Chapter 1

Biologically active Natural Products - An Overview with special reference to anticancer and antioxidant compounds from terrestrial sources

1.1 Introduction

Man has utilized materials from nature for his basic needs like food, clothing, shelter and medicine throughout the ages. Nature has been the source of medicines for the treatment of a wide spectrum of diseases all over the world and across wide spectrum of civilizations. Plant based sophisticated traditional systems of medicines were developed in many parts of the world like Egypt (Eberus Papyrus which dated from 1500 BCE documenting over 700 drugs), China (Chinese materia medica dating from about 1100 BCE) and India (Charaka and Sushruta Samhitas from before 1000 BCE) from ancient days. The indigenous people of South America derived medicines and poisons from thousands of plants. The rational development of modern medicine has its roots in such traditional medicines and therapies.

Many such drugs discovered early are still used in the modern system of medicine and many more carry the structural imprint of the parent molecular prototype or natural product which led to their discovery. It has been reported that nearly 120 compounds derived from 90 plant species can be considered as important drugs currently in use in one or more countries, with 77% of these being derived from plants used in traditional medicine.\(^1\) A large number of therapeutic activities are mediated by these drugs and a host of these drugs currently in use are still obtained from the plants in which they
are synthesized. Examples include steroids, cardiotonic glycosides (*Digitalis* glycosides), anticholinergics (belladonna type tropane alkaloids), analgesics and antitussives (opium alkaloids), antihypertensives (reserpine), cholinergics (physostigmine, pilocarpine), antimalarials (*Cinchona* alkaloids), antigout (colchicine), anesthetic (cocaine), skeletal muscle relaxant (tubocurarine) and anticancer (taxol) agents. Some of the important plant based drugs (1-15) are shown in Table 1.1.

**Table 1.1:** Prominent plant derived medicinal compounds

<table>
<thead>
<tr>
<th>No</th>
<th>Name</th>
<th>Source</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Guggulsterone</td>
<td><em>Commiphora mukul</em></td>
<td>Lowers cholesterol</td>
</tr>
<tr>
<td>2</td>
<td>Reserpine</td>
<td><em>Rauwolfia serpentina</em></td>
<td>Controls high blood pressure</td>
</tr>
<tr>
<td>3</td>
<td>Cocaine</td>
<td><em>Erythroxylon coca</em></td>
<td>Anesthetic</td>
</tr>
<tr>
<td>4</td>
<td>Pilocarpine</td>
<td><em>Pilocarpus jaborandi</em></td>
<td>Cures glaucoma</td>
</tr>
<tr>
<td>5</td>
<td>Atropine</td>
<td><em>Atropa belladonna</em></td>
<td>Ophthalmic treatment</td>
</tr>
<tr>
<td>6</td>
<td>Hyoscine</td>
<td><em>Hyoscyamus niger</em></td>
<td>Treats nausea</td>
</tr>
<tr>
<td>7</td>
<td>Digoxin</td>
<td><em>Digitalis lanata</em></td>
<td>To treat cardiac disorders</td>
</tr>
<tr>
<td>8</td>
<td>Colchicine</td>
<td><em>Colchicum autumnale</em></td>
<td>Cures rheumatism</td>
</tr>
<tr>
<td>9</td>
<td>Emetine</td>
<td><em>Psychotria ipecacuanha</em></td>
<td>Anti-amoebic</td>
</tr>
<tr>
<td>10</td>
<td>Vincristine</td>
<td><em>Catharanthus roseus</em></td>
<td>Cancer chemotherapy</td>
</tr>
<tr>
<td>11</td>
<td>Taxol/Paclitaxel</td>
<td><em>Taxus brevifolia</em></td>
<td>Cancer chemotherapy</td>
</tr>
<tr>
<td>12</td>
<td>Forskolin</td>
<td><em>Coleus forskohlii</em></td>
<td>Vasodilator</td>
</tr>
<tr>
<td>13</td>
<td>Calanolide A</td>
<td><em>Calophyllum lanigerum</em></td>
<td>Anti-HIV agent</td>
</tr>
<tr>
<td>14</td>
<td>Quinine</td>
<td><em>Cinchona officinalis</em></td>
<td>Antimalarial</td>
</tr>
<tr>
<td>15</td>
<td>Artemisinin</td>
<td><em>Artemesia annua</em></td>
<td>Antimalarial</td>
</tr>
</tbody>
</table>

Emergence of modern pharmaceutical industry is an outcome of different activities involving synthetic chemists, natural product chemists, pharmacologists, microbiologists and biochemists etc., which has led to the
development of potent single molecules with highly selective activity for a wide variety of ailments. Synthetic drugs were developed with improved properties as compared to the natural ones they were based on. For e.g., chloroquine, the synthetic anti-malarial drug is much less toxic than quinine 15, the white crystalline alkaloid extracted from the bark of South-American Cinchona tree. The most fascinating aspect of these plant based drugs is their structural variety as shown in chart 1.1.

**Chart 1.1:** Structures of some prominent plant based drugs
However, rapid growth of synthetic organic chemistry in the early and mid twentieth century made available a very large number of compounds, and random screening of such chemicals by pharmaceutical companies led to the development of many synthetic drugs like sulphonamides, isoniazids, synthetic anti-psychotics, anti-histamines and synthetic penicillin derivatives etc., which were highly useful. These successes from synthetic therapeutic drugs reduced interest in natural product based drug discovery and many major drug companies almost neglected natural product chemistry in the latter decades. In addition, the clinical efficacy of the botanical medications could not be evaluated/established de rigour. Thus, herbal medicines often reflected poor quality control for clinical efficacy.

Currently, the lag phase for botanical medicine is rapidly changing for a number of reasons. Problems with drug-resistant micro-organisms, side effects of modern synthetic drugs and emerging diseases where no medicines are available have stimulated renewed interest in plants as a significant source of new medicines. As a result, considerable research on pharmacognosy, chemistry, pharmacology and clinical therapeutics are being carried out on medicinal plants\(^2\) mainly based on the information from the traditional systems of medicine. A whole range of chronic and difficult to treat diseases such as cancer, cardiovascular diseases, diabetes, rheumatism and AIDS, all require new effective drugs.

**1.1.1 Current status**

It has been estimated that a large group of world population depends on crude plant drug preparations to tackle various health problems. In India, China and other countries with reputed traditional systems of medicine, plant based therapeutic agents occupy an important niche in health management. The last three decades witnessed new developments in natural products based drugs. Even in the economically developed countries, there is an ever growing interest in natural remedies, which have come to be known as
ʻphytomedicines’. These preparations are invariably single plant extracts or fractions thereof as distinct from pure chemical entities which may be called molecular drugs. The World Health Organization also has recognized the importance of traditional medicine and has been active in creating strategies, guidelines and standards for botanical medicines.\(^3\)

The mass screening of plants in the search for new drugs is vastly expensive and inefficient. However, such a programme was carried out for obtaining anticancer drugs by National Cancer Institute, USA and this effort led to the discovery of the anticancer drug Taxol (11). It would be cheaper and perhaps more productive to re-examine plant remedies described in different traditional medicine texts. i.e., Ethno-pharmacology could be used to identify drugs to alleviate human illness through a thorough analysis of plants known to be used by different human cultures throughout the world. Thus, there is opportunity for multidisciplinary research that joins the forces of natural products chemistry, molecular and cellular biology, synthetic and analytical chemistry, biochemistry and pharmacology to exploit the vast diversity of chemical structures and biological activities of natural products.

The traditional systems of medicine have a relatively organized database and a more exhaustive description of botanical material that can be tested using modern scientific methods. In India, the Ayurvedic system of medicine has an important role in the bioprospecting of new medicines.

1.1.2 Ayurveda

Ayurveda is an ancient system of health care that is native to the Indian subcontinent and is being practiced for thousands of years.\(^4\) The word Ayurveda is a compound of the Sanskrit words āyus meaning “life” and veda, which refers to “knowledge”. Thus, Ayurveda roughly translates as the “knowledge of life”. According to Charaka Samhita, an ancient Indian Ayurvedic text on internal medicine written by Charaka, “life” itself is defined
as the “combination of the body, sense organs, mind and soul, the factor responsible for preventing decay and death, which sustains the body over time and guides the processes of rebirth”. According to this perspective, Ayurveda is concerned with measures to protect āyus, which includes healthy living along with therapeutic measures that relate to physical, mental, social and spiritual harmony. Ayurveda is also one among the few traditional systems of medicine to contain a sophisticated system of surgery. Ayurveda is still being successfully used in many countries. Indian healthcare consists of medical pluralism and Ayurveda still remains dominant compared to modern medicine, particularly for treatment of a variety of chronic disease conditions.

Traditional Ayurvedic therapeutic formulations draw on an impressive array of plants, many of which have not been scrutinized thoroughly by modern scientific methods. India has about 45,000 plant species and several thousands of them have been found to be of medicinal use. The first Ayurvedic herb which attracted international attention was Rauwolfia serpentina when it was found that its constituent alkaloid, reserpine 2,5, had the twin effect of lowering high blood pressure and can act as a tranquilizer. In its traditional usage, this plant has been used for the treatment of snake bites, feverish illnesses and insanity for about 3000 years.6 In the classical Ayurvedic literature, several therapeutically useful plants which act as immunomodulators, memory enhancers, neuroprotectives, antiobesity, antiaging agents, etc., have been described and which are now receiving modern scientific attention.7 Some recent work in drug development taking advantage of the Ayurvedic knowledge relates to the species of Commiphora (hypolipidaemic agent), Picrorhiza (hepatoprotective), Bacopa (memory enhancer), Curcuma (anti-inflammatory) and Asclepias (cardiotonic).8 Numerous molecules have come out of Ayurvedic experiential base, examples of which include the rauwolfia alkaloids for hypertension, psoralens from
Psoralea corylifolia in vitiligo, holarrhena alkaloids in amoebiasis, guggulsterons as hypolipidemic agents, Mucuna pruriens for Parkinson’s disease, piperidines as bioavailability enhancers, baccosides in mental retention, picrosides in hepatic protection, phyllanthins as anti-virals, curcumines in inflammation, withanoloides and many other steroids, lactones and glycosides as immunomodulators.⁹

It is now generally believed that recapitulation and adaptation of this older science to modern drug discovery processes can bring renewed interest to the pharmaceutical world and offer unique therapeutic solutions for a wide range of human disorders. Eventhough time-tested evidences vouch immense therapeutic benefits for Ayurvedic herbs and formulations, several important issues are required to be resolved for successful implementation of Ayurvedic principles to present drug discovery methodologies. Additionally, clinical examination in the extent of efficacy, safety and drug interactions of newly developed Ayurvedic drugs and formulations are required to be carefully evaluated. A reverse-pharmacology approach focusing on the potential of Ayurvedic herbs and herbal products for different targets could perhaps bring tremendous leads to Ayurveda based drug discovery. Although several novel leads and drug molecules have already been discovered from Ayurvedic medicinal herbs, further scientific exploration in this arena along with verification and standardization according to the modern system of medicine is required.

