1. INTRODUCTION
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1.1 Plant based medicine

Fossil records date human use of plants as medicines at least to the middle Paleolithic age, some 60,000 years ago (Solecki and Shanidar, 1975). Human existence on earth has been made possible because of the vital role played by the plant kingdom in sustaining human life. Evidence on the effectiveness of plant in diagnosis, cure and prevention of disease states exists in every culture throughout the world. Through trial and error, early mankind has found medicinal properties in the seeds, leaves, barks and roots of plants. Medicines have come from various sources including terrestrial plants, terrestrial microorganisms, marine organisms and terrestrial vertebrates and invertebrates (Newman et al., 2000).

The traditional definition of medicinal plants is given in Ashtaanga Hrdaya (600 AD) Sutara sthana as: ‘jagatyevam anoushadham na kinchit vidyate dravyam vashaannaarthayagayoh’. “There is nothing in the universe, which is non medicinal, which cannot be made use of for many purposes and by many modes”. This definition rightly suggests that “in principle” all plants have a potential value, although in practice a plant is referred to as medicinal, when it is so used by some system of medicine. There is evidence since early Vedic period (Atharva Veda) of plants being used for a wide range of medicinal purposes. They have in fact been used in a continuous unbroken tradition for over four millennia. Medicinal plants use is still a living tradition. This is borne out by the fact that there exists around a million traditional, village based carriers of herbal medicine tradition in the form of traditional birth attendants, visha vaidyas, bone-setters, herbal healers and wondering monks. Apart from these specialized carriers, there are millions of women and elders who have a traditional knowledge of herbal home remedies and of food and nutrition.
Indian people had an incredible knowledge of plant based medicine, driven apparently by a tremendous passion for the study of medicinal plants. This is evident both in the living folk traditions in the rural communities as well as the scholarly traditions of the codified knowledge systems i.e. Ayurveda, Siddha, Unani and Tibetan. Indians obviously care for medicinal plants because they know so much about them and have done so much work on their applications. Probably, no other medical culture in the world has so extensive, detailed and deep in understanding about the medicinal value of the plants. The use of plants for medicine still vastly exceeds the use of modern synthetic drugs. The WHO estimates that 65-80% of the world’s population use traditional medicine as their primary form of healthcare and about 85% of traditional medicine involves the use of plant extracts. These medicines obtained from indigenous plants would be of immense benefit especially to inhabitants of developing countries, since the cost of these drugs would be within their means. Moreover, a drug prescribed by traditional medical practitioner would be more acceptable to the rural people in addition to being freely available.

It might have taken 5000 years, but the West has finally woken up to the possibilities of herbal medicines. The renewed interest in non-orthodox medicine in the West has dramatically increased the level of interest in plants with potential health benefits and fueled the sharply escalating commercial demand for herbal products in global markets. Presently, the global market is worth $180 billion a year. *Nutrition Business Journal* (NBJ) estimated sales in the mainstream market channel increased 6.6 percent in 2010 over 2009 sales to a level of a total estimated $936 million. Sales in the natural and health foods channel grew by about 2 percent in 2010 to $1.663 billion. The five top-selling single herbal supplements of 2010 in the natural and health foods channel, according to SPINS, were flaxseed and/or oil (*Linum usitatissimum*), grass (wheat or barley; *Triticum aestivum* or *Hordeum vulgare*, respectively), aloe (*Aloe vera*), turmeric (*Curcuma longa*)
and stevia (*Stevia rebaudiana*). The top-selling herbal singles of 2010 in the food, drug and mass market channel, according to IRI, were cranberry (*Vaccinium macrocarpon*), saw palmetto (*Serenoa repens*), soy (*Glycine max*), garlic (*Allium sativum*) and ginkgo (*Ginkgo biloba*). These rankings do not include combinations containing multiple herbs.

"Despite the general economic downturn, consumers continue to demonstrate their interest in and demand for natural ways to improve their health," said *Herbal Gram* Editor Mark Blumenthal. "These 2010 sales increase for herbal supplements tracks with strong demand in 2009, where the sales increased 4.8 percent over the previous year, even during the depths of the recession." The current global market for medicinal plants is growing annually at the rate of 7%. European share is nearly half of the total. Germany dominates the European trade ranking 3rd after Hong Kong and Japan as a world consumer. The Malaysian natural product industry is growing with an annual growth rate of 20%. According to Ayurvedic Drug Manufacturer’s Association (ADMA), the current of trade in Indian Systems of Medicine is around Rs. 4,205 crores, roughly close to US $1 billion.

All these data provide proof for the dramatic increase in the level of interest in plants and a virtual rediscovery of herbal medicines. In the past several drugs have been discovered from the medicinal plants. Interest in plants based drug discovery and development programs in developed countries, however, waned continuously through the 1960s, 1970s, and early 1980s (Farnsworth, 1988). This decline may be due to downward trend in prescribing medicinal plants and the very small percentage of newly approved drugs derived from higher plants in these countries. Hence, the subject of pharmacognosy has been curtailed or even removed from the curricula in pharmaceutical education in some western countries (Philipson and Anderson, 1989). Later public interest in medicinal plants grew exponentially (Tyler et al., 1988).
1.2 Cancer incidence and causes

Cancer is a disease of worldwide importance. Its incidence in the developed countries is rising and its mortality occupies second rank in the order of cause for death. About 12.7 million cancer cases and 7.6 million cancer deaths are estimated to have occurred in 2008 worldwide, with 56% of the cases and 64% of the deaths in the economically developing world. Breast cancer in females and lung cancer in males are the most frequently diagnosed cancers and the leading cause of cancer death for each sex in both economically developed and developing countries, except lung cancer is preceded by prostate cancer as the most frequent cancer among males in economically developed countries. These cancers were followed, without specific rank order, by stomach and liver cancers in males and cervix and lung cancers in females in economically developing countries and by colorectal and lung cancers in females and colorectal and lung or prostate cancers in males in the economically developed world.

The gradual improvement in the life expectancy is also associated with an elevated cancer incidence and mortality. Once considered a mysterious disease, cancer, however, has been eventually revealed to investigators (Trichopoulos et al., 1996). Disease development begins from a genetic alteration (mutation) of a cell within a tissue. This mutation allows the cell to proliferate at a very high rate and finally form a group of fast reproducing cells with an otherwise normal appearance (hyperplasia). Rarely, some hyperplastic cells will mutate again and produce abnormally looking descendants (dysplasia). Further mutations of dysplastic cells will eventually lead to the formation of a tumor, which can either remain localized at its place of origin, or invade neighbouring tissues (malignant tumor) and establish new tumors (metastases).
Cancer cells have some unique properties that help them to compete successfully against normal cells:

- Under appropriate conditions cancer cells are capable of dividing almost infinitely. Normal cells have a limited life span. For example, human epithelial cells cultured in vitro are commonly capable of sustaining division for no more than 50 times (Hayflick and Moorhead, 1961).

