Vegetables</strong>: potato, lettuce, spinach</td>
</tr>
<tr>
<td>Tannins</td>
<td>Others: coffee beans, tea, coffee, cider</td>
</tr>
<tr>
<td></td>
<td><strong>Fruits</strong>: grapes, peanuts</td>
</tr>
<tr>
<td></td>
<td><strong>Others</strong>: red wine</td>
</tr>
<tr>
<td></td>
<td><strong>Fruits</strong>: grapes, apple juice, strawberries, longan, raspberries, pomegranate, walnuts, peach, blackberry, olive, plum</td>
</tr>
<tr>
<td></td>
<td><strong>Vegetables</strong>: chick pea, black-eyed peas, lentils</td>
</tr>
<tr>
<td></td>
<td><strong>Cereal</strong>: haricot bean</td>
</tr>
<tr>
<td></td>
<td><strong>Others</strong>: wine, cocoa, chocolate, tea, cider, tea, coffee</td>
</tr>
</tbody>
</table>

**Table No. 2.5**: Polyphenolic Content in Some Natural Sources.  
(Source-Singh, et. al., 2012)
References:


175. World Conservation Monitoring Center (1992). *Global Biodiversity: Status*