1.2 Natural products as anticancer agents

As the current research work deals mainly with the anticancer and antioxidant activities of natural products, an introduction to both these properties is discussed in detail in the coming sections.
1.2.1 Cancer

Cancer may be considered as one of the worst form of human diseases prevailing now. Modern man is confronted with an increasing incidence of cancer and cancer death annually. Mortality that results even from the common forms of cancer is still unacceptably high. Statistics indicate that men are largely plagued by lung, colon, rectum and prostrate cancer whilst women increasingly suffer from breast, colon, rectum and stomach cancer. The cause for the occurrence of cancer is considered to be one among the three main reasons, viz., incorrect diet, genetic predisposition and via the environment.

Cancer is a disease in which disorder occurs in the normal process of cell division that are controlled by the genetic material (DNA) of the cell. For a cell to replicate, it must:

1. faithfully reproduce its DNA
2. manufacture sufficient cellular organelles, membranes, soluble proteins etc., to enable the daughter cells to survive and,
3. partition the DNA and cytoplasm equally to form two daughter cells.

This process requires a significant amount of feedback control to ensure that the molecular steps are sequentially and correctly oriented. Failure to control the cell cycle is believed to proceed through many stages over a number of years or even decades. The stages of carcinogenesis include initiation, promotion and progression.

1.2.1.1 Carcinogenesis

The substances that initiate cancer in human body are termed as carcinogens. Viruses, chemical carcinogens, chromosomal rearrangements or spontaneous transformations, inactivity of tumor suppressor genes etc., have been implicated as causes of cancer. Genetic predisposition to cancer lends itself to ~20% of cancer cases thus leaving the majority of cancers being
associated with a host of environmental carcinogens. Environmental carcinogens include both natural and manmade chemicals, radiations and viruses. The carcinogens may be divided into genotoxic carcinogens, procarcinogens, epigenetic carcinogens and unclassified carcinogens. Genotoxic carcinogens are those substances that react with nucleic acids. These can be directly acting carcinogens as they can directly affect cellular constituents. Procarcinogens are substances that require metabolic activation to induce carcinogenesis. Epigenetic carcinogens are carcinogens that are not genotoxic (Table 1.2).

Table 1.2: Types of carcinogens

<table>
<thead>
<tr>
<th>Type</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Genotoxic carcinogen</td>
<td></td>
</tr>
<tr>
<td>Primary, direct-acting alkylating agents</td>
<td>Dimethyl sulphate, ethylene imine, β-propiolactone</td>
</tr>
<tr>
<td>2. Procarcinogens</td>
<td></td>
</tr>
<tr>
<td>Polycyclic aromatic hydrocarbons</td>
<td>Benzo(a)pyrene</td>
</tr>
<tr>
<td>Nitrosamines</td>
<td>Dimethylnitrosamine</td>
</tr>
<tr>
<td>Hydrazine</td>
<td>1,2-Dimethylhydrazine</td>
</tr>
<tr>
<td>Inorganic</td>
<td>Cadmium, Plutonium</td>
</tr>
<tr>
<td>3. Epigenetic carcinogens</td>
<td></td>
</tr>
<tr>
<td>Promoters</td>
<td>Phorbol esters, saccharin, bile acids</td>
</tr>
<tr>
<td>Solid state</td>
<td>Asbestos, plastic</td>
</tr>
<tr>
<td>Hormones</td>
<td>Estrogens</td>
</tr>
<tr>
<td>Immunosuppressants</td>
<td>Purine analogues</td>
</tr>
<tr>
<td>Cocarcinogens</td>
<td>Catechol</td>
</tr>
<tr>
<td>4. Unclassified</td>
<td></td>
</tr>
<tr>
<td>Peroxisome proliferators</td>
<td>Clofibrate, phthalate esters</td>
</tr>
</tbody>
</table>

Molecular diversity of cancer initiating compounds range from metals to complex organic molecules with large variation in their potencies. The variation in structure and potency suggests that more than one mechanism is involved in carcinogenesis.

Carcinogens in the diet that trigger the initial stage include moulds and aflatoxins (in peanuts and maize), nitrosamines (in smoked meats and other cured products), rancid fats and cooking oils, alcohol and additives and preservatives. A combination of foods may have a cumulative effect and
when incorrect diet is added to a polluted environment, smoking, UV radiation, free radicals, lack of exercise and stress, the stage is set for DNA damage and cancer progression. On the protective side, a diet rich in fruits, vegetables and fibre is associated with a reduced risk of cancer at most sites. The elimination of environmental carcinogens or at least avoiding exposure to them offers the opportunity to prevent most cancers, which is the basis of primary prevention.

One of the most important mechanisms contributing to cancer is considered to be oxidative damage to the DNA. If a cell containing damaged DNA divides before it is repaired, the result is likely to be a permanent genetic alteration constituting a first step in carcinogenesis. Body cells that divide rapidly are more susceptible to carcinogenesis because there is less opportunity for DNA repair before cell division. Mutagenic changes in the components of signaling pathways also lead to cancer.

1.2.1.2 Chemo preventive agents

Chemo preventive agents used in anticancer therapy exert their protective effects in specific stages of multi step carcinogenesis. During the late 1960s and early 1970s, pace setting studies were performed by Dr. Lee W. Wattenberg and his associates at the University of Minnesota in which it was demonstrated that various compounds, especially those associated with fruits and vegetables such as indoles and isothiocyanates could inhibit chemically induced tumors.14 This was the advent of the “chemoprophylaxis of carcinogenesis” and the implications of these observations in terms of human health maintenance became immediately apparent. Subsequently, a series of hallmark studies performed with a myriad of retinoids, Dr. Michael B. Sporn demonstrated that “cancer chemoprevention” was possible.15 In general terms, cancer chemoprevention may be considered as the prevention of cancer in human populations by ingestion of chemical agents that prevent carcinogenesis.
According to the conventional classification of chemo preventive agents as proposed by Wattenberg, they are of two categories viz., the blocking agents and the suppressing agents. The classification of chemopreventive agents according to their mechanism of action is illustrated in figure 1.1.

**Figure 1.1**: Classification of chemo preventive phytochemicals based on their mode of action

Blocking agents are typically those compounds that can inhibit initiation either by inhibiting the formation of carcinogens from (i) precursor molecules, (ii) reactive metabolites from the parent carcinogens and those preventing the ultimate electrophilic and carcinogenic species from interacting with critical cellular target molecules, such as DNA, RNA and proteins. Suppressing agents are considered to inhibit malignant expression of initiated cells, in either the promotion or the progression stage. Certain chemo preventive agents such as curcumin and resveratrol have more than one
defined mechanism of action and hence possess both suppressing and blocking properties.\textsuperscript{18}

![Chemical structures 16 and 17](image)

The fragility of humans for the susceptibility of cancer presents an ongoing challenge for individuals who are involved in the discipline of therapeutic intervention. It is therefore very important for the full recognition of the benefits of disease prevention through therapeutic interventions and/or for aggressive implementation.

A vast variety of chemical compounds have been identified to elicit pronounced chemo preventive effects and many of them are of plant origin that are present naturally in our daily foods or have been used for traditional herbal medication.\textsuperscript{19} As such, many herbal medicines, botanicals, dietary supplements and edible plants have all been suggested as potentially important in cancer chemoprevention.\textsuperscript{20}

1.2.1.3 Natural products in anticancer therapy

Nature abounds with a rich potential heritage of therapeutic resource that has been exploited for effective and beneficial use against many human cancers, either in the prevention strategy or in therapeutic armamentaria to kill tumor cells. Many of the bioactive natural compounds might have evolved in the plants to counteract natural predators and for self defense. The potential of using natural products as anticancer agents was first recognized in the 1950s by the US National Cancer Institute (NCI) under the leadership of late Dr. Jonathan Hartwell and NCI has since made major contributions to the discovery of new naturally occurring anticancer agents.\textsuperscript{21} Several recent
reviews have provided data that document the importance of natural products as a source of bioactive compounds.

Literature studies reveal that many natural products are available as chemo protective agents against commonly occurring cancers. A major group of these compounds are the powerful antioxidants, others are phenolic in nature and the remainder include compounds bearing reactive groups that confer protective properties. Although the mechanism of the protective effect is unclear, the fact that the consumption of fruit and vegetables lowers the incidence of carcinogenesis at a wide variety of sites is broadly accepted. Of the many anticarcinogens already detected in plant foods, the antioxidants vitamin C and E and the provitamin β-carotene have received the most attention. In the last few decades, advances in cancer research have enhanced our understanding of cancer biology and genetics. Most important finding is that genes that control apoptosis have a major effect on malignancy through the disruption of the apoptotic process that leads to tumor initiation, progression and metastasis. Therefore, one mechanism of tumor suppression by natural products may be to induce apoptosis, thereby providing a genetic basis for cancer therapy by natural products. Many naturally occurring antioxidants, fatty acids, amino acids, flavonoids, resveratrol and alkaloids can play an important role in cancer prevention. A large number of plant, marine and microbial sources have been tested as leads and many compounds have survived those tests as potential leads. The chemistry and properties of some of the major plant derived anticancer drugs are discussed below.