- Normal cells adhere both to one another and to the extracellular matrix, the insoluble protein mesh that fills the space between cells. Cancer cells fail to adhere and, in addition, they possess the ability to migrate from the site where they began, invading nearby tissues and forming masses at distant sites in the body, via the bloodstream. This process is known as metastasis and examples include melanoma cells migrating to the lung, colorectal cancer cells to the liver and prostate cancer cells to bone. Although metastatic cells are indeed a small percentage of the total of cancer cells (e.g. $10^{-4}$ or 0.0001%), tumors composed of such malignant cells become more and more aggressive over time.

In a general sense, cancer arises due to specific effects of environmental factors (such as smoking or diet) on a certain genetic background. In the hormonally related cancers like breast and prostate cancer, genetics seem to be a much more powerful factor than lifestyle.

Two gene classes play major roles in cancer. Proto-oncogenes encourage growth, whereas tumor suppressor genes inhibit it. The coordinated action of these two gene classes normally prevents cells from uncontrolled proliferation; however, when mutated, oncogenes promote excessive cell division, while inactivated tumor suppressor genes fail to block the division mechanism. On a molecular level, control of cell division is maintained by the inhibitory action of various molecules, such as pRB, p15, p16, p21 and
p53 on proteins promoting cell division, essentially the complex between cyclins and cyclin-dependent kinases (CDKs) (Meijer et al., 1997). Under normal conditions, deregulation of the cell control mechanism leads to cellular suicide, the so-called apoptosis or programmed cell death. Cell death may also result from the gradual shortening of telomeres, the DNA segments at the ends of chromosomes. Most tumor cells, however, manage to preserve telomere length due to the presence of the enzyme telomerase, which is absent in normal cells.

Some oncogenes force cells to overproduce growth factors, such as the platelet-derived growth factor and the transforming growth factor alpha (sarcomas and gliomas). Alternatively, oncogenes such as the ras genes distort parts of the signal cascade within the cell (carcinoma of the colon, pancreas and lung) or alter the activity of transcription factors in the nucleus. In addition, suppressor factors may be disabled upon infection with viruses (e.g. a human papillomavirus).

Tumor development is a step-wise process in that it requires an accumulation of mutations in a number of these genes. Altered forms of other classes of genes may also participate in the creation of a malignancy, particularly in enabling the emergence of metastatic cancer forms.

Environmental causes of cancer comprise an extremely diverse group of factors that may act as carcinogens, either by mutating genes or by promoting abnormal cell proliferation (Sugimura, 1986; Wakabayashi et al., 1987; Greenwald, 1996). Most of these agents have been identified through epidemiological studies, although the exact nature of their activity on a biological level remains obscure. These factors include chemical substances (such as tobacco, asbestos, industrial waste and pesticides), diet (saturated fat, red meat, overweight), ionizing radiation, pathogens (such as the Epstein–Barr virus, the hepatitis B or C virus, papillomaviruses and Helicobacter pylori). In order for environmental factors
to have a significant effect, however, one must be exposed to them for a relatively long time. Cancer may also arise, or worsen, as a result of physiological stress. For example, a recent large-scale study in Israel demonstrated that survival rates declined for patients having lost at least one child in war (Anonymous, 2000).

Cancer may affect people of all ages, but risk tends to increase with age, due to the fact that DNA damage becomes more apparent in aging DNA. Statistics indicate that men are largely plagued by lung, colon, rectum, and prostate cancer, whilst women increasingly suffer from breast, colon, rectal, and stomach cancer. Despite many therapeutic advances in the understanding of the processes in carcinogenesis, overall mortality statistics are unlikely to change until, it is believed, there is a reorientation of the concepts for the use of natural products as new chemopreventive agents (Abdulla and Gurber, 2000).

It is believed that malignancy will be soon a global problem with its entire consecutive burden. Cancer therapy is, therefore, in the focus the world over. For the time being the treatment of any malignancy is based on surgery, radiotherapy and drug therapy. This complex approach is capable of curing approximately half of the cancer patients. while the other half of the affected individuals may have only prolonged survival or even no benefit at all from the treatment. While the results obtained by surgery and radiotherapy (which are locoregional interventions) are close to their maximum accomplishment, success of drug therapy, the only systemic approach, is far from satisfactory (Sandor, 2002)

1.3 Free radicals and their role in disease

A free radical is an atom or a molecule that contains one or more unpaired electrons (Halliwell and Gutteridge, 1989). Unpaired electrons alter the chemical reactivity of an atom or molecule and usually make it more reactive than the corresponding non-radical. The actual chemical reactivity of free radicals, however, varies enormously. The
hydrogen radical, which contains 1 proton and 1 electron (therefore unpaired), is the simplest free radical.

Free radicals in the body are generated by multiple mechanisms and are often initiated by removal of an H atom from other molecules (during lipid peroxidation). Living organisms are exposed to electromagnetic radiation from the environment, both natural (i.e. radon and cosmic radiation) and from man made sources. Low wavelength electromagnetic radiation (i.e. gamma rays) can split water in the body to generate hydroxyl radicals(OH). Hydroxyl radical has a very short in vivo half-life, reacting at its site of formation, usually leaving behind a legacy of free radical chain reactions (Halliwell and Gutteridge, 1989).

The body, through metabolic process, makes an oxygen radical called superoxide (\(\text{O}_2\)), where the unpaired electron is located on oxygen. Superoxide is made by adding one electron to the oxygen molecule, and is generally poorly reactive (Halliwell and Gutteridge, 1989; Liochev and Fridovich, 1994). Many molecules in the body react directly with oxygen to make superoxide, including the catecholamines, tetrahydrofolate, and some constituents of mitochondrial and other electron-transport chains (Halliwell and Gutteridge, 1989; Liochev and Fridovich, 1994). Even when this mode of superoxide generation is not available, activated phagocytes (neutrophils, monocytes, macrophages, eosinophils) generate large amounts of superoxide as part of the mechanism by which foreign organisms are killed (Babior and Woodman, 1990). During chronic inflammation, this normal protective mechanism may become damaging.

Another physiological free radical is nitric oxide (NO), which is made by vascular endothelium as a relaxing factor (Moncada and Higgs, 1993). Nitric oxide has many useful physiological functions, but excess nitric oxide can be toxic (Eiserich et al., 1994; Moncada and Higgs, 1993).
Neither superoxide nor nitric oxide is highly reactive chemically, but under certain circumstances they can generate more reactive toxic products (Eiserich et al., 1998; Halliwell and Gutteridge, 1989). These and other reactive species are listed in Table 1.1

