CHAPTER-I

Introduction and Review of Literature
1.1. Introduction:

The origin of disease is multifactorial in nature and is now being understood as vitiation in the basic homoeostatic balance phenomenon in the body. It is increasingly realized that the majority of the present day diseases are due to the shift in the balance of the pro-oxidant and the antioxidant homoeostatic phenomenon in the body. Pro-oxidant condition dominates either due to the increased generation of the free radicals caused by excessive oxidative stress of the current life, or due to the poor scavenging/quenching in the body caused by depletion of the dietary antioxidants (Rakesh Sharma et al., 2006). So it becomes very essential to aim at the balancing of oxidants in the body during the last few decades, there has been a considerable growth in the field of herbal medicine. It is becoming popularized in both developing and developed countries due to its natural origin and lesser side effects (Khopde et al., 2001, Naik et al., 2003). It is classified in the ancient Indian system of medicine (Ayurveda) as a Rasayana. A group of plant derived drugs that improves overall physical and mental health and puts off diseases by rejuvenating the body during the incapacitated conditions of disease or disorder.

Plants have been utilized as medicines for thousands of years. These medicines initially took the form of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations (Balick et al., 1997). The specific plants to be used 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, 2005).

Natural products have played a significant role in drug discovery and development especially for agents against cancer and infectious disease. An analysis
of new and approved drugs for cancer by the United States Food and Drug Administration over the period of 1981-2002 showed that 62% of these cancer drugs were of natural origin. Natural compounds possess highly diverse and complex molecular structures compared to small molecule synthetic drugs and often provide highly specific biological activities likely derived from the rigidity and high number of chiral centers. Ethno- traditional use of plant-derived natural products has been a major source for discovery of potential medicinal agents (Gonzales and Valerio, 2006).

However, it was not until the nineteenth century that scientists isolated active components from various medicinal plants. Friedrich Sertürner isolated morphine from Papaver somniferum in 1806, and since then natural products have been extensively screened for their medicinal purposes. Atropine obtained from Atropa belladonna, strychnine, a CNS stimulant, ziconotide, identified from a cone snail, Conus magus, and Taxol® obtained from the bark of the Pacific yew tree are a few examples of active components isolated from natural sources.

Natural drugs have found direct medical application as drug entities, but they also serve as chemical models or templates for the design, synthesis, and semi synthesis of novel substances, such as paclitaxel (Taxol®), vincristine (Oncovin®) and camptothecin, in the treatment of human cancer. Although there are some new approaches to drug discovery, such as a combination of chemistry and computer-based molecular modeling design, none of them can replace the important role of natural products in drug discovery and development (Amin et al., 2009).

\textit{Fluggea leucopyrus} Linn. (Euphorbiaceae) The plant is sweet, cooling, diuretic, aphrodisiac and tonic, and is useful in vitiated conditions of pitta, burning
sensation, strangury, seminal weakness and generally debility. The leaves act as a
disinfectant and its paste is used by tribes to extract any extraneous materials from
body tissues without surgery, leaves are eaten. The juice or paste of the leaves is used
along with tobacco to destroy worms in sores. The berry is sweet and edible. Slender
branches are used for masking wicker-baskets, and for thatching. Wood is pink, hard
and close-grained. It is used as fuel (wealth of India., 1952).

Anti ulcer activity of *fluggea microcarpa* was reported by (Goel, et al., 1997). Anti
microbial activity of this plant was reported by (Bakshu et al., 2001). However the
detailed mechanism of pharmacological activities of this plant has not been
thoroughly investigated. So far only few studies have been carried out on this plant.
Based on our preliminary findings, it was of interest to assess the in vivo efficacy of
flavonoids of *Fluggea leucopyrus* Linn. Hence in the present study, author reported
first time, the effect of new flavonoid molecule of *Fluggea leucopyrus* on in vivo
antitumor activity, and other pharmacological properties.
Hypothesis, Aim and Objective of this study:

Hypothesis:

*Fluggea leucopyrus* is under evaluated species of the Euphorbiaceae family and has cancer chemo preventive activity. It has antioxidant, anti-inflammatory, anti microbial and analgesic activity as well.

Aim of this study:

To investigate extracts of *Fluggea leucopyrus* for anti-tumor activity as well as to determine the chemical structure/s and activities of pure compound(s) isolated by bioassay guided fraction.

Objective of this study:

- To investigate anti-tumor activity and antioxidant activity.
- To isolate pure compound from extract of *Fluggea leucopyrus*

Envisaged contributions of this study:

- Determination of Anti-tumor activity.
- Isolated pure compound structure from the plant *Fluggea leucopyrus* elucidated with NMR, IR and LCMS data. This may contribute to existing knowledge about Euphorbiaceae’s phytochemistry.
- Screening of isolated pure compound for antioxidant, anti-inflammatory, anti microbial and analgesic activities.
- This knowledge may not only help in the discovery or development of new therapeutic agents, it will also contribute to the knowledge of where new sources of economic viable materials can be found.
1.2. General Overview:

1.2.1. Importance of Traditional medicine:

The use of traditional medicine has been recently encouraged by World Health Organization (WHO) and United Nation Children’s Fund (UNICEF) for its cultural role, greater availability and acceptability than the modern pharmaceutical agents. The therapeutic action of plants depends upon its chemical constituents. Plants have been reported to contain compounds such as tannins, saponins, phytate, fiber and phenolic compounds, which play prominent roles in medicinal activity (Falade et al., 2005).

1.2.2. History of plant medicine:

In India, the history of health care goes back to 5000 years B.C., when health care needs and diseases were noted in ancient literatures like ‘Rig-Veda’ and ‘Atharva-Veda’. Later, the texts like ‘Charak Samhita’ and ‘Sushruta Samhita’ were documented in about 1000 years B.C., which describe over 700 plants along with their classification, pharmacological and therapeutic properties and also use of plants and poly herbal formulations was highlighted for health care. Ayurveda remains one of the most ancient systems widely practiced in the Indian subcontinent and has a sound Philosophical, experiential and experimental basis. A considerable amount of research on Pharmacognosy, Chemistry, Pharmacology and Clinical therapeutics has been carried out on Ayurvedic medicinal plants. Numerous molecules have come out of Ayurvedic experimental base, including Rawolfia alkaloids in amoebiasis, guggulsterones as hypolipidemic agents, Mucuna purines for Parkinson’s disease, piperidines as bioavailability enhancers, baccosides for mental retention, picrosides for hepatic protection, phyllanthus as antiviral, curcumins for inflammation,
withanolides and many other steroidal lactones and their glycosides as immunomodulators (Patwardhan, 2005). The WHO estimates that about 80% of the population living in the developing countries relies on traditional medicine for their primary health care needs. In almost all the traditional systems of medicine, the medicinal plants play a major role and constitute their backbone. Indian Materia Medica includes about 2000 drugs of natural origin almost all of which are derived from different traditional systems and folklore practices (Narayana et al., 1998; Mukherjee and Wahile, 2006).

1.2.3. Current status of Plant based medicine:

It is difficult to get reliable figures for the total number of medicinal plants on earth; according to some estimation, around 35,000-70,000 plant species are being used worldwide in health care systems (Farnsworth and Soejarta, 1991). According to WHO estimations the populations in developing countries like India (70%), Uganda (60%), Tanzania (60%), Benin (80%) and Ethiopia (90%) extensively use traditional and alternative medicines for health care. Plants and plant-based products are an integrated part of most of the traditional and alternative systems of medicines worldwide. In developed countries like Belgium (31%), USA (42%), Australia (48%), France (49%), Canada (70%), a significant percentage of the population has used traditional and alternative remedies at least once for health care (WHO, 2002). The global market of trade related to medicinal plants is estimated around US $60 billion per year and is growing at the rate of 7% annually with varying shares of developed and developing countries (Dev, 1999; Laird and Pierce, 2002; Raskin et al., 2002). A study reveals that about 42% of the best selling pharmaceutical products in 1997 were biological or natural products or chemical entities derived from natural resources, worth of US $17.5 billion (Laird and Kate, 2002; Mukherjee and Wahile, 2006).
1.2.4. Current situation in Indian system of medicines:

Variations in geographical landscaping and biodiversities in the Indian subcontinent have helped to develop the use of a variety of plant species and other natural resources for health care and contributed to the Materia Medica of traditional systems of medicine. More than 25000 single or poly herbal formulations are used by the tribal and rural population in India. Ayurveda and Indian system of medicines (ISM) utilize a vast number of plant and their different parts in health care. Export-Import Bank reports reveal that the global trade of plant-derived and plant-originated products is around US $60 billion (with growth of 7% per annum) where India holds stake of US $1 billion (Raskin et al., 2002). Many important modern drugs are plant based or derived directly or indirectly from the plants. But only 6% of all therapeutically important species, which are noted in ancient literature, have been analyzed phyto-chemically for their therapeutic potential (Choudhary, 2002).