Camptothecin

![Chemical structure of Camptothecin](image)
The discovery of camptothecin (CPT, 18) by Wall and Wani as an anticancer drug in the early sixties added an entirely new dimension to the field of chemotherapy. Camptothecin, was first extracted\textsuperscript{24} from the stem wood of the Chinese ornamental tree *Camptotheca acuminata*. The molecule became so important and at present the first generation analogues of camptothecin, hycamptin (19, topotecan) and camptosar (CPT - II, 20, irinotecan) are used for the treatment of ovarian and colon cancers. Camptothecin is a member of the quinolinoalkaloid group. It consists of a pentacyclic ring structure that includes a pyrrole (3,4β) quinoline moiety and one asymmetric centre within the α-hydroxy lactone ring with 20(S) configuration (ring E). The planar pentacyclic ring structure (rings A–E) was suggested to be one of the most important structural features of this type of compounds. The stereochemistry at C-20 of CPT is very crucial for its activity, as 20(S) hydroxyl is active while the corresponding 20(R) hydroxyl compound is inactive.\textsuperscript{25} One of the major drawbacks observed in the use of CPT analogues in clinical studies was a marked loss of therapeutic activity due to their intrinsic instabilities resulting from the rapid hydrolysis of the lactone ring in the body. Apart form the above drawback, it is a potent cytotoxic agent. It shows anticancer activity mainly for solid tumors. It shows anticancer activity mainly against ovarian, colon and pancreatic cancer cells. But its analogues showed anticancer activity in breast, liver, prostate cancers etc. Camptothecin inhibits DNA topoisomerase I\textsuperscript{26} thereby preventing DNA replication. The development of synthetic and semisynthetic strategies has
facilitated the study of the CPT mechanism, as well as the identification of analogues with improved properties.

The most successful derivatives of CPT have been obtained due to modifications of rings A and B. To date, the only CPT analogues approved for clinical use\textsuperscript{27} are topotecan (19) and irinotecan (20). All the analogues of CPT have proved as potent cytotoxic agents by inhibiting cellular DNA topoisomerase I by a mechanism similar to CPT with similar or better activity. Continued studies on the camptothecin-DNA topoisomerase I interaction in addition to its detailed mechanism of action may suggest new directions in the synthesis of new camptothecins.

**Taxol**

\textbf{11} \hspace{1cm} \textbf{21}

Taxol (generic name paclitaxel, trade name taxol, \textbf{11}) is a complex polyoxygenated diterpenoid isolated from the Pacific yew, \textit{Taxus brevifolia}.\textsuperscript{28} Taxol is used for the treatment of refractory ovarian cancer, metastatic breast and lung cancer and Kaposi’s sarcoma. Taxotere (docetaxel, \textbf{21}), one of its semisynthetic derivatives, is now known as a better anticancer drug than taxol. Taxol has a basic [9.3.1.0\textsuperscript{38}] pentadecane, tetracyclic ring system. It has a N-benzoyl-\(\beta\)-phenylisoserine side chain attached at the C-13 hydroxyl as an ester linkage. This side chain is essentially required in taxol for anticancer activity and so is the C-2′-hydroxyl. Figure 1.2 depicts some of the interesting structure activity relationship (SAR) shown by taxol.
Figure 1.2: A brief description of SAR of taxol

Taxol has a unique mode of action. It acts as a microtubulin stabilizing agent. Tubulin polymerizes to microtubulin which in turn reverts back to tubulin. In a normal case, this process is in equilibrium. Taxol makes a microtubulin bundle larger in size than the normal bundle size required for the process of cell multiplication. Due to this, a defective polymerization occurs and the cells have unnatural bundles of microtubules with the absence of the mitotic spindle. The cancerous cells lack a check point to detect the absence of a spindle and attempts to continue the cell cycle which eventually lead to cell death. Because of this reason, taxol is also referred to as a “spindle poison”. A major drawback of taxol is that it has poor bio-availability due to its poor solubility in water.

Taxol is a drug tolerated by its recipients better than any other anticancer drugs used today. Many derivatives of taxol like taxotere and isotaxel having more advantages such as better potency, greater solubility and lesser side effects have been developed.
Combretastatin A-4

Combretastatins are mitotic agents isolated from the bark of the South African tree *Combretum caffrum*. Combretastatins A-1\(^{31}\) 22 and A-4\(^{32}\) 23 were isolated by Petit and coworkers in 1987 and 1989 respectively. Combretastatin A-4 (CA-4) is a simple stilbene that has been shown to compete with colchicines for binding sites on tubulin. Both are stilbene derivatives having two phenyl rings separated by a C-C double bond. It is concluded that a diaryl system separated through a double bond along with a trimethoxy system in one of the rings show cytotoxic activity.\(^{33}\)

CA-4 is a potent cytotoxic agent which strongly inhibits the polymerization of brain tubulin by binding to the colchicine site. It shows potent cytotoxicity against a wide variety of human cancer cell lines and is also an attractive lead molecule for the development of novel anticancer drugs.\(^{34}\) CA-4 is an investigation drug of the NCI. The compound is active against colon, lung and leukemia cancers. It is stated that this molecule is the most cytotoxic phytomolecule isolated so far. Varied modified analogues of CA-4 including modification in the functional group, aromatic ring and in the linear alkene have been synthesized and evaluated.\(^{35}\)

Podophyllotoxin
Podophyllotoxin (24) and deoxypodophyllotoxin (25) are two well known naturally occurring aryltetralin lignans. It was first isolated by Podwyssotzki from the North American plant *Podophyllum peltatum* (may Apple). Deoxypodophyllotoxin has been isolated from *Anthriscus sylvestris* and *Pulsatilla koreana*. Two of the semisynthetic derivatives of podophyllotoxin viz., etoposide 26 and teniposide 27 are currently used in frontline cancer chemotherapy against various cancers.

Chemically, podophyllotoxin is an aryltetralin lignan having a lactone ring. The SAR studies reveal that only the A and E rings of this compound is essential for its activity and the D-ring in lactone form enhances the activity. Also, introduction of bulky groups at the C-4 position in ring C enhances the activity.

Podophyllotoxin is effective in the treatment of Wilms tumors, various genital tumors and in non-Hodgkin’s and other lymphomas and lung cancer. Synthetic analogues such as epipodophyllotoxin, etoposide and tenetoposide have less toxic side effects than podophyllotoxin.

Over the years, a number of approaches have been developed for the discovery of new molecules for clinical use and as a result of this, a number of anticancer drugs have come out. The main problem with these molecules is the lack of specificity as these drugs also kill the healthy cells. Apart from this, drug resistance is another problem. The development of a safe, economic
and site specific anticancer drug is still a challenge. Perhaps it is necessary to look towards nature for other molecules with novel modes of action in order to tackle this dreadful disease.

1.3 Natural products as antioxidants

1.3.1 Introduction

Oxygen is an element indispensable for life. When cells use oxygen to generate energy, free radicals are created as a consequence of ATP (adenosine triphosphate) production by the mitochondria. A molecule with one or more unpaired electron in its outer shell is called a free radical. Free radicals are less stable than non-radical species, although they are more reactive. Free radicals are formed from molecules via (i) the breakage of a chemical bond such that each fragment keeps one electron, (ii) by cleavage of a radical to give another radical and, also via (iii) redox reactions. Oxygen free radicals or more generally, reactive oxygen species (ROS), as well as reactive nitrogen species (RNS) are products of normal cellular metabolism. ROS and RNS are the terms collectively describing free radicals and other non-radical reactive derivatives which are also called oxidants. Free radicals, as mentioned earlier, can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals. This unpaired electron(s) usually gives a considerable degree of reactivity to the free radical. Radicals derived from oxygen represent the most important class of radical species generated in living systems. Molecular oxygen (dioxygen, O₂) has a unique electronic configuration and is itself a radical (Figure 1.3) with unpaired electrons.

These ROS and RNS formed as the by products of normal cellular process, are well recognized for playing a dual role as both deleterious and beneficial species, since they can be either harmful or beneficial to living systems. These biological free radicals are highly unstable molecules that have electrons available to react with various organic substrates such as lipids,
proteins and DNA. The harmful effect of free radicals causing potential biological damage is termed oxidative stress and nitrosative stress. In other words, oxidative stress results from the metabolic reactions that use oxygen and represents a disturbance in the equilibrium status of pro-oxidant/antioxidant reactions in living organisms.\textsuperscript{41}

Fig. 1.3: MO diagram of $\text{O}_2$

The excess ROS can damage cellular lipids, proteins or DNA, inhibiting their normal function. Because of this, oxidative stress has been implicated in a number of human diseases as well as in the aging process. The delicate balance between beneficial and harmful effects of free radicals is a very important aspect of living organisms and is achieved by mechanisms called “redox regulation”. The process of “redox regulation” protects living organisms from various oxidative stresses and maintains “redox homeostasis” by controlling the redox status \textit{in vivo}. When free radicals are generated \textit{in vivo}, many antioxidants in the body act by defending the organism from oxidative damage. As a first line of defense, the preventive antioxidants such as peroxidases and metal chelating proteins suppress the generation of free radicals. Next, the radical-scavenging antioxidants such as vitamin C and vitamin E scavenge radicals to inhibit the oxidation chain initiation and prevent chain propagation as a second line of defense. This may also include
the termination of a chain by the reaction of two radicals. The repair and de novo enzymes act as the third line of defense by repairing damage and reconstituting membranes. 42