**Table 1.1: Reactive oxygen and nitrogen species of physiological importance**

<table>
<thead>
<tr>
<th>Reactive oxygen species (radicals):</th>
<th>Molecular oxygen (O₂), Superoxide ((\cdot O_2)), Peroxyl (RO₂), Hydroperoxyl (HO₂), Alkoxy (RO), Hydroxyl (OH).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive oxygen species (non radicals):</td>
<td>Singlet oxygen ((^1)O₂), Hydrogen peroxide (H₂O₂), Hypochlorous acid (HOCl), Ozone (O₃).</td>
</tr>
<tr>
<td>Reactive nitrogen species (radicals):</td>
<td>Nitric oxide (NO), Nitrogen dioxide (NO₂)</td>
</tr>
<tr>
<td>Reactive nitrogen species (non radicals):</td>
<td>Nitrous acid (HNO₂), Dinitrogen tetraoxide (N₂O₄), Dinitrogen trioxide (N₂O₃), Peroxynitrite (ONOO), Peroxynitrous acid (ONOOH), Nitronium cation (NO₂⁺), Alkyl peroxynitrates (ROONO).</td>
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When oxygen is reduced in the electron transport chain, oxygen-derived free radical intermediates are formed. The O₂ and H₂O₂ intermediates can escape from the system, and in the presence of transition metal ions (i.e. iron and copper) form the more reactive hydroxyl radicals (Halliwell and Gutteridge, 1989). While O₂ radicals are toxic to cells, the high reactivity of OH and O₂ renders these activated forms most cytotoxic due to deleterious peroxidation reactions with lipids, proteins and DNA. Lipid peroxidation is an example of this oxidative damage (Gutteridge, 1995). Free radicals may attack
polyunsaturated fatty acids (PUFA) within membranes, forming peroxyl radicals. These newly formed free radicals can then attack adjacent fatty acids within membranes causing a chain reaction of lipid peroxidation. The lipid hydroperoxide end products are also harmful, and may be responsible for some of the overall effect, which can lead to tissue and organ damage.

Oxidative stress is exerted by all peroxides, which can damage cells and tissues, or directly through their more reactive breakdown products such as malondialdehyde and hydroxynonenals (Chao et al., 1999). Moreover, metals such as iron and copper interact with free radicals which contribute to the propagation of the lipid peroxidation chain reaction (Akanmu et al., 1991; Schaich and Borg, 1988). It is evident then that a single initiating event, caused by a prooxidant, may cascade into a widespread chain reaction that produces many deleterious products in concentrations greater than that of the initiator (Ahmad, 1995). This is exemplified by the fact that thousands of PUFA molecules may be destroyed by a lipid peroxidation chain reaction initiated by a single radical (McCord, 1985). It is imperative that in order to prevent this vicious chain reaction, the $\cdot O_2$ radical cascade to $O_2$ and $H_2O_2$ must be attenuated, and the peroxides converted to innocuous metabolites. All aerobic organisms, therefore, possess elaborate defense mechanisms to prevent the formation of the toxic forms of oxygen and to remove any peroxides formed. Reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide, and hydroxyl, nitric oxide and peroxynitrite radicals, play an important role in the pathogenesis of various diseases. The constant attack by oxyradicals and reactive oxygen species (ROS) contributes to both the initiation and the progression of many major diseases. The oxidation of lipid, DNA, proteins, carbohydrate and other biological molecules by toxic ROS may cause mutation and damage to cells or tissues. The last
decade has yielded considerable evidence that implicates oxidative stress as a factor in the etiology and progression of a spectrum of diseases, which include atherosclerosis, cancer, eye disorders, parkinson disease, diabetes, gastric ulcers, liver diseases, etc. The underlying mechanism may differ in specific diseases, but generation of ROS is found in all cases (Liu Tsan and Arnold, 1998). Reactive oxygen Species (ROS) and Reactive Nitrogen Species (RNS) have been shown to possess many characteristics of carcinogens (Dizdaroglu et al., 2002). Mutagenesis by ROS/RNS could contribute to the initiation of cancer, in addition to being important in the promotion and progression phases, causing structural alteration in DNA, e.g. base pair mutations, rearrangements, deletions, insertion and sequence amplification. They affect cytoplasmic and nuclear signal transduction pathways (Schreck et al., 1992; Burdon et al., 1995) and modulate the activity of the proteins and genes that responds to stress and which act to regulate the genes that are related to cell proliferation, differentiation and apoptosis (Cerutti, 1994; Schreck et al., 1992; Burdon et al., 1995). There is considerable evidence that ROS/RNS are somehow involved in the link between chronic inflammation and cancer. Inflammation can accelerate the development of cancer (Oshima and Barlish, 1994; Rosin et al., 1994). A notable activity of tumor promoters is their ability to recruit inflammatory cells and to stimulate them to generate ROS/RNS.

1.4 Antioxidants

As already mentioned all aerobic forms of life maintain elaborate defense systems known as antioxidant systems to protect the body against free radical damage. The body needs to strike the right balance between the number of free radicals generated and the defense and repair mechanism available. The current view of cellular oxidant defenses can be categorized into primary and secondary defense systems (Davies, 1986; Sohal et al., 1986). The primary defenses consist of the broadly studied antioxidant compounds, such
as α-tocopherol, ascorbic acid, β-carotene and uric acid, along with a variety of antioxidant enzymes, where superoxide dimutase (SOD), catalase (CAT) and the glutathione peroxidase (GSH-Px) are notable examples. Some of the principal antioxidant and their interactions are shown in the Table 2.

**Table 1.2: Principal antioxidants and their interactions**

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>Interaction</th>
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<tbody>
<tr>
<td>Vitamin C</td>
<td>Regenerates active α-tocopherol (vitamin E) by reducing its radical form</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Transport and storage depend on selenium; absorption is reduced when vitamin A and β-carotene levels are high</td>
</tr>
<tr>
<td>β-carotene</td>
<td>Conversion to vitamin A requires vitamin E</td>
</tr>
<tr>
<td>Selenium</td>
<td>Synergistic with vitamin E</td>
</tr>
</tbody>
</table>

Data from Borek (Borek et al., 1997).

Secondary defenses are predominantly a series of enzyme systems that act to repair or eliminate molecules or cell components that were damaged by oxidants or free radical reactions, which escape the primary antioxidant defense (Davies, 1986; Halliwell, 1998; Halliwell, 1991; Prise et al., 1992).

Protecting normal cells from events that may trigger or promote cancer is a primary goal in maintaining health; it also serves to prevent long-term damage that may occur during cancer therapy and give rise to secondary malignancies in later years (Coia et al., 1998). Epidemiological data from more than 250 case control and cohort studies show that the risk of certain cancers is inversely related to the consumption of vegetables and fruit that contain essential antioxidant micronutrients, numerous other essential micronutrients, phytochemicals, and fiber (Greenwald et al., 2001).

While inverse risk is seen for cancers of the mouth and pharynx, esophagus, lung, stomach, colon, and rectum, the data for hormonally regulated cancers, including breast
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and prostate cancer, are not consistent. Reduction in breast cancer risk was often associated with consumption of carrots and green vegetables that are rich in carotenoids, and a reduced prostate cancer risk was seen within take of cruciferous vegetables, yellow vegetables, and tomatoes that are rich in lycopene. Intake of allium vegetables (garlic, onions) that contain high levels of antioxidant organosulfur compounds that are associated with reduced gastrointestinal cancer risk (Greenwald et al., 2001; Borek et al., 2001); green tea, which is rich in polyphenols, has been shown to significantly reduce the risk of breast cancer and ovarian cancer in Asian women (Wu et al., 2003; Zhang et al., 2002).