1.2.5. Plants in conventional western medicine:

Medicinal component from plants play an important role in conventional western medicine also. In 1984, at least 25% of the prescription drugs issued in the USA and Canada were derived from or modeled after plant natural products. In 1985, Farnsworth et al. identified 119 secondary plant metabolites that are used globally as drugs. It has been estimated that 14-28% of higher plant species are used medicinally, that only 15% of all angiosperms have been investigated chemically and that 74% of pharmacologically -active plant derived components discovered after following up on ethnomedical use of the plant (Eloff, 1998).
1.2.6. Serious underestimation of plant derived medicines:

The importance of plant-derived medicines is seriously underestimated in modern day medicine. Only approximately 15% of the angiosperms (flowering plants) have been chemically investigated for medical potential (Farnsworth, 1966; Farnsworth and Soejarto, 1991).

1.2.7. Tendency towards unrefined or “natural” assuming non-toxic:

The majority of people in developing countries like India are depending entirely on herbal medicines. This includes the almost all from rural communities along with some shack dwellers on the outskirts of cities depend entirely on herbal medicines for their health. The increased awareness about the toxicities of refined products among the modern city dwellers, individuals are increasingly focusing their attention towards the herbal medicines and herbal teas for better health. This is indicated by the steady increase in the number of retail outlets who solely merchandise herbal products and conventional pharmacies who stock herbal medicines. As the population growth is still increasing, the demand for natural resources will not decline, but maintain the current pressure of intrusion into phytomedicines. A greater demand for land in terms, pastoral agriculture and commercial development may reduce the available areas medicinal plants. A dynamic and clear-cut plan for cultivating and conserving the medicinal plants, as well as the more knowledge about the plant kingdom’s medicinal properties (many of which are undiscovered yet), urgently need to be implanted to ensure their future beneficial use. In addition to identifying phytomedicines which can offer solutions to modern day diseases like AIDS and cancers, increased knowledge about phytomedicines can;
• Serves as alternative solutions where other orthodox medicines have limitations, for example antibiotics (in case of antibacterial drug resistance) and anticancer drugs from plants, like tubulin polymerization inhibitors (which are less toxic than current anticancer drugs such as actinomycin D).

• Provide a necessary knowledge to avoid or minimize unwanted side effects from toxicities resulting from use of herbal medicines.

1.2.8. Ethno medical use of leads for discovery of modern drugs:

Folk or ethno medicinal uses represent leads that may guide pharmaceutical researchers to discover modern therapeutic drugs. Few researchers are still skeptic about the indigenous folk traditions that are transferred from generation to generations. However, many important modern compounds like atropine, digitoxin, ephedrine, morphine, reserpine and many other are of natural origin. Indeed some have been discovered through following up leads derived from ethnographic research into folk use and information on integration of the indigenous people with their ecosystem. For example the discovery of thiarubinn a, potent antibiotic from genus Aspillia (Compositae), took place by careful observation of chimpanzees dietary habit (Rodriguez et al., 1985). Winter (1955) investigated anti-microbial properties of two groups of plants. One group was randomly selected and the other obtained from herbal remedy sources, which was previously documented to have properties that are useful for the treatment of infections. Of the former randomly selected plants, only 29.5% exhibited the anti-microbial activity, while 65% of the latter selected group was active.
1.2.9. Expansion of human civilization poses a threat to plant biomass:

The rapid expansion of human civilization health care needs possess a threat to the plant biomass. Urbanization and industrial pollutions causing adverse affect on the survival of valuable medicinal plants. Their disappearance will be accompanied by the extinction of medicinal resources as well as folk knowledge about how to use them for existence and survival. Western Ghats is considered to be one of the twelve hot spots in biodiversity of the world. But there is a gradual decrease in the medicinal plants due to their replacement by agriculture and urban development. These activities may lead to the depletion of plant biomass from their resources and in feature they may virtually become extinct. There is an urgency to explore this plant wealth before they become extinct.

Plants defense mechanisms are sophisticated and which allow them to survive in the adverse environmental conditions. Their ability to survive against all odds is due to their capacity to synthesize the secondary metabolites already been isolated and their structures elucidated (Wink, 1999).

The main role of secondary metabolites has been identified to be:

- Defense against herbivores (insects, vertebrates).
- Defense against fungi and bacteria.
- Defense against viruses.
- Defense against other plants competing for light; water and nutrients.
- Signal compounds to attract pollinating and seed dispersing animals.
- Signals for communication between plants and symbiotic Micro Organisms (Nitrogen-fixing Rhizobia or Mycorrhiza fungi).
- Protection against UV-light or physical stress (Wink, 1999).
1.2.10. Bioactive properties derived from plants:

Large numbers of surveys have been conducted in which plant extracts have been evaluated for various biological activities. Only a small sample of species is listed in Table 1.

Table 1: Plant drugs used in traditional medicine which led to useful modern drugs (Gurib-Fakim, 2006).

<table>
<thead>
<tr>
<th>Botanical names</th>
<th>Origin</th>
<th>Indigenous use</th>
<th>Uses in biomedicine</th>
<th>Biologically active compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Adathoda vasica</em></td>
<td>India, Sri Lanka</td>
<td>Antispasmodic</td>
<td>Antispasmodic</td>
<td>Vasicin (lead molecule for Bromhexin and Ambroxal)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Antiseptic</td>
<td>Oxytocic</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Insecticide</td>
<td>Cough suppressant</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Fish poison</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Catharanthus roseus</em></td>
<td>Madagascar</td>
<td>Diabetes, fever</td>
<td>Cancer chemotherapy</td>
<td>Vincristine, Vinblastine</td>
</tr>
<tr>
<td><em>Condrodendron tomentosum</em></td>
<td>Brazil, Peru</td>
<td>Arrow poison</td>
<td>Muscular relaxation</td>
<td>D-Tubocurarine</td>
</tr>
<tr>
<td><em>Gingko biloba</em></td>
<td>Eastern China</td>
<td>Asthma, anthelmintic</td>
<td>Dementia, cerebral deficiencies</td>
<td>Gingkolides</td>
</tr>
<tr>
<td><em>Harpagophytum procumbens</em></td>
<td>South Africa</td>
<td>Fever, inflammatory conditions</td>
<td>Pain, rheumatism</td>
<td>Harpagoside, Caffeic acid</td>
</tr>
<tr>
<td><em>Piper methysticum</em></td>
<td>Polynesia</td>
<td>Ritual stimulant, tonic</td>
<td>Anxiolytic, mild stimulant</td>
<td>Kava pyrones</td>
</tr>
<tr>
<td><em>Podophyllum peltatum</em></td>
<td>North America</td>
<td>Laxative, skin infections</td>
<td>Cancer chemotherapy, warts</td>
<td>Phodophyllotoxin and lignans</td>
</tr>
<tr>
<td><em>Prunus africana</em></td>
<td>Tropical Africa</td>
<td>Laxative, Old man’s disease</td>
<td>Prostate hyperplasia</td>
<td>Sitosterol</td>
</tr>
</tbody>
</table>
1.2.11. Free radicals:

Free radicals are molecules containing unpaired electrons. The unpaired electron is highly reactive. It either burns a molecule (causes oxidative damage) or is passed from one molecule to another molecule. In that process it turns the recipient into a free radical and neutralizes the donor. Most of the free radicals are produced in the mitochondria and most of the free radical damage is done to mitochondrial membranes and mitochondrial DNA (Rakesh et al., 2006).