1.3.1.1 Reactive oxygen species (ROS)

The reactive oxygen species include hydroxyl (OH·), superoxide (O2·−), nitric oxide (NO·), nitrogen dioxide (NO2·), peroxyl (ROO·) and lipid peroxyl (LOO·). On the other hand, hydrogen peroxide (H2O2), ozone (O3), singlet oxygen (1O2), hypochlorous acid (HClO), nitrous acid (HNO2), peroxynitrite (ONOO·), dinitrogen trioxide (N2O3) and lipid peroxide (LOOH), generally called oxidants are not free radicals, but can easily lead to free radical reactions in living organisms. The addition of one electron to dioxygen forms the superoxide anion radical (O2·−). 43 Superoxide anion, arising either through metabolic processes or following oxygen “activation” by physical irradiation, is considered the “primary” ROS and can further interact with other molecules to generate “secondary” ROS, either directly or prevalently through enzyme- or metal-catalysed processes. 44 The production of superoxide occurs mostly within the mitochondria of a cell. 45 The mitochondrial electron transport chain is the main source of ATP in the mammalian cell and thus is essential for life. During energy transduction, a small number of electrons “leak” to oxygen prematurely, forming the oxygen free radical superoxide, which has been implicated in the pathophysiology of a variety of diseases. Another ROS, the hydroxyl radical, ·OH, is the neutral form of the hydroxide ion. The hydroxyl radical has high reactivity, making it a very dangerous radical with a very short in vivo half-life of approximately 9–10 s. 46 Thus when produced in vivo ·OH reacts close to its site of formation. Under stress conditions, an excess of superoxide releases “free iron” from iron-containing molecules. The released Fe2+ can participate in the Fenton reaction, generating highly reactive hydroxyl radical (Fe2+ + H2O2 → Fe3+ + ·OH + OH−). Thus under stress conditions, O2·− acts as an oxidant and facilitates
OR production from H₂O₂ by making Fe²⁺ available for the Fenton reaction. The superoxide radical participates in the Haber–Weiss reaction (O₂⁻ + H₂O₂ → O₂ + 'OH + OH⁻) which combines a Fenton reaction and the reduction of Fe³⁺ by superoxide, yielding Fe²⁺ and oxygen (Fe³⁺ +O₂⁻→ Fe²⁺ +O₂). Additional reactive radicals derived from oxygen that can be formed in living systems are peroxyl radicals (ROO'). The simplest peroxyl radical is HOO', which is the protonated form of superoxide (O₂⁻⁻⁻) and is usually termed either hydroperoxyl radical or perhydroxyl radical.

1.3.1.2 Reactive nitrogen species (RNS)

Nitric oxide (NO') is a small molecule that contains one unpaired electron on the antibonding 2π_y* orbital and is therefore, a radical. NO' is generated in biological tissues by specific nitric oxide synthases (NOSs), which metabolise arginine to citrulline with the formation of NO' via a five electron oxidative reaction.⁴⁷ NO' is an abundant reactive radical that acts as an important oxidative biological signaling molecule in a large variety of diverse physiological processes, including neurotransmission, blood pressure regulation, defense mechanisms, smooth muscle relaxation and immune regulation. Due to its extraordinary properties, NO' was acclaimed as the "molecule of the year" in Science Magazine in 1992.⁴⁷c NO' has a half-life of only a few seconds in an aqueous environment. NO' has greater stability in an environment with a lower oxygen concentration (half life >15 s). However, since it is soluble in both aqueous and lipid media, it readily diffuses through the cytoplasm and plasma membranes. NO’ has effects on neuronal transmission as well as on synaptic plasticity in the central nervous system. In the extracellular milieu, NO’ reacts with oxygen and water to form nitrate and nitrite anions. Overproduction of reactive nitrogen species is called nitrosative stress. This may occur when the generation of reactive nitrogen species in a system exceeds the system’s ability to neutralise and eliminate them.
Nitrosative stress may lead to nitrosylation reactions that can alter the structure of proteins and so inhibit their normal function.

Cells of the immune system produce both the superoxide anion and nitric oxide during the oxidative burst triggered during inflammatory processes. Under these conditions, nitric oxide and the superoxide anion may react together to produce significant amounts of a much more oxidatively active molecule, peroxynitrite anion (ONOO\textsuperscript{−}), which is a potent oxidising agent that can cause DNA fragmentation and lipid oxidation, NO\textsuperscript{−} + O_2\textsuperscript{−−} → ONOO\textsuperscript{−}. This reaction has one of the highest rate constants known for reactions of NO\textsuperscript{−}, viz., $7.0 \times 10^{9}$ M\textsuperscript{−1}s\textsuperscript{−1}. Thus NO\textsuperscript{−} toxicity is predominantly linked to its ability to combine with superoxide anions.

These ROS and RNS species play a dual role as both toxic and beneficial compounds. The delicate balance between their two antagonistic effects is clearly an important aspect of life.

1.3.1.3 Generation of free radicals and oxidants

Formation of ROS and RNS can occur in the cells either by enzymatic or by non-enzymatic reactions. Enzymatic reactions generating free radicals include those involved in the respiratory chain, the phagocytosis, the prostaglandin synthesis and the cytochrome P450 system.\textsuperscript{48} For example, the superoxide anion radical (O_2\textsuperscript{−−}) is generated via several cellular oxidase systems such as NADPH oxidase, xanthine oxidase and peroxidases. Once formed, it participates in several reactions yielding various ROS and RNS such as hydrogen peroxide, hydroxyl radical (·OH), peroxynitrite (ONOO\textsuperscript{−}), hypochlorous acid (HOCl), etc. H_2O_2 (a non radical) is produced by the action of several oxidase enzymes, including aminoacid oxidase and xanthine oxidase. Hydrogen peroxide catalyses the oxidation of hypoxanthine to xanthine and of xanthine to uric acid. Hydroxyl radical (·OH), the most reactive free radical \textit{in vivo}, is formed by the reaction of O_2\textsuperscript{−−} with H_2O_2 in the presence of Fe\textsuperscript{2+} or Cu\textsuperscript{+} (catalyst) as mentioned in section 1.3.1.1.
Hypochlorous acid (HOCl) is produced by the neutrophil-derived enzyme, myeloperoxidase, which oxidizes chloride ions in the presence of H₂O₂. Nitric oxide radical (NO') is formed in biological tissues from the oxidation of L-arginine to citrulline by nitric oxide synthase. Free radicals can be produced from non-enzymatic reactions of oxygen with organic compounds as well as those initiated by ionizing radiations. The non-enzymatic process can also occur during oxidative phosphorylation (i.e. aerobic respiration) in the mitochondria. ROS and RNS are generated from either endogenous or exogenous sources.

**Fig. 1.4: Summary of sources of free radicals**

Endogenous free radicals are generated from immune cell activation, inflammation, mental stress, excessive exercise, ischemia, infection, cancer or aging. Exogenous ROS/RNS result from air and water pollution, exposure to ultraviolet radiation, cigarette smoke, alcohol, heavy or transition metals (Cd, Hg, Pb, Fe, As), certain drugs (cyclosporine, tacrolimus, gentamycin, bleomycin), industrial solvents, cooking (smoked meat, used oil, fat), radiation etc (Figure 1.4). After penetration into the body by different routes, these exogenous compounds are decomposed or metabolized into free radicals.
1.3.1.4 Beneficial activities of free radicals and oxidants

At low or moderate concentrations, ROS and RNS are necessary for the maturation process of cellular structures and can act as weapons for the host defense system. Beneficial effects of ROS occur at low/moderate concentrations and involve physiological roles in cellular responses to noxia, as for example, in defense against infectious agents and in the function of a number of cellular signaling systems. At low/moderate concentrations ROS invokes induction of a mitogenic response too.

Indeed, phagocytes (neutrophils, macrophages, monocytes) release free radicals to destroy invading pathogenic microbes as part of the body’s defense mechanism against disease. The importance of ROS production by the immune system is clearly exemplified by patients with granulomatous disease. These patients have defective membrane-bound NADPH oxidase system which makes them unable to produce the superoxide anion radical (O\(_2^−\)), thereby resulting in multiple and persistent infection. Other beneficial effects of ROS and RNS involve their physiological roles in the function of a number of cellular signaling systems. Their production by non-phagocytic NADPH oxidase isoforms play a key role in the regulation of intracellular signaling cascades in various types of non-phagocytic cells including fibroblasts, endothelial cells, vascular smooth muscle cells, cardiac myocytes and thyroid tissue. For example, nitric oxide (NO\(^−\)) is an intercellular messenger for modulating blood flow, thrombosis and neural activity. NO\(^−\) is also important for nonspecific host defense and for killing intracellular pathogens and tumors. In brief, ROS/RNS at low or moderate levels are vital to human health.

1.3.1.5 Deleterious activities of free radicals and oxidants

Oxidative stress can arise when cells cannot adequately destroy the excess of free radicals formed. In other words, oxidative stress results from an
imbalance between formation and neutralization of ROS/RNS. For example, hydroxyl radical and peroxynitrite in excess can damage cell membranes and lipoproteins by a process called lipid peroxidation. This reaction leads to the formation of malondialdehyde (MDA) and conjugated diene compounds, which are cytotoxic and mutagenic. Lipid peroxidation occurs by a radical chain reaction, i.e. once started; it spreads rapidly and affects a great number of lipid molecules. Proteins may also be damaged by ROS/RNS, leading to structural changes and loss of enzyme activity. Oxidative damage to DNA leads to the formation of different oxidative DNA lesions which can cause mutations. Various oxidative stress induced diseases in humans is summarized in figure 1.5.

![Figure 1.5: Oxidative stress induced diseases in humans](source)

Our body has several mechanisms to counteract these attacks by using DNA repair enzymes and/or antioxidants. If not regulated properly, oxidative stress can induce a variety of chronic and degenerative diseases like cancer, cardiovascular diseases, neurological diseases, pulmonary diseases,
rheumatoid arthritis, nephropathy, ocular diseases, as well as the aging process and some acute pathology (trauma, stroke). Here comes the importance of antioxidants to fight against these oxidative stress induced diseases.