Although it is hard to ferret out with assurance the cancer-preventive compounds in fruits and vegetables, the major nutritional antioxidants, which cooperate closely with one another are deemed to have a major protective role against the disease. Animal and cell culture studies show that vitamin E, vitamin C, β-carotene (pro–vitamin A), and the mineral selenium prevent transformation of normal cells to cancer cells (Borek et al., 1997; Borek et al., 1986; Greenwald et al., 2001). In view of the suggested protective effects of antioxidants seen in observational studies, five large intervention trials have been carried out in the 1990s to evaluate the cancer-preventive effects of a variety of micronutrients: in a study in China on the effects of vitamin E, vitamin A, and β-carotene in various combinations and on lung cancer risk in smokers in the United States and Finland (Greenwald et al., 2001; Virtamo et al., 2003). While combined treatments with selenium (50µg), β-carotene (15 mg), and vitamin E (α-tocopherol, 30 mg) resulted in a lower incidence of cancer and a 10% reduction in cancer mortality from esophageal and gastric cancer in the China study; vitamin A and β-carotene as well as α-tocopherol did not reduce lung cancer risk in smokers. Vitamin E, however, significantly reduced the risk of prostate cancer by 32% and reduced mortality from colorectal cancer (Greenwald
et al., 2001; Virtamo et al., 2003). New studies have addressed the role of vitamin E, β-carotene, and selenium in reducing cancer risk (Helzlsouer et al., 2000; Li H. et al., 2004; Zhuo et al., 2004; Clark et al., 1996). A landmark multicenter clinical trial by Clark and colleagues showed that subjects with a history of skin cancer who were supplemented with 200 µg of selenium for a mean of 4.5 years (SD = 2.8 years) had a significant reduction in total cancer (all sites combined) and lung, prostate, and colorectal cancer (though not skin cancer), indicating that once skin cancer had occurred, selenium could not reduce the risk of recurrence (Clark et al., 1996). In a study designed to test the association between levels of tocopherols (α and γ) and selenium and subsequent prostate cancer, investigators have found a decline in prostate cancer with increasing concentration of α-tocopherol, and a 5-fold reduction in the risk of developing prostate cancer with increased levels of γ-tocopherol (Helzlsouer et al., 2000). Selenium showed protection, and supplementation with high levels of selenium and α-tocopherol was associated with a significant decrease in prostate cancer only when γ-tocopherol concentrations were high, suggesting a more important role for γ-tocopherol than previously considered (Helzlsouer et al., 2000). Selenium alone has been shown to reduce the risk of prostate cancer and lung cancer (Li H. et al., 2004; Zhuo et al., 2004; Clark et al., 1996).

The growing evidence that selenium and vitamin E may reduce prostate cancer risk has led to the design of the Selenium and Vitamin E Cancer Prevention Trial (SELECT), an ongoing large-scale randomized and controlled phase III trial, to investigate further the role of selenium and vitamin E in prostate cancer prevention. Enrollment began in 2001, with final results anticipated in 2013 (Klein et al., 2003). β-Carotene, which had no chemopreventive effects on lung cancer in smokers showed promise in a study on breast cancer (Virtamo et al., 2003). A nested case-control study investigated the association
between serum and plasma concentration of retinol, retinyl palmitate, α - and β carotene, β-cryptoxanthin, lutein, lycopene, α and γ -tocopherol and subsequent development of breast cancer. The results showed about a 50% reduction in breast cancer risk in women with high levels of β -carotene, lycopene, and total carotene compared to those with a low level of these micronutrients (Sato et al., 2002).

1.5 Role of medicinal plants as antioxidants

The widespread use of traditional herbs and medicinal plants has been traced to the occurrence of natural products with medicinal properties. In recent years, the traditional medicine the world over has been revalued by an extensive activity of research on different plant species and their therapeutic principles. Many studies have been performed to identify antioxidant compounds with pharmacological activity with limited toxicity. A whole range of plant derived dietary supplements, phytochemicals and pro-vitamins that assist in maintaining good health and combating disease are now being described as functional foods, nutriceuticals and nutraceuticals (Ivonova et al., 2005).

Potential sources of antioxidant compounds have been searched in many types of plant materials such as fruits, leaves seeds, etc. As plants produce a lot of antioxidants to control the oxidative stress caused by sunbeams and oxygen, they can represent a source of new compounds with antioxidant activity. Phytochemicals like tannic acid, flavonoids, tocopherol, curcumin, ascorbate, carotenoids, polyphenols, etc. were reported to have potent antioxidant properties (Gutteridge, 1995).

The biological activities of medicinal plants are attributed mostly to their bioactive chemicals. Many of the common fruits and vegetables contain bioactive compounds with antioxidant activities that may potentially be chemoprotective against a variety of cancers (Wargovich et al., 2001). Polyphenols such as resveratrol modulate the cell signaling pathways, inhibition of angiogenesis, induction of apoptosis and type II programmed cell
death. Curcumin neutralizes ROS by induction of detoxifying enzymes, inhibition of cyclooxygenase (COX-1 and COX-2) and phospholipase A₂ (PLA₂) enzymes, induction of apoptosis and down regulation of β-catenin. Catechins show their antioxidant activity by modulation of cell signaling pathways, inhibition of COX-2 and iNOS enzymes, antiangiogenic and induction of apoptosis (Etherton et al., 2002). In polyphenols the hydroxyl groups attached to aromatic rings create an electron-rich environment that traps the ROS precluding them from reacting with nucleophilic centers in cellular proteins and DNA.

Studies in cell cultures show that vitamin E, vitamin C, selenium, and some polyphenols selectively induce apoptosis in cancer cells while sparing normal cells (Sigounas et al., 1997; Taper et al., 2004). Other findings, in a model of metastatic growth, show that vitamin C is an angiostatic factor and may have potential in aiding host resistance to tumor growth and invasiveness (Ashino et al., 2003). Antioxidants also show promise in cancer therapy by their palliative action, reducing painful side effects associated with treatment (Kennedy et al., 2001).

1.6 Plant derived anticancer agents in use and clinical development

Plants have a long history of use in the treatment of cancer (Hartwell, 1982). Natural product secondary metabolites from plants and microbes in particular, have played a very important role in the amelioration of cancer. A major group of these products are the powerful antioxidants, others are phenolic in nature, and the remainder includes reactive groups that confer protective properties. Several plant-derived compounds are currently employed successfully in cancer treatment.

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* G. Don. (Apocynaceae), which was used by various cultures for the treatment of
diabetes (Gueritte and Fahy, 2005). While under investigation as a source of potential oral hypoglycemic agents, it was noted that extracts reduced white blood cell counts and caused bone marrow depression in rats, and subsequently found to be active against lymphocytic leukemia in mice. This led to the isolation of VLB and VCR as the active agents. Their discovery may be indirectly attributed to the observation of an unrelated medicinal use of the source plant. It is interesting to note that though the plant was originally endemic to Madagascar, the samples used in the discovery of VLB and VCR were collected from Jamaica and the Philippines. More recent semisynthetic analogs 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, including leukemias, lymphomas, advanced testicular cancer, breast and lung cancers, and Kaposi’s sarcoma.