Free radicals can also be produced by many cells as a protective mechanism. Neutrophils produce free radicals to attack and destroy pathogens, while the liver uses free radicals for detoxification (Lunec et al., 2002). However, the presence of free radicals within the body can also have a significant role in the development and progression of many disease processes like heart disease, congestive heart failure, hypertension, cerebrovascular accidents, and diabetic complications (Chen et al., 2002). Excess free radicals can result from tissue damage and hypoxia, overexposure to environmental factors (eg: smoking, ultraviolet radiation, and pollutants) and due to lack of antioxidants or destruction of free radical scavengers. When the production of damaging free radicals exceed the capacity of the body's antioxidant defenses to detoxify them, a condition known as oxidative stress occurs (Kohen and Nyska, 2002).

As free radicals play an important role in the diseases scenario of an individual, a thorough understanding of the various physiologically significant free radicals is of paramount importance (Rakesh, Sharma and Rajesh Arora., 2006).
a. Preventive antioxidants: First Line Defense:

The first line defense comprises preventive antioxidants that act by quenching of O₂, decomposition of H₂O₂ and sequestration of metal ions. The antioxidants belonging to this category are enzymes, like superoxide dismutase (SOD), catalase, glutathione peroxidase, and glutathione reductase and non-enzymatic molecules like minerals and some proteins. Superoxide dismutase mainly acts by quenching of superoxide (O₂), an active oxygen radical, produced in different aerobic metabolism. Catalase is a tetrameric enzyme, present in most of the cells and acts by catalyzing the decomposition of H₂O₂ to water and oxygen. Glutathione peroxidase is a selenium containing enzyme which catalyses the reduction of H₂O₂ and lipid hydroperoxide, generated during lipid peroxidation, to water using reduced glutathione as substrate (MacMillan- Crow et al., 1998; Yamakura et al., 1998; Irshad and Chaudhuri, 2002).

b. Radical Scavenging Antioxidants: Second Line Defense:

The antioxidants belonging to second line defense include glutathione (GSH), vitamin C, uric acid, albumin, bilirubin, vitamin E (mainly- tocopherol), carotenoids, flavonoids and ubiquinol. Glutathione (GSH gamma glutamyl cysteinylglycine) is the most abundant non-protein thiol, synthesized in the liver and acts as a substrate for glutathione peroxidase enzyme. This also serves as a scavenger of different free radicals. Similarly carotene (Pro-vitamin A), vitamin C and vitamin E are some important scavenging antioxidant vitamins which cannot be synthesized by most mammals including human beings and therefore, are required from diet (Halliwell and Gutteridge, 1999; Irshad and Chaudhuri, 2002).
c. Repair and De-Novo Enzymes: Third Line Defense:

Third line antioxidants are a complex group of enzymes for repair of damaged DNA (Henle and Linn, 1997), damaged protein, oxidized lipids and peroxides and also to stop chain propagation of peroxy lipid radical. These enzymes repair the damage to biomolecules and reconstitute the damaged cell membrane eg. Lipase, proteases, DNA repair enzymes, transferase, methionine sulfoxide reductase etc (Irshad and Chaudhuri, 2002).

1.2.12. Processes of free radical formation:

Radical formation by electron transfer: \( A + e^- \rightarrow A^- \)

Radical formation by homolytic fission: \( X: Y \rightarrow X + Y^- \)

Ion formation by heterolytic fission: \( X: Y \rightarrow X^- + Y^+ \)

1.2.13. Types: Reactive oxygen and nitrogen free radicals include superoxide (\( O_2^- \)), hydroxyl (OH), peroxyl (\( RO_2^- \)), alkoxyl (\( RO^• \)), hydroperoxyl (\( HO_2^• \)), nitric oxide (NO) and nitrogen dioxide (\( NO_2^• \)) radicals. Oxygen and nitrogen free radicals can be converted to other non-radical reactive species, such as hydrogen peroxide (\( H_2O_2 \)), hypochlorous acid (\( HOCl \)), and peroxynitrite (\( ONOO^- \)). The reactive oxygen species (ROS) are continuously produced during normal physiological events and are removed by antioxidant defense mechanisms. There is a balance between the generation of ROS and inactivation of ROS by the antioxidant system in organisms.

Under pathological conditions, the imbalance between ROS and antioxidant defence mechanisms leads to oxidative modification in cellular membrane or intracellular molecules (Duh et al., 1999). Consequently, antioxidants that can neutralize direct ROS attacks and terminate free radical-mediated oxidative reaction would have beneficial effect in protecting the human body from various diseases.
The most important free radicals in biological systems are radical derivatives of oxygen. Reduction of oxygen by the transfer of single electron will produce the superoxide free radical anion (super oxide).

\[ \text{O}_2 + e^- \rightarrow \text{O}_2^- . \]

A two electron reduction of oxygen would yield hydrogen peroxide:

\[ \text{O}_2 + 2e^- + 2H^+ \rightarrow \text{H}_2\text{O}_2 \]

a. Superoxide: Components as a precursor of more reactive oxidative species, such as single oxygen and hydroxyl radicals (Aurand and Boonme, 1977). Furthermore, superoxide radical is considered to play an important role in the peroxidation of lipids (Dahl and Richardson, 1978). At low pH value superoxide will protonate to form the perhydroxyl radical (\(\text{HO}_2^-\)), a more reactive oxidizing species but at physiological pH less than 1% will be in protonated form.

b. Hydrogen peroxide: Although not a radical species, \(\text{H}_2\text{O}_2\) is an important ROS contributing to oxidative stress. The generation of even low levels of \(\text{H}_2\text{O}_2\) in biological systems may be important, since naturally occurring iron complexes are believed to react with \(\text{H}_2\text{O}_2\) in vivo to generate the highly reactive hydroxyl radicals in a superoxide driven Fenton reaction. Hydrogen peroxide is an oxidizing agent but not specially reactive. Its main significance lies in it being a source of hydroxyl radicals in the presence of reactive transition metal ions. Hydrogen peroxide is often generated in biological systems via the production of superoxide, two superoxide molecules can react together to form hydrogen peroxide and oxygen.

\[ 2\text{O}_2^- + 2\text{H}^+ \xrightarrow{\text{SOD}} \text{H}_2\text{O}_2 + \text{O}_2 \] (dismutase reaction)

Here hydrogen peroxide is the non-radical product. \(\text{H}_2\text{O}_2\) is not a free radical but falls into the category of “reactive oxygen species” (ROS) that includes not only oxygen free radicals but also non-radical oxygen derivatives that are involved in
oxygen radical production. Hydrogen peroxide is an important compound in free radical biochemistry because it can rather easily break down, particularly in the presence of transition metal ions, to produce the most reactive and damaging of the oxygen free radicals, the hydroxyl radical (OH).

\[ \text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \text{OH} + \text{OH}^- + \text{Fe}^{3+} \] (Fenton's reaction)

\[ \text{O}_2^- + \text{H}_2\text{O}_2 \rightarrow \cdot\text{OH} + \text{OH}^- + \text{O}_2 \] (Haber-weiss reaction)

\[ \text{O}_2^- + \text{Fe}^{3+} \leftrightarrow \text{Fe}^{2+} + \text{O}_2 \]

\[ \text{O}_2^- + \text{Cu}^{2+} \leftrightarrow \text{Cu}^{+} + \text{O}_2 \]

Ferrous (Fe\(^{2+}\)) iron and cuprous (Cu\(^+\)) copper are much more reactive with H\(_2\)O\(_2\) than their oxidized counterparts Ferric (Fe\(^{3+}\)) and cupric (Cu\(^{2+}\)), respectively. In the absence of metal catalysts, superoxide and H\(_2\)O\(_2\) also readily removed and are virtually harmless.