1.3.2 ANTIOXIDANTS

The word antioxidant has become popular in modern society as it gained publicity through mass media coverage of its health benefits. The dictionary definition of antioxidant is rather straightforward viz., “a substance that opposes oxidation or inhibits reactions promoted by oxygen or peroxides”. A more biologically relevant definition of antioxidant is “a synthetic or natural substance added to products to prevent or delay their deterioration by action of oxygen in air”. In biochemistry and medicine, “antioxidants are enzymes or other substances such as Vit. E or β-carotene that are capable of counteracting the damaging effects of oxidation in animal tissues”. The most important and widely accepted explanation of an antioxidant is that defined by Halliwell and Gutteridge as “any substance that, when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate.” Antioxidants fight against the free radicals generated in vivo, thus preventing the organism against oxidative damage. Hence, the media attention on their health benefits.

1.3.2.1 Antioxidant classification

Antioxidants in cells can be classified as enzymatic antioxidants and non-enzymatic antioxidants. The major enzymatic antioxidants directly involved in the neutralization of ROS and RNS are: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GRx). SOD, the first line of defense against free radicals, catalyzes the dismutation of superoxide anion radical (O₂⁻) into hydrogen peroxide (H₂O₂) by reduction. The oxidant formed (H₂O₂) is transformed into
water and oxygen (O₂) by catalase (CAT) or glutathione peroxidase (GPx). The selenoprotein GPx enzyme removes H₂O₂ by using it to oxidize reduced glutathione (GSH) into oxidized glutathione (GSSG). Glutathione reductase, a flavoprotein enzyme, regenerates GSH from GSSG, with NADPH as a source of reducing power. Besides hydrogen peroxide, GPx also reduces lipid or nonlipid hydroperoxides while oxidizing glutathione (GSH). The nonenzymatic antioxidants are divided into metabolic antioxidants and nutrient antioxidants. Metabolic antioxidants are endogenous antioxidants, produced by metabolism in the body, such as lipoid acid, glutathione, L-arginine, coenzyme Q₁₀, melatonin, uric acid, bilirubin, metal-chelating proteins, transferrin, etc. Nutrient antioxidants are exogenous antioxidants. They are compounds which cannot be produced in the body and must be provided through foods or supplements, such as vitamin E, vitamin C, carotenoids, trace metals (selenium, manganese, zinc), flavonoids, omega-3 and omega-6 fatty acids, etc.

1.3.2.2 Endogenous Antioxidants

Antioxidants that are produced within the body for defense as a result of normal metabolic processes are called endogenous antioxidants. There is a vast network of intracellular and extracellular antioxidants with diverse roles within each area of defense. As already mentioned, catalase converts H₂O₂ to O₂ and H₂O while superoxide dismutase (SOD) converts the superoxide radical to H₂O₂ and O₂. Some of the antioxidant enzymes exist in several forms. For example, membrane, cytosolic and plasma forms of glutathione peroxidase have been isolated and SOD has membrane, cytosolic and extracellular forms. The levels and locations of these antioxidants must be tightly regulated for cell survival. The antioxidant enzymes, SOD, glutathione peroxidase (GPx) and catalase (CAT), work within the cells to remove most superoxides and peroxides before they react with metal ions to form more reactive free radicals. Peroxidative chain reactions initiated by free radicals
that escaped the antioxidant defenses are terminated by chain-breaking water or lipid soluble antioxidants.52

1.3.2.3 Exogenous Antioxidants

Antioxidant compounds supplied through diet is termed as exogenous antioxidants. Diet plays a vital role in the production of the antioxidant defense system by providing essential nutrient antioxidants such as vitamin E, C and β-carotene, other antioxidant plant phenols including flavonoids and essential minerals that form important antioxidant enzymes. Diet also plays an important role in the oxidation process by affecting the substrates that are subject to oxidation. The best example is the oxidation of lipids. Polyunsaturated fatty acids (PUFA) having two or more double bonds are increasingly susceptible to free radical attack as the number of double bonds increases. Antioxidants available at the site of radical attack break the chain of oxidation by being preferentially oxidized by the attacking radical, thereby preventing oxidation of the adjacent fatty acid.

1.3.3 Antioxidant Process

When an antioxidant destroys a free radical, this antioxidant itself becomes oxidized. Therefore, the antioxidant resources must be constantly restored in the body. Thus, while in one particular system an antioxidant is effective against free radicals, in other systems the same antioxidant could become ineffective. Also, in certain circumstances, an antioxidant may even act as a pro-oxidant e.g., it can generate toxic ROS/RNS.49a The antioxidant process can function in one of two ways: chain-breaking or prevention. When a radical releases or steals an electron, a second radical is formed. This exerts the same action on another molecule and the process continues until either the free radical formed is stabilized by a chain-breaking antioxidant (vitamin C, E, carotenoids, etc.), or it simply disintegrates into an inoffensive product. The classic example of such a chain reaction is lipid peroxidation which will be discussed in detail in Chapter 2.
For the preventive way, antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase prevent oxidation by reducing the rate of chain initiation, e.g., either by scavenging initiating free radicals or by stabilizing transition metal radicals such as copper and iron. The groups of antioxidants and their actions are presented in figure 1.6.

![Antioxidant groups and actions](image)

**Fig 1.6:** Antioxidant groups and actions

Certain compounds have shown antioxidant properties *in vitro*, but uncertain *in vivo*. Such compounds include bilirubin, α-keto acids, melatonin, coenzyme Q, lipoic acid, carnosine, anserine and melanins. A majority of compounds have proved their role in the antioxidant defense mechanisms either directly or indirectly in human system. These include both the endogenous and the exogenous antioxidants.

The major components of the antioxidant defense system with their mode of antioxidant action are summarized in Table 1.3.
**Table 1.3:** Major components of antioxidant defense system

<table>
<thead>
<tr>
<th>Components</th>
<th>Antioxidant action</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enzymes</strong></td>
<td></td>
</tr>
<tr>
<td>Superoxide dismutase (SOD)</td>
<td>Removal of superoxide radical</td>
</tr>
<tr>
<td>Catalase</td>
<td>Reduction of H2O2 to water</td>
</tr>
<tr>
<td>Glutathione peroxidase</td>
<td>Reduction of H2O2 to water</td>
</tr>
<tr>
<td>Thioredoxin</td>
<td>Reduction of peroxides</td>
</tr>
<tr>
<td>Metal ion sequestration</td>
<td></td>
</tr>
<tr>
<td>Metallothionein</td>
<td>Chelates Zn, Ag, Cu, Cd, Hg</td>
</tr>
<tr>
<td>Phytochelatin</td>
<td>Chelates Cd, Zn, Cu</td>
</tr>
<tr>
<td>Transferrin</td>
<td>Chelate Fe</td>
</tr>
<tr>
<td>Albumin</td>
<td>Chelates Fe and Cu</td>
</tr>
<tr>
<td><strong>Low molecular mass (endogenous)</strong></td>
<td></td>
</tr>
<tr>
<td>Low molecular mass (endogenous)</td>
<td>Scavenges NO₂</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>Spares tocopherol, scavenges free radicals</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Scavenges peroxyl radicals, most important chain breaking inhibitor of lipid peroxidation</td>
</tr>
<tr>
<td>Carotenoids</td>
<td><em>In vivo</em> antioxidant role uncertain</td>
</tr>
<tr>
<td>Plant phenols</td>
<td>Suggested, but not proven to inhibit LDL oxidation <em>in vivo</em></td>
</tr>
</tbody>
</table>

**1.3.4 NATURAL PRODUCTS AS ANTIOXIDANTS**

Natural antioxidants (from the diet) play an important role in helping endogenous antioxidants for the neutralization of oxidative stress. Nutrient antioxidant deficiency is considered to be among the causes of numerous chronic and degenerative pathologies. Each nutrient is unique in terms of its structure and antioxidant function. The properties of some of the important exogenous (natural) antioxidants are summarized below.
Vitamin E

Vitamin E is a fat-soluble vitamin with high antioxidant potency. It is a chiral compound with eight stereoisomers: \( \alpha, \beta, \gamma, \delta \) tocopherol and \( \alpha, \beta, \gamma, \delta \) tocotrienol (with double bonds in side chain). \( \alpha \)-Tocopherol is the most bioactive form in humans.\(^5^4\) As it is fat-soluble, \( \alpha \)-tocopherol safeguards cell membranes from damage by free radicals. Its antioxidant function mainly resides in the protection against lipid peroxidation.

Vitamin E has been proposed for the prevention against colon, prostate and breast cancers, some cardiovascular diseases, ischemia, cataract, arthritis and certain neurological disorders. The dietary sources of vitamin E are vegetable oils, wheat germ oil, whole grains, nuts, cereals, fruits, eggs, poultry, meat etc.

Vitamin C

Vitamin C, also known as ascorbic acid is a water-soluble vitamin. It is essential for collagen, carnitine and neurotransmitters biosynthesis.\(^5^5\) Health benefits of vitamin C are as antioxidant, anti-atherogenic, anti-carcinogenic and as an immunomodulator. The positive effect of vitamin C resides in
reducing the incidence of stomach cancer and in preventing lung and colorectal cancer. Vitamin C works synergistically with vitamin E to quench free radicals and also regenerates the reduced form of vitamin E. Natural sources of vitamin C are acidic fruits such as lemon and orange, green vegetables, tomatoes etc.

**β -Carotene**

β -Carotene is a fat soluble member of the carotenoids which are considered pro-vitamins because they can be converted to active vitamin A. β -Carotene is converted to retinol, which is essential for vision. It is a strong antioxidant and is the best quencher of singlet oxygen. β -Carotene is present in many fruits, grains, oil and vegetables (carrots, green plants, squash, spinach etc.).

**Lycopene**

Lycopene, a carotenoid, possesses antioxidant and antiproliferative properties. Lycopene has been found to be very protective, particularly for prostate cancer. The major dietary source of lycopene is tomatoes, with the lycopene in cooked tomatoes, tomato juice and tomato sauce included, being more bio-available than that in raw tomatoes.

**Selenium (Se)**

Se is a trace mineral found in soil, water, vegetables (garlic, onion, grains, nuts and soybean), sea food, meat, liver and yeast. It forms the active site of several antioxidant enzymes including glutathione peroxidase. At low
dose, health benefits of Se are antioxidant, anti-carcinogenic and as an immunomodulator. Selenium is also necessary for the thyroid function. In China, people in the area with Se poor soil have developed a fatal cardiomyopathy called *Keshan* disease which was cured with Se supplement.