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), may be considered as being more closely linked to a plant originally used for the treatment of “cancer” (Lee and Xiao, 2005). The *Podophyllum* species (Podophyllaceae), *Podophyllum peltatum* Linnaeus (commonly known as the American mandrake or Mayapple), and *Podophyllum emodi* Wallich from the Indian subcontinent, have a long history of medicinal use, including the treatment of skin cancers and warts. The major active constituent, podophyllotoxin, was first isolated in 1880, but its correct structure was only reported in the 1950s. Many closely related podophyllotoxin-like lignans were also isolated, and several of them were introduced into clinical trials, only to be dropped due to lack of efficacy and unacceptable toxicity. Extensive research led to the development of etoposide and teniposide as clinically effective agents which are used in the treatment of lymphomas and bronchial and testicular cancer.
A more recent addition to the armamentarium of plant derived chemotherapeutic agents, are the taxanes (Kingston, 2005). Paclitaxel (taxol®) initially was isolated from the bark of the Pacific Yew, *Taxus brevifolia* Nutt. (Taxaceae), as part of a random collection program for the National cancer Institute (NCI) by the U.S. Department of Agriculture (USDA). The use of various parts of *Taxus brevifolia* and other *Taxus* species (e.g., *Taxus Canadensis* Marshall, *Taxus baccata* L.) by several Native American tribes for the treatment of some non-cancerous conditions has been reported, while the leaves of *Taxus baccata* are used in the traditional Asiatic Indian (Ayurvedic) medicine system, with one reported use in the treatment of “cancer” (Hartwell, 1982). Paclitaxel, along with several key precursors (the baccatins), occurs in the leaves of various *Taxus* species, and the ready semi-synthetic conversion of the relatively abundant baccatins to paclitaxel, as well as active paclitaxel analogs, such as docetaxel (Taxotere®), has provided a major and renewable natural source of this important class of drugs. Paclitaxel is used in the treatment of breast, ovarian and non-small cell lung cancer (NSCLC), and has also shown efficacy against Kaposi sarcoma, while docetaxel is primarily used in the treatment of breast cancer and NSCLC. Paclitaxel has also attracted attention in the potential treatment of multiple sclerosis, psoriasis and rheumatoid arthritis. In addition, 23 taxanes are in preclinical development as potential anti-cancer agents.

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 (Nyssaceae) (Rahier et al., 2005). Camptothecin (as its sodium salt) was advanced to clinical trials by the NCI in the 1970s, but was dropped because of severe bladder toxicity. Extensive research later led to the development of more effective derivatives, Topotecan and Irinotecan (CPT-11; Camptosar). Topotecan is used for the treatment of ovarian and small cell lung cancers,
while Irinotecan is used for the treatment of colorectal cancers. Other plant-derived agents in clinical use are homoharringtonine, isolated from the Chinese tree, *Cephalotaxus harringtonia* var. *drupacea* (Sieb and Zucc.) (Cephalotaxaceae) (Itokawa et al., 2005), and elliptinium, a derivative of ellipticine, isolated from species of several genera of the Apocynaceae family, including *Bleekeria vitensis* A.C. Sm., a Fijian medicinal plant with reputed anti-cancer properties. A racemic mixture of harringtonine and homoharringtonine (HHT) has been used successfully in China for the treatment of acute myelogenous leukemia and chronic myelogenous leukemia. Purified HHT has shown efficacy against various leukemias, including some resistant to standard treatment, and has been reported to produce complete hematologic remission (CHR) in patients with late chronic phase chronic myelogenous leukemia (CML). Elliptinium is marketed in France for the treatment of breast cancer.

Flavopiridol is totally synthetic, but the basis for its novel flavonoid structure is a natural product, rohitukine, isolated as the constituent responsible for anti-inflammatory and immunomodulatory activity from *Dysoxylum binecetariferum* Hook. f. (Meliaceae), which is phylogenetically related to the Ayurvedic plant, *Dysoxylum malabaricum* Bedd., used for rheumatoid arthritis. Flavopiridol is one of the over 100 analogs synthesized during structure–activity studies, and 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 has also shown broad spectrum *in vivo* activity against human tumor xenografts in mice, which led to its selection for preclinical and clinical studies by the NCI in collaboration with the company, Hoechst. It is currently in 18 Phase I and Phase II clinical trials, either alone or in combination with other anticancer agents, against a broad range of tumors, including leukemias, lymphomas and solid tumors. The combretastatins were isolated from the South African “bush willow”, *Combretum caffrum*
(Eckl and Zeyh.) Kuntze (Combretaceae), in the 1970s as part of a random collection program for the NCI by the USDA, working in collaboration with the Botanical Research Institute of South Africa (Pinney et al., 2005). Species of the *Combretum* and *Terminalia* genera, both of which belong to the Combretaceae family, are used in African and Indian traditional medicine for the treatment of a variety of diseases, including hepatitis and malaria. Several *Terminalia* species have reportedly been used in the treatment of “cancer”.

The combretastatins are a family of stilbenes which act as anti-angiogenic agents, causing vascular shutdown in tumors resulting in tumor necrosis. A water-soluble analog, combretastatin A4 phosphate (CA4), has shown promise in early clinical trials, and hence a number of combretastatin (CA4) mimics are 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 analogs 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, and provides 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).

Another synthetic agent based on a natural product model is roscovitine which is derived from olomucine, originally isolated from the cotyledons of the radish, *Raphanus sativus* L. (Brassicaceae) (Meijer and Raymond, 2003). Olomucine was shown to inhibit cyclin-dependent kinases (Cdk), proteins which play a major role in cell cycle progression, and chemical modification resulted in the more potent inhibitor, roscovitine, which is currently 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).
1.7 Challenges to natural products-based drug discovery

In spite of the success of the traditional approach to drug discovery by the bioactivity-directed fractionation of plant and marine extracts, this approach has not fared well in recent years, particularly in terms of funding from the major granting agencies in the U.S. and Europe and in the support of this research within major pharmaceutical companies. The major reasons for this are the incompatibility of Crude Natural Product Extracts with High-Throughput Screening (HTS). Drug discovery within the pharmaceutical industry, with few exceptions, is based HTS of tens of thousands of compounds a week, using enzyme- or receptor-based assays designed to uncover compounds with specific mechanisms of action (Koehn, 2008). This poses a dual problem for natural products screening. In the first place, crude natural product extracts are complex mixtures, containing hundreds of compounds, often including polyphenolic compounds such as plant tannins. Tannins act as promiscuous protein binders and thus give false positive readouts in HTS, so that crude plant extracts cannot be used in HTS. Although this problem was solvable in principle by detanninization procedures, later come a second problem (Wall et al., 1996). Once a lead extract has been identified in natural products drug discovery, in the classical approach the active compound must be isolated by a process of bioactivity-directed fractionation, which can take weeks or months. HTS is not a good mechanism to use for this approach, because a typical HTS assay may be online for only a few weeks, and so the fractionation would need to be supported by another assay, adding cost to the process.