c. Hydroxyl radical: The reactive oxygen radicals are unstable and react readily with other groups or substances in the body, resulting in cell damage and hence human diseases (Halliwell and Gutteridge, 1989). Among the oxygen radicals specifically, the hydroxyl radical is the most reactive. It severely damages adjacent biomolecules such as all proteins, DNA, PUFA, nucleic acid, and almost any biological molecule it touches. This damage causes ageing, cancer and several diseases (Aruoma, 1998). Therefore, the removal of hydroxyl radical is probably one of the most effective defenses of a living body against various diseases.

d. DPPH: The DPPH radicals were widely used to investigate the scavenging activity of some natural compounds. In the DPPH assay, the antioxidants were able to reduce the stable radical DPPH to the yellow colored diphenyl-picrylhydrazine. The method
is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H by the reaction. With this method it is possible to determine the antiradical power of an antioxidant by measuring of a decrease in the absorbance of DPPH at 517 nm. Resulting a color change from purple to yellow, the absorbance decreased when the DPPH was scavenged by an antioxidant through donation of hydrogen to form a stable DPPH molecule. In the radical form, this molecule had an absorbance at 517 nm which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule (Visioli et al., 1998).

e. Hypochlorous acid: ROS production is important in inflammatory processes, allowing phagocytes to kill some bacterial strains. ROS produced in vivo by activated phagocytic cells include O$_2^-$, H$_2$O$_2$ and HOCl. Hypochlorous acid is produced by the oxidation of Cl$^-$ ions catalysed by neutrophil-derived myeloperoxidase, in the presence of H$_2$O$_2$ (Aruoma et al., 1989). Thiol groups are easily oxidized by HOCl (Ching et al., 1994). HOCl damages and induces target cell lysis, caused by sulphydryl oxidation in plasma membrane proteins (Cochrane, 1991), inactivates a$_1$-antiprotease, activates collagenase and gelatinase, depletes antioxidant vitamins such as ascorbic acid and inactivates antioxidant enzymes like catalase (Paya et al., 1992,).

f. Lipid Peroxidation: Lipid peroxidation is a complex process that occurs in aerobic cells and reflects the interaction between molecular oxygen and unsaturated fatty acids. This produces and propagates the Lipid radical (L°), uptake of O$_2$, generation of lipid alkoxyl (LO°), lipid peroxyl radicals (LOD°), rearrangement of double bonds, lipid hydroperoxide (LOOH) as well as a number of degradation products. Two paths are known for the formation of lipid peroxide in vivo. One occurs through autoxidation of compounds such as catecholamines, quinines, thiols and redox
reactions of myoglobin and oxyhaemoglobin, and the other from active oxygen by the action of NADPH oxidase, xanthine oxidase, catalase, superoxide dismutase and glutathione peroxide. The disturbance of balance between the formation of free radicals (lipid radicals) and antioxidants leads to oxidative stress. (Ciddi Veeresham and Kaleab Asres, 2005).

g. Nitric oxide: Nitric oxide (NO) an intracellular messenger, is produced from oxygen by various nitric oxide synthases.

\[
O_2 + \text{arginine} + \text{NADPH} \rightarrow \text{NO} + \text{Citrulline} + H_2O + \text{NADP}
\]

This free radical, by reacting with superoxide (another radical) produces the strong oxidant peroxynitrite (ONOO).

\[
O_2^- + \text{NO} \rightarrow \text{ONOO}^-
\]

h. Peroxyl and alkoxyl radicals (Yun-zhong Fang et al., 2002) Apart from oxygen free Radicals and other free Radicals are of importance of wide range of carbon-centered radicals \( R \), that arise from the attack of an oxidizing radical (Eg: \( \cdot \text{OH} \)) on a biological molecule \( RH \) such as lipid, nucleic acid, Carbohydrate or protein.

\[
RH + \cdot \text{OH} \rightarrow R^+ + H_2O
\]

These react very rapidly with oxygen to form the corresponding peroxyl radicals \( \text{ROO}^+ \)

\[
R^+ + O_2 \rightarrow \text{ROO}^+
\]

These in turn participate in reactions to generate alkoxyl radicals \( \text{RO}^- \).

i. Thiol radicals: Sulphur atoms can also be the centre for free radicals, eg: Thiol radicals \( \text{RS}^- \) are formed by the oxidation of glutathione.
Table.2: Selected human diseases/diseased states & free radicals implicated

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Disease/Diseased State</th>
<th>Free Radical Implicated</th>
<th>Damage inflicted</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Cancer (Dreher and Junod, 1996)</td>
<td>OH¹</td>
<td>Oxidative DNA damage</td>
</tr>
<tr>
<td>2.</td>
<td>Myocardial damage injury (Chan, 1996)</td>
<td>OFR</td>
<td>Myocardial reperfusion</td>
</tr>
<tr>
<td>3.</td>
<td>Atherosclerosis (Terasawa et al., 2000)</td>
<td>OFR</td>
<td>Oxidative modification of LDL</td>
</tr>
<tr>
<td>4.</td>
<td>Parkinson’s disease (Evans, 1992)</td>
<td>OFR</td>
<td>Mediates neuronal loss</td>
</tr>
<tr>
<td>5.</td>
<td>Alzheimer’s disease (Pedersen et al., 2001)</td>
<td>OFR</td>
<td>Promotes neuro-degenerative damage</td>
</tr>
<tr>
<td>6.</td>
<td>Ischemic hepatitis (Poli, 1992)</td>
<td>OFR</td>
<td>Acute lethal damage of hepatocytes</td>
</tr>
<tr>
<td>7.</td>
<td>Lung disease (Ryrfeldt et al., 1992)</td>
<td>ROS</td>
<td>Lipid peroxidation leading to lung fibrosis</td>
</tr>
</tbody>
</table>

OFR = Oxygen Free Radicals      ROS = Reactive Oxygen Species

1.2.14. Phytoconstituents with antioxidant activity:

In the present era natural products have a crucial role in therapeutics and human clinical trials. A number of plant constituents possessing antioxidant potential have been reported.
a. Phenols:

The vast majority of plant based aromatic natural products are phenols. Numerous categories of those compounds exists viz., simple phenols, phenyl propanoids, flavonoids, tannins and quinines.

b. Simple phenols:

Most of the simple phenols are mono numeric compounds of the polymeric polyphenols and acids which make up plant tissues, including lignin, melanin, flavanols and tannins. These individual components are obtained by acid hydrolysis of tissues.

c. Flavonoids:

Flavonoids are a large group of naturally occurring phenolic compounds ubiquitously distributed in the plant kingdom. They are the pigments responsible for the autumnal explosion of plant colors, and for the shades of yellow, orange and red in flowering plants. Most flavonoids occur naturally in conjugated form, mostly as glycosides but aglycones are also found. Flavonoids may directly scavenge some radical species by acting as chain breaking antioxidant such as tocopherols by donating a hydrogen atom to tocopherol radical. Transition minerals such as ferric and copper are important pro-oxidants and flavonoids. They chelate divalent metal ions, thus preventing free radicals formation.

The various classes of flavonoids differ in the level of oxidation of ring C of the basic benzo-y-pyrone structure (Peterson and Dwyer, 1998; Cotelle, 2001; Amic et al., 2003). Flavonoids are important components of the human diet. The intake of flavonoids can range between 50 and 800 mg/day, depending on the consumption of vegetables and fruits (Hollman and Katan, 1999; Yang et al., 2001; Lugasi et al.,
2003). Many studies have suggested that flavonoids exhibit biological activities, including antiallergenic, antiviral, anti-inflammatory, hepatoprotective, antioxidant, antithrombolic, vasodilating, antiviral and anticarcinogenic activities. However, most interest has been devoted to their antioxidant activity, which is due to their ability to reduce free radical formation and also to scavenge free radicals (Miller, 1996; Pietta, 2000; Majzisova and Kuchta, 2001; Knekt et al., 2002). Comprehensive accounts on the medicinal significance of flavonoids are provided by (Middleton et al., 2000, Narayana et al., 2001 Havsteen et al., 2002).