**Omega-3 and omega-6 fatty acids**

They are essential long-chain polyunsaturated fatty acids because the human body cannot synthesize them. Therefore, they are only derived from food. Omega-3 fatty acids can be found in fatty fish (salmon, tuna, halibut, sardines, pollock), algae, walnut, nut oils and flaxseed. There are three major dietary types of omega-3 fatty acids: eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and alpha-linolenic acid (ALA). EPA and DHA are abundant in fish and are directly used by the body; while ALA is found in nuts and has to be converted to DHA and EPA by the body. Dietary sources of omega-6 fatty acids (linoleic acid) include vegetable oils, nuts, cereals, eggs, poultry. It is important to maintain an appropriate balance of omega-3s and omega-6s in the diet, as these two substances work together to promote health. Omega-3s reduce inflammation and prevent chronic ailments such as heart disease, stroke, memory loss, depression, arthritis, cataract and cancer.
Omega-6s improve diabetic neuropathy, eczema, psoriasis, osteoporosis and aid in cancer treatment.

**Flavonoids**

Flavonoids have been reported to prevent or delay a number of chronic and degenerative ailments such as cancer, cardiovascular diseases, arthritis, aging, cataract, memory loss, stroke, Alzheimer’s disease, inflammation and infection. The main natural sources of flavonoids include green tea, grapes (red wine), apple, cocoa (chocolate), *Ginkgo biloba*, soybean, curcuma, berries, onion, broccoli, etc.

As the major work of this thesis deals with the flavonoids and their biological activities, a detailed introduction on flavonoids is portrayed here.

1.4 FLAVONOIDS – An introduction

Flavonoids are a group of more than 4000 polyphenolic compounds that occur naturally in foods of plant origin. These compounds possess a common phenylbenzopyrone structure. Flavonoids are most commonly divided into six sub-classes, based on the connection position of B and C rings as well as the degree of saturation, oxidation and hydroxylation of the C ring as flavonols, flavones, flavanones, flavan-3-ols (or catechins), isoflavones and anthocyanidines. The basic structure with numbering system of flavonoids and the structures of the different types of flavonoids are given in figures 1.7 and 1.8 respectively. The subclasses and dietary sources of flavonoids with a few representative examples are given in Table 1.4.

![Fig 1.7: Basic flavonoid structure](image)
Flavonoids have probably existed in the plant kingdom for over one billion years. They are synthesized by all vascular plants and are present in practically all dietary sources like fruits, nuts, vegetables, herbs, whole grains etc. It is estimated that the human intake of all flavonoids is a few hundreds of milligrams per day. Additionally, flavonoids are found in several medicinal plants and herbal remedies containing flavonoids have been used in folk
medicine around the world. Within the plant, flavonoids are involved in electron transport during photosynthesis, serve as antioxidants against the pro-oxidant effects of ultraviolet light and act against bacterial, fungal and viral pathogens as well as some insect predators.62

Resistance of plants to UV-B (280 – 315 nm) may take many forms, but one type of resistance lies in the flavonoid pigments, which are known to be almost universally present in green leaves. These flavonoids generally absorb in the 280-315 nm region and thus are capable of acting as UV filters, thereby protecting the underlying photosynthetic tissues from damage. One of the undisputed functions of flavonoids and related polyphenols is their role in protecting plants against microbial invasion. This not only involves the presence of flavonoids in plants as constitutive agents but also their accumulation as phytoalexins in response to microbial attack.63 There is an ever increasing interest in plant flavonoids for treating human diseases and especially for controlling the immunodeficiency virus which is the causative agent of AIDS. The presence of a phenolic group in a natural flavonoid would be expected to provide antimicrobial activity and the addition of further phenolic groups might be expected to enhance this activity.64

1.4.1 Biosynthesis of flavonoids

All flavonoids derive their 15-carbon skeletons from two basic metabolites, malonyl-CoA and p-coumaroyl-CoA.65 Basically, flavonoids are derivatives of 1,3-diphenylpropan-1-one (C6–C3–C6). The crucial biosynthetic reaction is the condensation of three molecules of malonyl-CoA with one molecule of p-coumaroyl-CoA to a chalcone intermediate. Chalcones and dihydrochalcones are classes of flavonoids that consist of two phenolic groups which are connected by an open three carbon bridge. Derived from the chalcone structure, a flavonoid-class containing three rings, the flavanones, can be formed. Here, the three-carbon bridge is part of an additional heterocyclic six-membered ring that involves one of the phenolic
groups on the adjacent ring. Based on these flavanones all other flavonoid-
classes are generated, including isoflavones, flavanols, anthocyanidines, 
flavonols and flavones (Figure 1.9).

Figure 1.9: Scheme of general flavonoid pathway.
Enzymes are abbreviated as follows: CHS, chalcone synthase; CHKR, chalcone polyketide reductase; 
CHI, chalcone isomerase; FHT, flavanone 3-β-hydroxylase; DFR, dihydroflavonol-4-reductase; 
ANS, anthocyanidin synthase; FGT, flavonoid glycosyltransferase; FNS, flavone synthase; FLS, 
flavonol synthase; LAR, leucoanthocyanidin reductase; ANR, anthocyanidin reductase; IFS, 
isoflavone synthase; IFD, isoflavone dehydratase.

1.4.2 Biological activity of flavonoids

Flavonoids display a remarkable spectrum of biological activities 
including those that might be able to influence processes that are deregulated 
during cancer development. These include, for example, antiallergic, anti-
inflammatory, antioxidant, antimutagenic, anticarcinogenic and modulation of 
enzymatic activities. They may therefore have beneficial health effects and 
can be considered possible chemopreventive or therapeutic agents against 
cancer.
1.4.3 Flavonoids in anticancer therapy

Impressive epidemiological evidence exists for the protective effect of flavonoids against cancer. A large number of such epidemiological studies suggest that high flavonoid intake may be correlated with a decreased risk of cancer.\textsuperscript{68} Recently, population based studies on the protective effects of flavonoids against cancer conducted in Shanghai, Finland and Hawai have also provided evidence for the protective role of flavonoids against cancer.\textsuperscript{69} Studies on the potential anticancer activity of flavonoids in diverse cell systems have demonstrated their ability to inhibit carcinogenesis \textit{in vitro} and substantial evidence indicates that they can also do so \textit{in vivo}.\textsuperscript{70} For example, Hirano \textit{et al}\textsuperscript{70d} examined anticancer efficacy of 28 flavonoids on human acute myeloid leukemia cell line HL-60 and compared with those of four clinical anticancer agents. Eight of the 28 flavonoids showed considerable suppressive effects on HL-60 cell growth. Kuntz \textit{et al}\textsuperscript{70e} screened more than 30 flavonoids for their effects on cell proliferation and potential cytotoxicity in human colon cancer cell lines Caco-2 and HT-29. Almost all compounds displayed antiproliferative activity without cytotoxicity. There was no obvious SAR in the antiproliferative effects either on the basis of subclasses or with respect to kind or position of substituents within a class. An array of 55 flavones having a variety of substituents was evaluated by Cushman and Nagarathnam\textsuperscript{70f} for cytotoxicity in five cancer cell cultures, A-549 lung carcinoma, MCF-7 breast carcinoma, HT-29 colon adenocarcinoma, SKMEL-5 melanoma and MLM melanoma. Fifteen of the 55 flavone derivatives were significantly active against at least one of these cell cultures. In addition, seven of the 27 citrus flavonoids examined were observed to inhibit the proliferation of tumor cells, while being less active against normal human cells.\textsuperscript{70g}

Flavonoids may inhibit carcinogenesis by affecting the molecular events in the initiation, promotion and progression stages. Animal studies and investigations using different cellular models suggested that certain
flavonoids could inhibit tumor initiation as well as tumor progression.\textsuperscript{71} The encouraging results of anticancer effects in preclinical studies have stimulated the clinical trials of some flavonoids in human beings. Based on the studies \textit{in vivo} and \textit{in vitro}, many mechanisms of action have been proposed. These include, carcinogen inactivation, antiproliferation, cell cycle arrest, induction of apoptosis and differentiation, inhibition of angiogenesis, antioxidation and reversal of multi drug resistance or a combination of these mechanisms.

Flavonoids may interfere in several of the steps that lead to the development of malignant tumors, including protecting DNA from oxidative damage, inhibiting carcinogen activation and activating carcinogen detoxifying systems. Dietary phenolics have also been shown to act as prooxidants in systems containing redox-active metals. i.e., In the presence of O\textsubscript{2}, transition metals such as copper (Cu) and iron (Fe) catalyze the redox cycling of phenolics, leading to the formation of reactive oxygen species (ROS) and phenoxy radicals that can damage DNA, lipids and other biological molecules.\textsuperscript{72} Thus phenolic antioxidants can be both pro-oxidative and antioxidative (Figure 1.10), which suggests that flavonoids/phenolics also have the potential to lead to oxidative risk. Therefore, consumption of large amounts of flavonoids in the form of a concentrated supplement might not be considered safe until their \textit{in vivo} potential for oxidative stress is evaluated fully.

\textbf{Fig. 1.10:} Diagram representing the balance between antioxidant and pro-oxidant characteristics of flavonoids
1.4.3.1 Pro-oxidant behavior and anticancer property

The beneficial effects of flavonoids in cancer therapy have often been linked to their ability to act as antioxidants, which includes their reducing capacities and ROS-scavenging capabilities. The chemopreventive properties of flavonoids are generally believed to reflect their ability to scavenge endogenous ROS. However, the pro-oxidant action of plant-derived phenolics rather than their antioxidant action may be an important mechanism for their anticancer and apoptosis-inducing properties, as ROS can mediate apoptotic DNA fragmentation. Certain properties of dietary phenolic compounds, such as binding and cleavage of DNA and the generation of ROS in the presence of transition metal ions, are similar to those of known anticancer drugs. Another mechanism proposed for the anticancer and tumor cell apoptosis-inducing properties of flavonoids is that their pro-oxidant phenoxy radicals cause mitochondrial toxicity by collapsing the mitochondrial membrane potential. Apoptosis (programmed cell death) is required to maintain a balance between cell proliferation and cell loss. Misregulation of this balance can lead to malignant transformation, whereas induction of apoptosis suppresses the development of cancer. Various diet-derived compounds, e.g., resveratrol, have been shown to induce apoptosis in malignant cells and provide a promising natural strategy to prevent cancer. Pro-oxidant activity is thought to be directly proportional to the total number of hydroxyl groups present especially in the flavonoid B-ring. Glycosylation and methylation of OH groups attenuate the pro-oxidant behavior of flavonoids.