i. Diversion of resources to combinatorial chemistry

The increasing availability and sophistication of HTS from the early 1990s created the opportunity to screen libraries of hundreds of thousands of compounds, far larger than the existing compound libraries at most major pharmaceutical companies. This naturally
created a demand for compounds to satiate the maw of the screening monster, and combinatorial chemistry provided the perfect fit, with its ability to generate libraries of tens of thousands of compounds. It was seemingly a marriage made in heaven. Sadly, this approach has not been the panacea that it was hoped to be, very few drugs were discovered by the combination of HTS and combinatorial chemistry. This lack of productivity is in part responsible for the decline in new drugs, with only 20 new drugs approved in the U.S. in 2007, down from an average of about 40 a year from 1981 to 2005 (Li et al., 2009). Although the productivity of combinatorial chemistry as a drug discovery tool eventually improved, recently considerable importance is being placed on making “natural product-like” compounds by diversity oriented synthesis (Grabowski et al., 2008; Schreiber, 2000). The situation, however, has not changed significantly since 2004, when Ortholand and Ganesan could write, “The early years of combinatorial chemistry suffered from an excess of hype and the major victim was natural-product screening. Many organizations went through an irreversible shift in policy, and prematurely discontinued their efforts in this area. We are now seeing the backlash from this knee-jerk reaction. The early combinatorial strategies were flawed and unproven, and have yet to deliver any blockbuster drugs. Meanwhile, we have lost the uniqueness of screening natural-product space as a complement to synthetic compounds. If past indicators are any guide, there are undoubtedly many more unique and potent biologically active natural products waiting to be discovered.”(Ortholand, J.-Y et al., 2004). A recent review by Ganesan concludes, “One can only hope that natural products that have served as important source of drugs in the past will not be overlooked in 21st century drug discovery” (Ganesan, 2008).

ii. Technical difficulties

In addition to the problems with HTS noted above, the isolation of bioactive compounds from plants and marine organisms faces a number of technical challenges. These include
the variability of the source material (since an activity found in one collection may be absent in another), the difficulty of isolating the active constituents, the possibility that the active compound is a known compound (thus not protectable by composition-of-matter patents), and the costs of collection. However, as will be discussed below, new methods and techniques offer exciting opportunities to avoid or at least ameliorate many of these difficulties.

iii. **Resupply problems**

A further level of difficulty is encountered once a particular natural product has been isolated and identified as a lead compound, since this raises the large issue of compound supply. Depending on the potency of the compound and its target, several grams to hundreds of grams are needed for preclinical development, and multi kilogram quantities would be needed for clinical use. Probably the classic case of the problem of compound supply was with the anticancer drug paclitaxel, then known as taxol. The clinical activity of this compound against ovarian cancer was reported in 1989, and this touched off an intensive search for supplies for clinical use in what has been called the “taxol supply crisis” (McGuire et al., 1989; Cragg et al., 1993). The problem was especially acute in the case of taxol because it treated a life-threatening disease but was obtainable at that time only from the bark of the western yew, *Taxus brevifolia*, which grew predominantly in the old-growth forests of the Pacific Northwest, home to the endangered spotted owl. The solution to this problem initially involved synthetic chemistry, as described below. A different kind of resupply problem arises when the plant itself is used as the medicinal agent, as is still the case for a large percentage of the world’s population. In this case there is a real danger that nonsustainable harvesting will result in depletion of these critical resources, and initiatives are needed to commercialize the cultivation of the major
species involved. This aspect of the supply problem is discussed in more detail by Cordell (Cordell, 2009).

iv. Policy issues

The access and benefit sharing (ABS) provisions of the Convention on Biological Diversity (CBD) could be construed as an impediment to making natural product collections outside the researcher’s home country, and it cannot be denied that the legal requirements involved in meeting its terms can be time-consuming. There is also concern that these provisions will limit academic researchers interested in noncommercial studies such as taxonomy, ecology, and evolutionary biology (Martinez et al., 2010). However, these provisions should be viewed as an opportunity to carry out natural products research in an ethical way, within an agreed legal framework. In this sense it protects the institution or company involved from charges of biopiracy and, in addition, provides the possibility of doing some real good for a developing country.

v. Financial pressures

On top of all the problems noted above, the pharmaceutical industry in general, and particularly in the U.S., is undergoing a massive retrenchment, with major cuts in pharmaceutical research and development. As one analysis put it, “Big pharma’s path through the recession is littered with job and program cuts and plant closures,” and lists numerous examples to back up this statement (Jarvis, 2010). These financial pressures make it very difficult for “Big Pharma” to invest the resources that would be needed to regain the effectiveness of their former natural product discovery programs. This in turn implies that developing nations cannot rely on “Big Pharma” to discover and develop their medicinal natural product resources; this task must be undertaken by smaller and more nimble companies and by academic researchers.
vi. **Compound supply**

As noted above, paclitaxel was the subject of a major supply issue. The problem was initially solved by a combination of intensive bark collection and a semisynthetic approach. The bark collection was carried out primarily by Hauser Chemical Research, operating under contract from Bristol-Myers Squibb (BMS). Following an inventory of *T. brevifolia* on government lands, they were able to collect over 730 tonnes of bark in 1991, yielding 130 kg of paclitaxel in 1992 (Cragg et al., 1993). This work was then superseded by the development of a practical semisynthetic route to paclitaxel from a protected baccatin III, available from the needles of *T. baccata* and other yew species, and a β-lactam (Jarvis et al., 2010; Holton et al., 1995). In a final development, Bristol-Myers Squibb is now producing paclitaxel by plant tissue culture, although other companies are still producing it by semisynthesis or by direct isolation from natural sources (Walker et al., 2005; Leistner, 2005).

The halichondrins, exemplified by halichondrin B, are complex natural products that were originally isolated from the western Pacific sponge *Halichondria okadai* (Hirata et al., 1986). Halichondrin B showed excellent activity in the NCI 60-cell-line panel, acted as an inhibitor of tubulin polymerization, and was active in various animal models, so it was clearly a candidate for clinical development (Bai et al., 1991; Luduena et al., 1993; Hirata et al., 1986). The major stumbling block was compound supply, since it was available only in miniscule amounts from its marine sources. The problem was solved by the discovery by scientists at Eisai Research Institute (ERI), based on synthetic work done by the Kishi group, showing that truncated halichondrin B analogues retained much of the activity of the parent compound (Aicher et al., 1992). This led eventually to the design and synthesis of eribulin mesylate as a clinical candidate for advanced breast cancer, and this is now in phase III clinical trials.
The synthesis of eribulin was achieved in a convergent manner, but still required over 70 steps, although recent improvements have been made (Seletsky et al., 2004; Zheng et al., 2004; Yang et al., 2009). The successful synthesis of eribulin as a clinical candidate demonstrates the power of organic synthesis to generate even highly complex compounds in adequate quantities for clinical use.