In very recent years, flavonoids as potent free radical scavengers have attracted a tremendous interest as possible therapeutics against free radical mediated diseases (Wall, 1992; Amic et al., 2003; Soobrattee et al., 2005). Flavonoids like many other polyphenols are excellent free radical scavengers (chain-breaking antioxidants) because they are highly reactive as hydrogen or electron donors (Cotelle, 2001; Kaur and Kapoor, 2001; Pannala et al., 2001; Yang, 2001; Blokhina et al., 2003). Various structure-activity relationship (SAR) studies of flavonoids have pointed to the importance of the number and location of the phenolic OH groups present, for effective radical scavenging activity (Van-Acker et al., 1998; Yokozawa et al., 1998; Middleton et al., 2000; Pannala et al., 2001; Chen et al., 2002; Heim et al., 2002; Amic et al., 2003). Despite a number of consistent lines of evidence supporting the roles of few specific structural components in their radical-scavenging activity (RSA), the correlation between flavonoids’ RSA and their chemical structures has stayed elusive (Cotelle, 2001; Amic et al., 2003). In addition, the explanation for the underlying molecular phenomena has not been adequate. One of the major reasons for these set-backs appears to be the strict adherence to classical concepts of SAR. The objective of this work is to establish the structural requirements of flavonoids for
appreciable RSA. An attempt has been made to design a consistent and comprehensive model for the RSA of flavonoids. A prediction mechanism for the radical scavenging potentials of flavonoids was established by first designing prediction mechanisms for the hydrogen donating capacity and the ease of termination of flavonoids radicals. To this end experimental data, the theoretically calculated relative change in energy ($\Delta H_f$) associated with the formation of various radicals from flavonoidal and other phenolic structures and also the spin density distribution in these radicals were considered. Ultimately, hydroxylation patterns anticipated to impart appreciable radical scavenging capability have been identified (Seyoum et al., 2006).

Some of the phytoconstituents isolated and reported having antioxidant activity are, Chebulagic acid-Termonslia catappa (Kinoshita et al., 2007), the following plants are reported to have antioxidant activity and shown in Table-3.
Table 3: Plants with Antioxidant Activity: (Ciddi Veeresham 2005)

<table>
<thead>
<tr>
<th>Plant name</th>
<th>Plant name</th>
<th>Part reported</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acacia catechu</em></td>
<td><em>Emblica officinalis</em>,</td>
<td>(Fruits),</td>
</tr>
<tr>
<td><em>Acanthus ilicifolius</em></td>
<td><em>Emilia sonchifolia</em></td>
<td>(Leaves),</td>
</tr>
<tr>
<td><em>Aconitum heterophylum</em></td>
<td><em>Fagopyrum</em></td>
<td>(Seeds),</td>
</tr>
<tr>
<td><em>Acorus calamus</em>,</td>
<td><em>esculentum</em></td>
<td>(Bark),</td>
</tr>
<tr>
<td><em>Alchornea laxiflora</em></td>
<td><em>Ficus bengalensis</em>,</td>
<td>(Root, Leaves, Trunk, Stem</td>
</tr>
<tr>
<td><em>Allium sativum</em></td>
<td><em>Garcinia atroviridis</em></td>
<td>Bark),</td>
</tr>
<tr>
<td><em>Allium vienale</em></td>
<td><em>Glycyrriza glabra</em></td>
<td>Roots</td>
</tr>
<tr>
<td><em>Aloe vera</em></td>
<td><em>Helichrysum</em></td>
<td>(Flowers),</td>
</tr>
<tr>
<td><em>Alpinia sp.</em></td>
<td><em>arenarium</em></td>
<td>(Entire plant),</td>
</tr>
<tr>
<td><em>Anthriscus cerefolium</em></td>
<td><em>Hemidesmus indicus</em></td>
<td>shoots</td>
</tr>
<tr>
<td></td>
<td><em>Hypericum</em></td>
<td></td>
</tr>
</tbody>
</table>

1.2.15. Flavonoids as anticancer agents:

Plant-derived flavonoids are a large group of naturally occurring phenylchromones found in fruits, vegetables, tea, and wine. The daily human intake in western countries of flavonoids was recently estimated to be about 23 mg/day (Hertog et al., 1993). However, this may be elevated to as much as 2-3/day with a high dietary intake of herbs and spices (Hertog et al., 1992). Flavonoids have been shown to have a wide range of biological activities, including antiallergic,
antibacterial, anti-inflammatory, antimutagenic, antioxidant, antiproliferative, antithrombotic, antiviral, and hepatoprotective effects (Bors and Saran, 1987; Robak and Gryglewski, 1988; Wall, 1992; Meddleton and Kandaswami, 1994).

Recently, there has been a tremendous increase in the number of studies on flavonoids as potential cancer chemopreventive agents. Certain flavonoids have been shown to interact in the genesis of cancer in both of the defined stages of initiation and promotion/progression (Ito and Imaida, 1992). For example, they have been shown to prevent carcinogens from reacting with cells at the initiation stage (Wattenberg and Leong, 1970) and to block the promotion stage by inhibiting ornithine decarboxylase (ODC) synthesis (Fujika et al., 1986). The major subclasses of flavonoids so far demonstrated with this type of biological activity include chalcones, flavanones, flavanols, flavones, flavonols, and isoflavones.

1.2.16. Free radicals and cancer:

The oxidants/free radicals are species with very short half-life, high reactivity and damaging activity towards macromolecules like proteins, DNA and lipids. These species may be either oxygen derived (ROS, Reactive oxygen species,) or nitrogen derived (RNS, Reactive nitrogen species). The oxygen-derived species include O₂ (super oxide), HO (Hydroxyl), HO₂ (Hydroperoxy), ROO (Peroxyl), RO (alkoxyl) as free radicals and H₂O₂ (Hydrogen peroxide), HOCL (Hypochlorous acid), O₃ (Ozone), and O₂ (Singlet oxygen) (Frei, 1994) as non-radicals. Similarly, nitrogen derived oxidant species are mainly NO (Nitric oxide), and ONOO (Peroxynitrite), NO₂ (Nitrogen dioxide) and N₂O₃ (Dinitrogen trioxide)(Evans and Halliwell, 1999).
a. Free radicals may be involved in metabolism required for the activation of procarcinogen.

b. Reactions involving metabolic activation and/or degradation of carcinogen may release free radicals that can attack DNA.

c. Metabolites of carcinogen may themselves become free radicals and may interact directly with DNA.

d. Following binding of ultimate carcinogen to DNA, DNA-carcinogen adducts may produce free radicals in vicinity of DNA (Athar, 2002).

1.2.17. Role of Oxygen Free Radicals in tumor promotion:

Following evidences support the involvement of free radicals in tumor promotion. A number of free radical-generating compounds are found to be tumor promoters in various animal model systems.

a. ROS generating systems can mimic the biochemical action of tumor promoters.

b. Some tumor promoters stimulate the production of ROS.

c. Tumor promoters modulate the cellular antioxidant defense systems.

d. Free radical scavengers, detoxifiers and antioxidants inhibit the process of tumor promotion (Salga et al., 1995; Athar 2002).

1.2.18. Incidence of Cancer:

The World Cancer Report documents that cancer rates are set to increase at an alarming rate globally. Cancer rates could increase by 50% new cases for the year 2020(WHO). This year, about 562,340 Americans (Table.4.) are expected to die of cancer, more than 1,500 people a day. Cancer is the second most common cause of
death in the US, exceeded only by heart disease. In the US, cancer accounts for nearly 1 of every 4 deaths.

Though the cancer incidence rate in India is less than that of the Western countries but due to the large population size, number of cases is more prevalent at any time (Nair and Sankaranarayanan, 1991). It is shown that in India 8.7 million Disease Adjusted Life Years lost from cancer was second to ischemic heart disease (Sarin, 2005; Marimuthu, 2008). The Cancer Atlas published by Indian Council of Medical Research (ICMR) indicates that the age adjusted incidence of gall bladder cancer in women in New Delhi is 10.6 per 100,000 population is the world's highest rate for women. From the same source it is reported that Thyroid cancer is more prevalent in the coastal areas of Kerala and Karnataka (ICMR, 2004).