While these experiences strengthen the notion that flavonoids could be useful as anticancer agents, to date only few clinical studies have demonstrated that these bioflavonoids retain anticancer properties in humans in vivo. Considering the fact that many chemotherapeutic agents against tumor cells kill without sparing normal cells remains a major obstacle and
development of multidrug resistance further limits chemotherapy in cancer, the promising results on flavonoids have stimulated further study of these compounds for cancer chemoprevention and chemotherapy.

1.4.4 Flavonoids as antioxidants

Owing to the incomplete efficiency of our endogenous defense systems and the existence of some physiopathological situations (cigarette smoke, air pollutants, UV radiation, high polyunsaturated fatty acid diet, inflammation, ischemia/reperfusion, etc.) in which ROS are produced in excess and at the wrong time and place, dietary antioxidants are needed for diminishing the cumulative effects of oxidative damage over the life span. According to Halliwell and Gutteridge, mechanisms of antioxidant action can include (1) suppressing reactive oxygen species formation either by inhibition of enzymes or chelating trace elements involved in free radical production; (2) scavenging reactive oxygen species; and (3) upregulating or protecting antioxidant defenses. Flavonoids inhibit the enzymes responsible for superoxide anion production, such as xanthine oxidase and protein kinase C. Flavonoids have been also shown to inhibit cyclooxygenase, lipoxygenase, microsomal monooxygenase, glutathione S-transferase, mitochondrial succinoxidase and NADH oxidase, all involved in reactive oxygen species generation. A number of flavonoids efficiently chelate trace metals, which play an important role in oxygen metabolism. Free iron and copper are potential enhancers of reactive oxygen species formation, as exemplified by the reduction of hydrogen peroxide with generation of the highly aggressive hydroxyl radical, $\text{H}_2\text{O}_2 + \text{Fe}^{2+} (\text{Cu}^+) \rightarrow \cdot \text{OH} + \text{OH}^- + \text{Fe}^{3+} (\text{Cu}^{2+})$ or by the copper-mediated LDL (low-density lipoprotein) oxidation, $\text{LH} \rightarrow \text{L}^- \rightarrow \text{LOO}^-$, where LH represents LDL. Nevertheless, it has to be remembered that these metal ions are essential for many physiological functions, as constituents of hemoproteins and cofactors of different enzymes, including those involved (iron for catalase, copper for ceruloplasmin and Cu,Zn-
superoxide dismutase) in the antioxidant defense. The proposed binding sites for trace metals to flavonoids are the catechol moiety in ring B, the 3-hydroxyl, 4-oxo groups in the heterocyclic ring and the 4-oxo, 5-hydroxyl groups between the heterocyclic and the A rings (Figure 1.11).

![Figure 1.11: Binding sites for trace metals](image)

However, the major contribution to metal chelation is due to the catechol moiety. Due to their lower redox potentials, flavonoids are thermodynamically able to reduce highly oxidizing free radicals, such as superoxide, peroxyl, alkoxyl and hydroxyl radicals by hydrogen atom donation, via the reaction, Fl-OH + R• → Fl-O• + RH, where R• represents superoxide anion, peroxyl, alkoxyl and hydroxyl radicals. The aroxyl radical (Fl-O•) may react with a second radical, acquiring a stable quinone structure (Figure 1.12).

![Figure 1.12: Scavenging of ROS (R') by flavonoids](image)

The aroxyl radicals could interact with oxygen, generating quinones and superoxide anion, rather than terminating chain reactions. The last
reaction may take place in the presence of high levels of transient metal ions and is responsible for the sometimes undesired pro-oxidant effect of flavonoids.\textsuperscript{84} Thus, the overall capacity of flavonoids to act as antioxidants depends not only on the redox potential of the couple Fl-O'/Fl-OH but also on possible side reactions of the aroxyl radical. Scavenging of superoxide is particularly important, because this radical is ubiquitous in aerobic cells and, despite its mild reactivity, is a potential precursor of the aggressive hydroxyl radical in the Fenton and Haber-Weiss reactions. Besides scavenging, flavonoids also stabilize free radicals involved in oxidative processes by complexing with them.\textsuperscript{85}

1.4.4.1 Structural features and antioxidant activity of flavonoids

The antioxidant activity of flavonoids and their metabolites \textit{in vitro} depends upon the arrangement of functional groups about the nuclear structure.\textsuperscript{86} Consistent with most polyphenolic antioxidants, both the configuration and total number of hydroxyl groups substantially influence several mechanisms of antioxidant activity. Free radical scavenging capacity is primarily attributed to the high reactivities of hydroxyl substituents that participate in Fl-OH + R' → Fl-O' + RH reaction. The B-ring hydroxyl configuration is the most significant determinant of scavenging of ROS and RNS.\textsuperscript{87} Hydroxyl groups on the B-ring donate hydrogen and an electron to hydroxyl, peroxyl and peroxynitrite radicals, stabilizing them and giving rise to a relatively stable flavonoid radical. A 3',4'-catechol structure in the B-ring as in quercetin 28, strongly enhances lipid peroxidation inhibition. This arrangement is a salient feature of the most potent scavengers of peroxyl, superoxide and peroxynitrite radicals.\textsuperscript{88} The significance of other hydroxyl configurations is less clear, but beyond increasing total number of hydroxyl groups, A-ring substitution correlates little with antioxidant activity. Compared to the B-ring hydroxylation pattern, the impact of the A-ring
arrangement on antioxidant activity is of questionable significance. The flavonoid heterocycle contributes to antioxidant activity by (i) the presence of a free 3-OH and (ii) permitting conjugation between the aromatic rings. The closed C-ring itself may not be critical to the activity of flavonoids, given that chalcones are active antioxidants. Free radical scavenging by flavonoids is highly dependent on the presence of a free 3-OH. Flavonoids with a 3-OH and 3',4'-catechol are reported to be 10-fold more potent than ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one; a known RNS scavenger) against peroxynitrite. The torsion angle of the B-ring with respect to the rest of the molecule strongly influences free radical scavenging ability. Flavonols and flavanols with a 3-OH are planar, while the flavones and flavanones, lacking this feature, are slightly twisted. Planarity permits conjugation, electron dislocation and a corresponding increase in flavonoid phenoxy radical stability. Removal of a 3-OH abrogates coplanarity and conjugation, thereby compromising scavenging ability.

Substitution of 3-OH by a methyl or glycosyl group completely abolishes the activity. It is postulated that B-ring hydroxyl groups form hydrogen bonds with the 3-OH, aligning the B-ring with the heterocycle and A-ring. Eliminating this hydrogen bond effects a minor twist of the B-ring, compromising electron delocalization capacity. Due to this intramolecular hydrogen bonding, the influence of a 3-OH is potentiated by the presence of a 3',4'-catechol, explaining the potent antioxidant activity of flavan-3-ols and flavon-3-ols that possess the latter feature.

The differences in antioxidant activity between polyhydroxylated and polymethoxylated flavonoids are most likely due to differences in both hydrophobicity and molecular planarity. Suppression of antioxidant activity by O-methylation may reflect steric effects that perturb planarity. Flavonoids with a 2–3 double bond in conjugation with a 4-carbonyl group exhibit stronger antioxidant activity. The majority of research supports that
flavonoids lacking one or both of these features are less potent antioxidants than those with both elements. Conjugation between the A and B rings permits a resonance effect of the aromatic nucleus that lends stability to the flavonoid radical and is therefore critical in optimizing the phenoxy radical-stabilizing effect of a 3',4'-catechol. The premise that flavanols are more effective free radical scavengers than flavones\(^9^4\) may be ascribed to the greater number of hydroxyl groups and 3-OH in the former. Aglycones are more potent antioxidants than their corresponding glycosides.\(^9^5\) i.e., glycosylation in a flavonoid decreases antioxidant activity.

Tea and soy flavonoid are also one among the major sources of dietary antioxidants. Tea is rich in antioxidant polyphenols such as catechins, flavonols, theaflavins and thearubigins. Tea flavonoids have many health benefits. Tea flavonoids reduce the oxidation of low-density lipoprotein, lowers the blood levels of cholesterol and triglycerides. Soy flavonoids (isoflavones) can also reduce blood cholesterol and can help to prevent osteoporis. Soy flavonoids are also used to ease menopausal symptoms.