A natural product that was made available for clinical use by synthesis is the anticancer compound trabectedin (Yondelis). Originally isolated from the Caribbean tunicate Ecteinascidia turbinata as ecteinascidin-743, trabectedin was found to be a DNA-binding agent that binds to the minor groove of DNA and forms covalent adducts with the N2 of guanine (Rinehart et al., 1990; Pommier et al., 1996; Zewail-Foote et al., 2001). It showed strong anticancer activity and has been approved in Europe for treatment of soft tissue sarcoma; it is also being developed for treatment of ovarian and other cancers (Cuevas et al., 2009). The supply of trabectedin for preclinical development was initially met by aquaculture of Ecteinascidia turbinata, but this resource proved to be inadequate for clinical supplies, and a viable semisynthetic route was developed from the microbial metabolite cyanosafracin B (Cuevas et al., 2009). These examples, taken together, demonstrate that even complex natural products like paclitaxel that have 11 stereocenters can be obtained in the amounts needed for clinical use by synthesis. Synthetic organic chemistry played a major role in each case, assisted for paclitaxel and trabectedin by the availability of suitable naturally occurring precursors, and it will continue to make key contributions to drug development from natural products. Biological approaches will also play an important role, as exemplified by the fact that plant tissue culture now provides access to paclitaxel and that aquaculture has the potential to provide access to some marine metabolites.
1.8 Natural products as lead compounds for medicinal chemistry

In addition to their direct use as drugs, many natural products have served as lead compounds for medicinal chemistry. In the anticancer area the lead compound podophyllotoxin led to the clinical drugs etoposide and teniposide, and the lead compound camptothecin spawned the drugs topotecan and irinotecan (Lee et al., 2005; Rahier et al., 2005). The unique paclitaxel skeleton has led to hundreds of new derivatives and several compounds in various stages of clinical trials (Kingston and Newman, 2007). In the antimalarial area artemisinin has been modified to give the water-soluble analogue artesunate, which is suitable for injection (Wells et al., 2009). Many other analogues have been developed, including a fluoroanilide that cured malaria-infected mice in a single combination dose with mefloquine hydrochloride (Woodard et al., 2009). Even common natural products have been successfully modified to make lead compounds; thus the betulinic acid derivative bevirimat and related compounds have been shown to be specific inhibitors of HIV-1 entry (Qian et al., 2010). The importance of “diverted total synthesis” as a way of optimizing the value of natural products has been emphasized by Danishefsky, who says, “natural products, a proven long-term source of pharma discovery, re-emerge as potentially valuable elements for synthesis-driven pharma exploitation” (Danishefsky, 2010).

1.9 New approaches to natural products drug discovery

i. New extract selection and preparation strategies

As noted earlier, the difficulty of screening crude plant and marine extracts by HTS is one of the reasons for the current decline in interest in natural products among the major pharmaceutical companies. This problem is conceptually rather simple to solve, like detanninization step can remove the offending interfering tannins (Wall et al., 1996). A more sophisticated approach to the problem, however, is the creation of “peak libraries” in
which a crude extract is prefractionated into a series of pure or almost pure compounds that can be rapidly screened by HTS. Once a fraction is identified as bioactive in the selected assay, it can easily be purified if it is not already pure, and its structure determined (Schmid et al., 1999). This approach has been systematized by Sequoia Sciences in the U.S., with an extensive library of plant-derived compounds and partially purified extracts, and by Analyticon in Germany, with a library of plant and microbial natural products available for screening (Eldridge et al., 2002; Bindseil et al., 2001; Wolf et al., 2007). The high upfront costs involved prevent most academic laboratories from making extensive peak libraries, but some laboratories have moved in this direction, as exemplified by the preparation of a marine natural product library characterized online by mass spectrometry, and a simple method for high-throughput extract prefractionation has been described (Bugni et al., 2008; Bugni et al., 2008A; Appleton et al., 2007).

The major danger in preparing extract libraries of pure compounds is that minor compounds may be missed, and hence a combination of crude extracts, prefractionated extracts, and pure compounds is recommended for a well-balanced natural product discovery program (Buss et al., 2004). An alternate approach to preparing extracts or extract libraries with high potential, is by the preselection of extracts using ethnopharmacological or ethnobotanical data (Rollinger et al., 2006; McClatchey et al., 2009). It has been suggested that the rate of anticancer drug discovery can be enhanced by the screening of natural products from ancient species, since these species may harbor compounds that have contributed to their lower rate of evolution (Ma, X. and Wang, 2009). A survey correlating cytotoxicity with plant taxonomy indicated that the genera Aglaia, Casearia, Exostema, Mallotus, and Trichosanthes have yielded higher hit rates, suggesting that plants of these genera could be fruitful starting points for future collections (Balunas et al., 2006). The use of ecological clues for natural products
discovery has also been demonstrated by the isolation of novel sesquiterpene quinones from a Fijian macroalga (Lane et al., 2010). The use of data mining strategies has also been developed. Databases of known natural products can be screened against a set of pharmacophore models to identify promising lead compounds, or a set of compounds isolated from a plant without reference to bioactivity can be screened \textit{in silico} for potential bioactive compounds; the most promising hits can then be evaluated in relevant bioassays. In one example, 16 secondary metabolites from Ruta graveolens were screened \textit{in silico} and compounds with activity against three targets were identified (Langer et al., 2007; Rollinger et al., 2009).

\textbf{ii. Broader bioassays}

The preparation of natural product extracts or peak libraries can be a costly and time-consuming operation. As an example of the costs involved, a report from a longtime collector for the NCI, states that the cost of collecting plant samples rose from $5.00 per sample in 1978 to $30 per sample in 1978 to nearly $200 per sample in 2008 (Spjut, 2010). With costs such as these it is important to extract the maximum value from each collection and, thus, to screen it in as many assay systems as possible. In this respect the decision of the NCI to open up its Natural Product Extract Repository to scientists interested in diseases other than cancer is highly laudable, since it ensures that this unique and very valuable resource can be screened against a large range of assays and possible disease targets (http://dtp.nci.gov/branches/npb/repository.html. Accessed September 30, 2010). In the case of the International Cooperative Biodiversity Group (ICBG) programs, all of them involve partnerships between academic and industrial groups, ensuring that extracts are screened against several targets. In the case of the Madagascar ICBG, for example, extracts are screened for anticancer, immunological, CNS, insecticidal,
antimarial, and fungicidal activities by one or other of the collaborating group members.

New screening approaches are also beginning to make an impact in natural products isolation. One helpful approach is the use of cell-based assays targeted toward specific mechanisms of action. Such assays combine the advantages of cell-based assays, such as robustness, selectivity for cell-permeable compounds, and noninterference by tannins, with the selectivity of enzyme and receptor-based assays. Examples include a cell-based screen for antimitotic agents, an engineered cell line for screening for modulators of the multidrug transporter protein component ABCG-2, a G2 checkpoint inhibition assay, and an automated cell-based assay for inhibitors of mTORC1 signaling (Roberge et al., 2000; Henrich et al., 2007; Roberge et al., 1998; Balgi et al., 2009). The use of genetically manipulated yeast cells to uncover cellular targets has been reviewed, and yeast-based assays were used in work on the isolation of potential mechanism-based anticancer agents (Sturgeon et al., 2006; Gunatilaka et al., 1994). The use of whole cell assays as well as target-based assays was instrumental in the work at Merck to discover the novel antimicrobial agent platensimycin (Singh et al., 2006). In non-cell-based approaches, screening by NMR is becoming an important method. Its use on natural product extracts is limited because of the complexity of these extracts, but it can be used on prefractionated natural product libraries, and $^1$HNMR spectroscopy was used to identify a G-quadruplex ligand in two different plant extracts by observing the difference in the imino region of ligand-bound and free G-quadruplexes (Koehn, 2008; Zhou et al., 2008).