From the Population-based cancer registry data of 1982-84 it is demonstrated that the total number of incident cases in males could have increased (from 0.29 million) to 0.43 million and in females the incident cases of cancer could have increased (from 0.32) to 0.42 million by 2001 (Murthy et al., 1990). The increasing trend of cancer incidence has forced the humanity to work more on the cancer prevention and treatments.
<table>
<thead>
<tr>
<th>Cancer</th>
<th>Estimated New Cases</th>
<th>Estimated Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Both Sexes</td>
<td>Male</td>
</tr>
<tr>
<td>Oral cavity &amp; pharynx</td>
<td>35,720</td>
<td>25,240</td>
</tr>
<tr>
<td>Digestive system</td>
<td>275,720</td>
<td>150,020</td>
</tr>
<tr>
<td>Respiratory system</td>
<td>236,990</td>
<td>129,710</td>
</tr>
<tr>
<td>Bones &amp; joints</td>
<td>2,570</td>
<td>1,430</td>
</tr>
<tr>
<td>Soft tissue (including heart)</td>
<td>10,660</td>
<td>5,780</td>
</tr>
<tr>
<td>Skin (excluding basal &amp; squamous)</td>
<td>74,610</td>
<td>42,920</td>
</tr>
<tr>
<td>Breast</td>
<td>194,280</td>
<td>1,910</td>
</tr>
<tr>
<td>Genital system</td>
<td>282,690</td>
<td>201,970</td>
</tr>
<tr>
<td>Urinary system</td>
<td>131,010</td>
<td>89,640</td>
</tr>
<tr>
<td>Eye &amp; orbit</td>
<td>2,350</td>
<td>1,200</td>
</tr>
<tr>
<td>Brain &amp; other nervous system</td>
<td>22,070</td>
<td>12,010</td>
</tr>
<tr>
<td>Endocrine system</td>
<td>39,330</td>
<td>11,070</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>74,490</td>
<td>40,630</td>
</tr>
<tr>
<td>Myeloma</td>
<td>20,580</td>
<td>11,680</td>
</tr>
<tr>
<td>Leukemia</td>
<td>44,790</td>
<td>25,630</td>
</tr>
</tbody>
</table>

* Rounded to the nearest 10; estimated new cases exclude basal and squamous cell skin cancers and in situ carcinomas except urinary bladder. About 62,280 female carcinoma in situ of the breast and 53,120 melanoma in situ will be newly diagnosed in 2009.

Source: Estimated new cases are based on 1995-2005 incidence rates from 41 states and the District of Columbia as reported by the North American Association of Central Cancer Registries (NAACCR), representing about 85% of the US population. Estimated deaths are based on data from US Mortality Data, 1969-2009. (National Center for Health Statistics, Centers for Disease Control and Prevention, 2009).
1.2.19. Underlying Cause in many types of cancer:

The underlying cause in many types of cancer seems to be free radical damage of the DNA of cells, triggering their altered behavior. Since Reactive Oxygen Radicals (ROR) play an important role in carcinogens, it would be important to develop a course of action to prevent these free radicals from causing damage. Intake of antioxidant nutrients and supplements provide protection against free radical activity, therefore antioxidants present in consumable fruits, vegetables, neutraceuticals and beverages have received considerable attention as cancer chemopreventive agents (Muktar et al., 1994). The balance between one's intake of antioxidants and exposure to free radicals may literally be the balance between life and death (Holford, 1997). Plants make protective compounds (phytochemicals) to protect themselves from radiation, insects and humans. Ingestion of plant rich diet confers the chemopreventive effect of phyto-chemical to man. Currently the value of taking antioxidants during chemotherapy is researched. Cancer itself creates oxidative stress and impairs antioxidant status in the organisms as whole.

1.2.20. Chemotherapy:

Some confusion exists in the use of the terms cytotoxicity, antineoplastic and anti-tumor. The National Cancer Institute (NCI) has defined these terms: cytotoxic refers to in vitro toxicity of tumor cells, which antineoplastic and anti-tumor should refer to in vivo activity in experimental systems (Ghisalberti, 1993). Chemotherapy is an effective treatment against cancers either singly or in combination with surgery and/or radiotherapy. In chemotherapy, drugs like cisplatin, carboplatin, oxorubucin, melphalan, mitomycin-C, gemcitabine, etc have been used for the treatment of cancers. (Rosangkima and Prasad, 2004). Various classes promising cancer chemopreventive agents have been listed in Fig.1.
Fig. 1: Promising cancer chemo-preventive agents.

Anticancer drugs

Act on DNA

Damage DNA

Inhibitors of Chromatin Function

Inhibit Synthesis

Topoisomerase Inhibitors

Microtubule Inhibitors

Act on Steroid Hormone Receptors

Agonists

Antagonists

DNA-binding Drugs

Antimetabolites

Alkylation

Free radicals/Intercalation

1.2.21. Plant compounds role in the treatment of cancer:

Conventional anticancer drugs are derived to arrest and kill rapidly dividing cancer cells. However, therapeutic efficacies of most of them are limited due to the development of various side effects in the host and/or the acquired drug resistance by the cancer cells. In an attempt to abate these side effects and better remedy against various malignancies, many plant derivatives have been used with varying success. Between 1955 & 1982, NCI screened 35,000 plant species representing 1551 genera comprising 114000 extracts for in vitro cytotoxicity and in vivo activity against various animal tumor systems (Hamburger et al., 1991). Estimates indicate that there are approximately 2,50,000 terrestrial species of higher plants. Existing in vivo test
systems, for example, xenograft on immunodeficient mice, are far too slow, complex, expensive and probably immoral to be used as a mass screen. Using cells derived from human cancers is an in vitro setting, on the other hand, is quite compatible with the desired goal (Lednicer and Narayan, 1993). Plant constituents able to kill cancer cells, and hence described as being cytotoxic exhibit a very large range of structural types. (Cordell et al., 1993).

1.2.22. Plant-Derived Anti-Cancer Agents in Clinical Use:

The first agents to advance into clinical use were the so-called vinca alkaloids, vinblastine (VLB) and vincristine (VCR), isolated from the Madagascar periwinkle, *Catharanthus roseus* (Apocynaceae), which was used by various cultures for the treatment of diabetes (Guritte and Fahy, 2005). More recent semi-synthetic analogues of these agents are vinorelbine (VRLB) and vindesine (VDS). These agents are primarily used in combination with other cancer chemotherapeutic drugs for the treatment of a variety of cancers. VLB is used for the treatment of leukemias, lymphomas, advanced testicular cancer, breast and lung cancers, and Kaposi’s sarcoma, and VCR, in addition to the treatment of lymphomas, also shows efficacy against leukemias, particularly acute lymphocytic leukemia in childhood. VRLB has shown activity against non-small-cell lung cancer and advanced breast cancer (Cragg and Newman, 2005).

The two clinically-active agents, etoposide (VM 26) and teniposide (VP 16-213), which are semi-synthetic derivatives of the natural product, epipodophyllotoxin (an isomer of podophyllotoxin) (Fig.2), may be considered as being more closely linked to a plant originally used for the treatment of "cancer" (Lee and Xiao, 2005).
A more recent addition to the armamentarium of plant-derived chemotherapeutic agents is the class of molecules called taxanes (Kingston, 2005). Paclitaxel (taxol) initially was isolated from the bark of *Taxus brevifolia* Nutt. (Taxaceae). Docetaxel is primarily used in the treatment of breast cancer and non-small cell lung cancer (NSCLC). In addition, of 2069 cancer clinical trials recorded by the NCI as being in progress as of July 2004, 248 or close to 12% are listed as involving taxane-derived drugs, including 134 with paclitaxel (Taxol), 105 with docetaxel (Taxotere), and 10 with miscellaneous taxanes, either as single agents or in combination with other anti-cancer agents (Cragg and Newman, 2005).