### 1.5 Spices as antioxidants

Spices and herbs that are widely used in ethnic foods are major natural antioxidants. Oxidation processes caused by ROS are a major cause of deterioration of various food products. Oxidation of food products is associated with loss of quality. Significant changes can occur in flavour, colour and texture and finally, can lead to loss of nutritive value or complete spoilage. In order to prevent these processes, antioxidants are used. The use of synthetic antioxidants in food products like butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) is under strict regulation owing to uncertainty about their safety.\(^9^6\) Therefore there is a demand for the discovery of safe antioxidants especially from natural origin and many groups are currently actively involved in the search for natural antioxidants.
Since ancient times, spices like ginger and turmeric have been added to different types of food to improve their flavour. Some of the early scientific investigations carried out by Sehti and Aggarwal,\(^9^7\) reported the improved storage stability of groundnut oil after the addition of different spices. Chipault \textit{et al}\(^9^8\) investigated the antioxidant activity of several spices. Since the early work of Chipault \textit{et al}, the interest in the antioxidative activity of spices has increased and has led to a large amount of data/information about the compounds present in them and the mechanisms involved. Because of their strong antioxidant and antimicrobial properties which are more appreciable than many currently used natural and synthetic antioxidants, there is continuous efforts among industry and scientific circles in the study of spices and herbs. These advantageous properties are due to many substances, including some vitamins, flavonoids, terpenoids, carotenoids, phytoestrogens, minerals, etc., which render spices/herbs or their antioxidant components the ability to function as preservative agents in food.\(^9^9\) Apart from the antioxidants like \(\beta\)-carotene, tocopherols, vitamin C etc., there are specific compounds that are characteristic to each of the aromatic herbs and spices. Some examples of specific antioxidants from spices include biflorin \(^2^9\), eugenol \(^3^0\) and eugenyl acetate in clove;\(^1^0^0\) carnosol \(^3^1\), carnosic acid \(^3^2\), rosmanol \(^3^3\), rosmaridiphenol, rosmadial and rosmariquinone \(^3^4\) and various methyl and ethyl esters of these substances in rosemary and sage;\(^1^0^1\) diarylheptanoid, gingerol \(^3^5\) and zingerone \(^3^6\) in ginger;\(^1^0^2\) curcumin \(^1^6\) and tetrahydrocurcumin \(^3^7\) in turmeric;\(^1^0^3\) flavonoids, ferulic acid \(^3^8\), piperine \(^3^9\), phenolic amide feruperine \(^4^0\) in black pepper;\(^1^0^4\) thymol \(^4^1\) and carvacrol \(^4^2\) in essential oils from \textit{Algerian origanum}\(^1^0^5\) etc (Chart 1.2). Modern health conscious consumers often ask for natural ingredients, free of synthetic additives. Therefore, the application of natural antioxidants will probably gain even more interest the future and it will be necessary to study their effect and interactions in more detail. It is of interest to note that in Kerala, several
species of Zingiberaceae are used as spices and also as important constituents in formulations under the Ayurvedic system of medicines.

1.6 The Zingiberaceae: General and Botanic Aspects

The Zingiberaceae is a large family of rhizomatous plants originating from Asia and far-east which has been cultivated for centuries. It is one of the largest families of the plant kingdom. Plants belonging to Zingiberaceae are distributed mostly in tropical and subtropical areas. It is an important natural source that provides many useful products for food, spices, medicines, dyes and perfumes to man. Zingiberaceae plants are perennial rhizomatous herbs. Leaves are simple and distichous. Inflorescence is terminal on the leafy shoot or on the lateral shoot. Flowers are delicate, ephemeral and highly modified. All parts of the plant are aromatic and the fruits are capsules. All Zingiberaceae are ground plants mostly growing in damp and humid shady places. However, few rare species can tolerate full exposure to the sun while a small number grow at high elevation also.

Zingiberaceae comprises of 53 genera and 1400 species. In India, 21 genera and 190 species are found. In the North Eastern region of India, 19 genera and 70 species are present. In South India, the family is represented by 11 genera and 60 species. Zingiberaceae family shows high endemism in India. Two genera and 70 species are endemic to India. The name Zingiber, originated from the Greek word ‘Zingiberis’ which in turn originated from the Sanskrit word ‘Singa Verni’ meaning ‘deer horn shaped’ presumably in allusion to the sprouting rhizomes. There is another argument that the word Zingiber originated from the Malayalam word of ginger—“Inchiver”. The rhizomes of these plants are fleshy with nodes and internodes and sometimes with scaly leaf sheaths.

Zingiberaceae is broadly divided into four tribes namely,

- HEDYCHIEAE
- ZINGIBEREA
ALPINIEAE and GLOBBAAE

The species under each tribe is given in Table 1.5.

Table 1.5: Classification of the Zingiberaceae family

<table>
<thead>
<tr>
<th>Tribe</th>
<th>Type of species</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEDYCHIEAE</td>
<td>o Bosenbergia</td>
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<tr>
<td></td>
<td>o Caulokaempferia</td>
</tr>
<tr>
<td></td>
<td>o Cautleya</td>
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<tr>
<td></td>
<td>o Curcuma</td>
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<tr>
<td></td>
<td>o Curcumorpha</td>
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<tr>
<td></td>
<td>o Haniffia</td>
</tr>
<tr>
<td></td>
<td>o Hedychium</td>
</tr>
<tr>
<td></td>
<td>o Kaempferia</td>
</tr>
<tr>
<td></td>
<td>o Scaphochlamys</td>
</tr>
<tr>
<td></td>
<td>o Stahlianthus</td>
</tr>
<tr>
<td>ZINGIBEREA</td>
<td>• Zingiber</td>
</tr>
<tr>
<td>ALPINIEAE</td>
<td>• Alpinia</td>
</tr>
<tr>
<td></td>
<td>• Amomum</td>
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<tr>
<td></td>
<td>• Elettariopsis</td>
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<td></td>
<td>• Etlingera</td>
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<td></td>
<td>• Geostachys</td>
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<td></td>
<td>• Hornstedtia</td>
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<tr>
<td></td>
<td>• Pomereschia</td>
</tr>
<tr>
<td>GLOBBEAE</td>
<td>✓ Gagnepainia</td>
</tr>
<tr>
<td></td>
<td>✓ Globba</td>
</tr>
</tbody>
</table>

Zingiberaceae is also considered as an economically important family as the source of important spice plants such as Curcuma domestica, Curcuma longa (turmeric), Elettaria cardamomum (cardamom) and Zingiber officinale (ginger). While most Hedychium species are cultivated as ornamental plants for their flowers, few Alpinia are grown for their rhizomes that are used as spices and in traditional medicines. Various pharmacological properties of many species of Zingiberaceae are reported. Ginger and turmeric are widely used in Indian system of medicine. Ginger, the rhizome of Zingiber officinale is one of the most familiar spices and one of the most frequently used medicinal plant in traditional medicinal systems of India and...
South East Asia. It is used as carminative, stimulant and in the treatment of gastrointestinal and respiratory diseases in India. The rhizomes have antipyretic effect. Zingiberene 43 (Chart 1.2), a major compound from the rhizome of Zingiber officinale has antiviral, antiulcer and antifertility effects. Numerous chemical investigations of the flavor and bioactive compounds of ginger have led to the isolation of a large number of terpenoids and phenylalkanoids. Many Zingibers are used by tribals for treatment of several ailments. The plants exhibit much diverse pharmacological and insecticidal activities. For eg., curcumin 16, the yellow bioactive component of turmeric has been shown to have a wide spectrum of biological actions. These include its antiinflammatory, antioxidant, anticarcinogenic, antimutagenic, anticoagulant, antifertility, antidiabetic, antibacterial, antifungal, antiprotozoal, antiviral, antifibrotic, antivenom, antiulcer, hypotensive and hypocholesteremic activities. Its anticancer effect is mainly mediated through induction of apoptosis. Its anti-inflammatory, anticancer and antioxidant roles may be clinically exploited to control rheumatism, carcinogenesis and oxidative stress-related pathogenesis. Clinically, curcumin has already been used to reduce post-operative inflammation.

Chart 1.2
A literature search on Zingiberaceae showed that a variety of biologically active compounds belonging to different structural types are present in them. These structural types includes flavonoids, flavonols, steroidal saponins, hydroxyl phenyl alkanones, phenolic esters, labdane type diterpenoids, stilbenes, mono and bicyclic sesquiterpenes, diarylheptanoids, phenylbutanoids and their dimers, carabane type sesquiterpenes, oxygenated bisabolanes, chalcones, steroid glycosides, triterpenoids, cyclohexane diepoxides etc.

1.7 Objectives and Organization of the thesis

The preceding pages clearly portray the importance of natural products and the heightened awareness about the potential of medicinal plants used in traditional systems of medicine in the bioprospecting for drugs, drug leads and neutraceuticals. Ayurvedic therapeutic formulations are used extensively in India and have proven efficacy for treatment of chronic diseases such as rheumatism, atherosclerosis etc. Since several Zingiberaceae plants are used in such formulations, and it is now well known that reactive oxygen species play a major role in the initiation of these chronic diseases, it appeared timely and relevant to study these plants in detail. Among the important Zingiberaceae plants, only Zingiber officinale and Curcuma longa have been studied by many groups. Other genuses of this family, which are also of much importance are Alpinia and Kaempferia. Accordingly, a detailed investigation of the three plants, Alpinia galanga, Alpinia calcarata and Kaempferia pulchra have been undertaken during this PhD programme.
It is impossible to give a comprehensive overview of natural products in a brief manner. But an attempt has been made to bring out the importance of biologically active natural products with special reference to anticancer and antioxidant compounds from terrestrial plants in Chapter 1.

Natural product chemistry has undergone a renaissance in the last few decades. Sophistication in separation and analytical techniques has added momentum to the discovery of new molecules. Even though the principal motivation for searching for new substances remains one of discovering new pharmacologically useful materials, the field of “chemosystematics” has also played a major role in the isolation and structural elucidation of a large number of natural products.

A detailed discussion on the genus *Alpinia* of the Zingiberaceae family with special emphasis on the phytochemicals present in them is given as a preamble to Chapter 2. *Alpinia galanga* commonly known as ‘greater galangal’ is the subject matter of Chapter 2, describing the compounds isolated from the rhizomes and their biological activity (anticancer and antioxidant activities). Chapter 3 deals with the phytochemical investigation and biological activity studies of the rhizomes of *Alpinia calcarata*. In Chapter 4, phytochemical investigation of *Kaempferia pulchra* belonging to the genus *Kaempferia* is discussed. The search for biologically active compounds especially anticancer compounds from natural products prompted us to investigate the leaves of the most important and common Indian medicinal plant *Azadirachta indica* (Neem). This resulted in the isolation of nimbolide from its leaves which is discussed in the final chapter, viz., Chapter 5. Here, a short discussion of the plant *Azadirachta indica*, the compound nimbolide isolated from the leaves and its anticancer and the antioxidant properties are discussed.
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