Another important addition to the anti-cancer drug armamentarium is the class of clinically-active agents derived from camptothecin, which is isolated from the Chinese ornamental tree, *Camptotheca acuminata* Decne (Rahir et al., 2005). Topotecan (Hycamtin) and Irinotecan (CPT-11; Camptosar), are now in clinical use. Topotecan is used for the treatment of ovarian and small-cell lung cancers, while Irinotecan is used for the treatment of colorectal cancers.

### 1.2.23. Plant-Derived Anticancer Agents in Clinical Development:

The flavone, flavopiridol (Fig.3) is totally synthetic, but the basis for its novel structure is a natural product, rohitukine, in the early 1990s from *Dysoxylum binectariferum* (Meliaceae), which is phylogenetically related to the Ayurvedic plant, *D. malabaricum* Bedd. Used for rheumatoid arthritis. A total synthesis was undertaken, and one of the over 100 analogues synthesized during structure-activity studies was flavopiridol, which was found to possess tyrosine kinase activity and potent growth inhibitory activity against a series of breast and lung carcinoma cell lines (Sausville et al., 1999). It also showed broad spectrum in vivo activity against
human tumor xenografts in mice, and this led to its selection for preclinical and clinical studies by the NCI in collaboration with Hoechst. It is currently in Phase I and Phase II clinical trials, either alone or in combination with other anti-cancer agents, against a broad range of tumors, including leukemias, lymphomas and solid tumors (Cragg and Newman, 2005).

The combretastatins were isolated from the South African “bush willow”, *Combretum caffrum* (Pinney et al., 2005). A water-soluble analogue, combretastatin A-4 phosphate (CA4), has shown promise in early clinical trials. The number of combretastatin (CA4) mimics being developed. Three are in clinical trials, while 11 are in preclinical development. This chemical class has served as a model for the synthesis of a host of analogues containing the essential trimethoxy aryl moiety linked to substituted aromatic moieties through a variety of two or three atom bridges including heterocyclic rings and sulfonamides. This is an impressive display of the power of a relatively simple natural product structure to spawn a prolific output of medicinal and combinatorial chemistry (Li and Sham, 2002).

An interesting agent in early clinical development is roscovitine which is derived from olomucine, originally isolated from the cotyledons of the radish, *Raphanus sativus* L. (Brassicaceae) (Meijer and Raymond, 2003). Chemical modification of olomucine resulted in the more potent inhibitor, roscovitine, which is in clinical development under the code CYC202 by Cyclacel in Dundee, Scotland, and currently is in Phase II clinical trials in Europe. Further development of this series, following synthesis of a focused library via combinatorial chemistry techniques, has led to the purvalanols which were even more potent, and are in preclinical development (Chang et al., 1999).
Fig. 2: Plant-Derived Anti-Cancer Agents in Clinical Use.

Camptothecin

Topotecan

Irinotecan

Taxol

Docetaxel

Vinblastine; R = CH₃
Vincristine; R = CHO

Podophyllotoxin

Etoposide

Teniposide

Elliptinium

Homoharringtonine
1.2.24. Plant-Derived Antitumor Agents in Preclinical Development:

A number of naturally-derived agents were entered into clinical trials and were terminated due to lack of efficacy or unacceptable toxicity. The case of maytansine illustrates how the emergence of novel technologies can revive interest in these "older" agents. It is also worth remembering that the development of effective drugs, such as paclitaxel (taxol) and the camptothecin derivatives, topotecan and irinotecan required 20 to 30 years of dedicated research and patience, and considerable resources, to ultimately prove their efficacy as clinical agents.

Another example of an "old" drug is bruceantin (Fig.4) which was first isolated from a tree, *Brucea antidysenterica* J. F. Mill. (Simaroubaceae), used in Ethiopia for the treatment of "cancer" (Cuendet and Pezzuto, 2004). Interest has been revived by the observation of significant activity against panels of leukemia, lymphoma and myeloma cell lines, as well as in animal models bearing early and advanced stages of the same cancers. This activity has been associated with the down-regulation of a key oncoprotein (*c-myc*), and these data are being presented as strong evidence supporting the development of bruceantin as an agent for the treatment of hematological malignancies (Cragg and Newman, 2005).
The pervilleines isolated from the Madagascar plant, *Erythroxylum pervillei* Baillon (Erythroxylaceae), have shown promising MDR activity both *in vitro* and *in vivo*, and pervilleine A is currently in preclinical development (Mi *et al.*, 2003).

**Fig.4: Plant-Derived Antitumor Agents in Preclinical Development.**

![Chemical structures](image)

1.3. **Pharmacological Properties of Anticancer Drugs:**

Prostaglandins (PGs) have been shown to mediate the inflammatory response locally and modulate a variety of physiological responses systematically (Davies *et al.*, 1994). The conversion of arachidonic acid to prostaglandins is mediated by the two step action of prostaglandin H synthase (PGHS), commonly known as cyclooxygenase (COX). The first committed step in this process is the oxidative cyclisation of arachidonic acid to PGG$_2$, which is followed by peroxide reduction to
PGH₂ at the second distinct binding site. The moieties are then converted to PGD₂, PGE₃, PGF₂, and PG₁₂ or thromboxanes (TBX). The specific prostaglandin produced is determined in part, by the particular cell type under consideration. In the past it was thought that COX was a single enzyme present constitutively in most cells. This led to the widely held notion that inhibition of cyclooxygenase would unavoidably lead to both beneficial and detrimental effects. However, recently it was observed that COX activity dramatically increased in inflammatory states and that cellular COX activity could be induced by inflammatory cytokines and endotoxins. This suggested that a second form COX existed, an inducible form (COX-2), which is expressed during inflammatory conditions. The constitutive form (COX-1) produces physiologically important PG’s and is present in tissues such as the gastrointestinal tract and kidney. Despite recognition of cyclooxygenase’s mechanism of action thirty years ago, the enzyme, COX-1 was first cloned only in 1988. The second isoform, COX-2 was reported three years later. Both the isozymes have about the same affinity (Kₘ) and capacity (Vₘₐₓ) to convert arachidonic acid to PGH₂, COX-2 is able to metabolize C₁₈ and C₂₀:₃ fatty acids, additionally. Protective PGs that preserve the integrity of the stomach lining and maintain normal renal function in a compromised kidney are synthesized by COX-1. It is also constitutively expressed in cultured endothelial and vascular smooth-muscle cells. Although, COX-1 is constitutively expressed playing a housekeeping role, in many tissues under basal conditions, its expression may also be regulated. Conversely, COX-2 was thought to be an inducible enzyme, generally not present (or minimally so) in most tissues. Rather, its expression is considered to be associated with an inflammatory response and other pathophysiological states. More recent investigations, however, reveal that COX-2 plays a key role in a wide range of physiological processes including organogenesis, brain and nerve function,
reproduction, bone metabolism, salt and water handling, rennin release, angiogenesis and apoptosis. It is present constitutively in the brain and the spinal cord, where it may be involved in nerve transmission, particularly for pain and fever. PGs made by COX-2 are also important in ovulation and in the birth process (Ebehart et al., 1994). Thus the dichotomy of a constitutive COX-1, with solely “house-keeping” functions under physiological conditions, and an inducible-COX-2, accounting for prostanoid production in disease states, is an over simplification of biological reality.

During the past few years, selective inhibitors of COX-2 enzyme have emerged as important pharmacological tools for the treatment of pain and inflammation. But the recent findings of the involvement of COX-2 enzyme in some key physiological functions, as described above, have complicated the scene regarding the use of selective /specific COX-2 inhibitors as anti-inflammatory agents. At the same time, these findings have opened new doors for the treatment of certain other ailments as well. Further, higher concentrations of COX-2 have been implicated in several other disease states other than the inflammation, such as colonic polyposis (Ebehart et al., 1994), various forms of cancer (Tsujii et al., 1997), Alzheimer’s disease and vascular retinosis following angioplasty. Prevalence of higher concentrations of COX-2 enzyme than the normal in these diseases states has opened avenues for the possible therapeutic mitigation of these diseases. Involvement of COX-2 expression in these diseases and the role COX-2 inhibitors could play as potential therapeutic agents in future.

1.3.1. COX-2 in Cancer:

Several lines of evidence suggest that COX-2 enzyme plays an important role in carcinogenesis. Increased amounts of COX-2 are commonly found in both pre-
malignant tissues and malignant cancers of the head and neck, esophagus and lung (Wilson et al., 1998, Wolff et al., 1998). COX-2 expression is induced in a variety of cells leading to high levels of prostaglandin production. Such aberrant expression of COX-2 has been reported in murine and human breast cancer, human colon cancer, lung cancer, head and neck cancer and pancreatic cancer (Tsujii et al., 1997). There is extensive evidence, beyond the findings that COX-2 is commonly over expressed in tumors, to suggest that COX-2 is mechanistically linked to the development of cancer. COX-2 can affect multiple mechanisms that are important in carcinogenesis. It may aid in carcinogenesis, by altering the normal cellular processes like angiogenesis, cell proliferation, immunomodulation, carcinogenic metabolism and apoptosis.

Angiogenesis is essential for the proper nourishment and oxygen supply to highly dividing and proliferating cells. Normal blood vessel endothelium expresses COX-1 isozyme whereas angiogenic blood vessel endothelium expresses COX-2 isozyme. COX-2 and thromboxane A2 receptor dependent signaling pathways have been shown to activate cellular invasion and angiogenesis. Recently, levels of COX-2 were found to correlate with both VEGF expression and tumor vascularisation in head and neck cancers. This finding in human tissues is consistent with prior evidence that over expression of COX-2 in epithelial tissues leads enhanced production of vascular growth factors and the formation of capillary like networks. Evidence has been provided that COX-2 derived prostaglandins contribute to tumor growth (Maferrer et al., 2000) by inducing newly formed blood vessels (neo angiogenesis) that sustain tumor cell viability and growth (Wolff et al., 1998). Prostaglandins appears to increase cell proliferation with the help of biological modifiers like polyamines. Increased polyamine levels are associated with DNA synthesis that results from ornithine decarboxylase activity. Over expression of COX-2 in colorectal tumor cell
lines caca-2 results in increased metastatic potential (Tsujii et al., 1997). The growth of tumors typically is associated with immune suppression. Colony stimulating factors released by tumor cells activate monocytes and macrophages to synthesize PGE₂ that inhibits the production of immune lymphokines, T and B cell proliferation and the cytotoxic activity of natural killer cells. PGE₂ also inhibits the production of tumor-necrosis factor while inducing the production of IL-10, which has immunosuppressive effects. High concentrations of certain prostaglandins like PGE₂ attenuate host’s immune response preventing the killing of malignant cells. PGE₂ shows a potent immunosuppressive effect by acting as a negative feedback inhibitor, thereby inhibiting T cell activity, lymphocytic mitogenesis, macrophage activity, antibody production, production of cytokines by immune cells and natural killer cell activity.

COX-2 inhibitors, when given immediately after tumor transplant in animal models decrease PGE₂ levels and tumor growth. The peroxidase component of COX isozymes has broad specificity and can oxidize a variety of xenobiotics including certain procarcinogens and carcinogens. Classes of compounds like aflatoxins, halogenated pesticides, polycyclic hydrocarbons, heterocyclic amines etc. are acted upon by the peroxidase component of COX to form mutagenic carcinogens. The byproducts of the oxidation of arachidonic acid like malondialdehyde, are highly reactive and form adducts with DNA that may initiate cancer. COX inhibitors along with antioxidants may play a role to protect the cells and DNA from the damage (Chinery et al., 1998).

Overproduction of prostaglandins like PGE₂ by COX-2 may also send improper signals in the cells thereby stimulating cell growth inappropriately or reducing apoptosis. Bcl-2 gene is an important gene in regulating apoptosis. Murine intestinal cell lines like RIE-S showed that over expression of COX-2 in cancerous cells was associated with the over expression of Bcl-2 gene, which prolongs cell’s life by
inhibiting apoptosis. The exact relationship between COX-2 over expression, production of prostaglandins and expression of Bcl-2 gene in cancer is yet to be investigated. Inhibition of COX isozymes results in decreased production of PGs from their substrates like arachidonic acid, leading to the accumulation of the substrate. Arachidonic acid when present in increased concentration in the cells is supposed to stimulate the fragmentation of DNA and conversion of sphingomyelin to ceramide in the cells, which is a known inducer of apoptosis.

Selective COX-2 inhibitors have the desired property of interfering with tumorigenesis in experimental systems. Inhibition of COX-2 by celecoxib delays tumor growth and metastasis in xenograft tumor models as well as suppresses basic fibroblast growth factor 2 (FGF-2)-induced neovascularisation of the rodent cornea (Masfeerer et al., 2000).
1.4. *Fluggea leucopyrus* linn:

1.4.1. Past work on the plant *F. leucopyrus*:

An up-to-date literature survey has been carried out on *F. leucopyrus* and described their phytoconstituents and biological properties as below:

1.4.2. List of *F. leucopyrus* species: (www.ipni.org).

- *Securinega leucopyrus* (Willd.) Mull. Willd.
- *Securinega ramiflora* Mull.
- *Securinega suffruticosa* (Pall.) (Pall.) Baill.
- *Securinega tinctoria* (L.) Rothm.. Webster
- *Securinega virosa* (Roxb. ex Willd.) Baill.

1.4.3. Botanical description: (*Wealth of India, 1952*).

*F. leucopyrus*, a large, rigid, thorny bush with straggling branches, usually ending in sharp thorns; leaves simple, alternate, ovate or obcordate or elliptic, reticulately veined beneath; flowers very small, male greenish yellow in cluster, female reddish, also in clusters; fruits globose, white fleshy berries. A thorny, dioecious woody shrub or a small tree, with white branchlets usually ending in sharp thorns, distributed from Rajasthan to Punjab and Uttar Pradesh, and in peninsular India, ascending up to an altitude of 1500m. It is also grown for hedges. Leaves obvate, elliptic or obcordate; flowers very small, male greenish clusters; female in red-flowered clusters; berries small, fleshy, globose, white, and smooth.
1.4.4. Taxonomical Classification *F. leucopyrus* (www.ipni.org).

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Malpighiales

Family: Phyllanthaceae

Tribe: Phyllantheae

Subtribe: Securineginae

Genus: Securinega


Eng: Spinous fluggea

Hin: Hartto, Ainta

Kan: Bilculi, Gudahale, Huli gida

Mal: Ampuripaccila, mulpulanni

San: Panduraphalli, bhuriphalli

Tam: Mullupulatti, Vellaippulanci

Tel: Tellapulisara, pulugudu, puli, caiamunta
Fig. 5. Fluggea leucopyrus plant.
1.4.6. Morphological characters *F. leucopyrus* (*Wealth of India, 1952*).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>simple, alternate, ovate or obcordate or elliptic, reticulately veined</td>
</tr>
<tr>
<td>Leaf blade</td>
<td>Obvate, elliptic or obcordate.</td>
</tr>
<tr>
<td>Size</td>
<td>20cm long, 5cm wide.</td>
</tr>
<tr>
<td>Apex</td>
<td>Acute to acuminate.</td>
</tr>
<tr>
<td>Flowers</td>
<td>Very small, male greenish yellow in cluster, female reddish, also in Clusters.</td>
</tr>
<tr>
<td>Fruits</td>
<td>Capsule or berry.</td>
</tr>
<tr>
<td>Seeds</td>
<td>Numerous, very small, ellipsoid and compressed.</td>
</tr>
</tbody>
</table>
1.5. References:


Duh PD, Tu YY and Yen GC. Antioxidant activity of water extract of harnngiyur (Chrysanthemum morifolium Ramat) Lebens Wiss Technol 1999; 32: 269-277.


Fridovich I. superoxidae dismustase; a contaminant of Bovine catalase Biochem j.1969; 135,-379-381.


Kozarich JW, Stubbe J. Mechanism of bleomycin: evidence for a rate-determining $4^\pi$-hydrogen abstraction from poly (dA-dU) associated with the formation of both free base and free propane, *Biochem* 1985; 24: 7562-7568.