In some cases bioactivity can be detected online in an LC-BCD (biochemical detection) approach, and microarrays of natural product extracts have been used to detect binding interactions with rapamycin (Van Elswijk et al., 2003; Schmitz, K et al., 2007). The various approaches to tracking bioactivity during the fractionation of natural products have been described in a review, and the importance of stringent end point criteria,
appropriate controls, and selectivity criteria has been emphasized (Potterat et al., 2006; Cos et al., 2006).

iii. Dereplication

One of the biggest concerns in natural products research is that after so much study many of the compounds in a given extract may well be known compounds, leading to much wasted effort in the search for new bioactive compounds. Dereplication, or the rapid identification of known compounds in an extract, is thus an important part of the process. The most useful methods employ HPLC in combination with either MS, MS/MS, NMR, or a combination of these methods, coupled with the availability of reference libraries of natural products (Cordell et al., 1999; Constant et al., 1995; Konishi et al., 2007; Wolfender et al., 2001; Wolfender et al., 2006; Lang et al., 2008; Staerk et al., 2009; Bitzer et al., 2007). These approaches can also usefully be coupled with bioassay. As one example, a combination of HPLC, collection of the fractions in a microtiter plate, and bioassay enabled the rapid identification of camptothecin and 9-methoxycamptothecin in an extract of a Didymochlaena sp (Cao et al., 2006).

iv. Isolation and structure elucidation

The bioassay-guided isolation of a bioactive natural product requires a strong collaboration between a biologist and a chemist, such that the desired active compounds are obtained efficiently. Isolation methods have improved significantly in recent years, with a myriad of chromatographic and liquid-liquid partition methods routinely available (Sticher, 2008). Major improvements have also been made in the area of bioassay, as noted above, such that very sophisticated assays can often be run on even crude extracts. The use of NMR on crude spider venom has been shown to allow an impartial view of the extract composition and thus allow for improved purification procedures (Taggi and Schroeder, 2004). New natural products have been identified and isolated from a series of fungal extract libraries.
with initial identification of new metabolites by 2D NMR on unfractionated extracts (Schroeder et al., 2007). Major improvements have also been made in the area of structure elucidation. The major tools continue to be NMR and mass spectrometry, but IR and UV also play significant roles. One industrial group uses IR in combination with MS to dereplicate known compounds from its database and reports that 20% of compounds can be identified by these two methods alone (Moss et al., 2007). NMR methods continue to improve, and a useful summary of methods for the structure analysis of natural products has been published (Fukushi, 2006). Methods for automated structure elucidation also continue to advance, with one group achieving a 90% success rate in structure confirmation by a combination of 1H and 2D HSQC spectra (Golotvin et al., 2007). Microscale NMR using a capillary flow detector has proved to be an excellent tool for work with the minute amounts of compounds often obtained in natural products work. As in one example, 13 steroids were isolated and identified from fireflies, and additional examples are given in a review of microcoil probes (Koehn, 2008; Gronquist et al., 2005; Schroeder et al., 2006). Superconducting micro-cryoprobes are also available and have found use in the structure elucidation of water-soluble microbial metabolites (Carrieri et al., 2009). A recent review of microscale methods in natural products discovery claims that “the era for the exploration of rare natural products at the ‘nanomole-scale’ has arrived” and demonstrates the truth of this claim with examples of several complex natural products for which structures were elucidated on samples of 1 mg or less (Molinski, 2009). It can thus be concluded that advances in techniques, especially in NMR methods, means that isolation and structure elucidation are no longer the limiting step in the discovery of bioactive natural products. One of the problems with work on the microscale is that it is difficult or impossible to obtain accurate weights of samples by normal weighing procedures for quantitative studies such as UV spectroscopy or bioassay. This problem
can, however, be addressed by NMR studies using solvent $^{13}$C NMR satellites (Dalisay, 2009).

In summary, advances in analytical instrumentation for both separation and structure elucidation, coupled with the increasing sophistication of bioassays, means that it is now possible to isolate, identify, and obtain bioassay information on much smaller amounts of crude extract than was possible even 10 years ago.

1.10 Natural products as lead compounds for combinatorial chemistry

Natural products can also be used as templates for combinatorial chemistry, thus linking the unique topology of a natural product with the ability of combinatorial chemistry to generate large numbers of analogues. Although natural products have served as the inspiration for the design of many combinatorial libraries, there are relatively few examples of the use of combinatorial chemistry to prepare libraries from actual natural product scaffolds, as opposed to natural product-like scaffolds (Ortholand et al., 2004; Abel et al., 2002; Grabowski et al., 2008; Boldi, 2004). Examples include the synthesis of a library of betulinic acid derivatives as HIV-2 inhibitors, a library of 3,17-hydroxysteroid dehydrogenase inhibitors, a library based on a tambjamine template, and several libraries based on paclitaxel (Dang et al., 2009; Qian et al., 2009; Maltais et al., 2001; Davis et al., 2001; Xiao et al., 1997; Jagtap et al., 2002; Bhat et al., 1998; Liu et al., 2002).

1.11 Natural products and biodiversity conservation

The ratification of the Convention on Biological Diversity marked a turning point in the search for drugs from natural sources. Before the CBD, individuals and companies were free to collect and evaluate plant, marine, and microbial sources from around the world as potential drug sources, and many did so. It was not uncommon for pharmaceutical company employees going on vacation overseas to be asked to “bring back a bit of dirt” as a source of new microbial species, and some companies such as SmithKline French
Laboratories (now part of Glaxo-Wellcome) had full-scale plant collection projects in place, making extensive collections of alkaloid-bearing plants in the southern hemisphere under the direction of Robert Raffauf in the 1960s (Raffauf, 1962). The origin of the Biodiversity Convention is the realization that the Earth’s biodiversity which is a global asset is fast disappearing, became accepted wisdom during the 1980s, thanks to the efforts of activists and environmental scientists. Biodiversity loss has been documented on many occasions; one estimate is that only about half of the tropical humid forests of the world, or about 7 million square kilometers, remain, with continuing losses of 1 million square kilometers every 5 to 10 years (Pimm and Raven, 2000). Clearly such losses are serious, and a prediction made in 2003 is that by 2050 “a considerable number of species extinctions will have taken place” and that “existing large blocks of tropical forest will be much reduced and fragmented” (Jenkins, 2003). Various approaches to identifying regions at greatest risk for biodiversity loss have been proposed, with the greatest attention focused on so-called “biodiversity hot spots”; several of these approaches have been compared to assist global conservation planning (Myers et al., 2000; Brooks et al., 2006).

Considerations such as these led the United Nations Environment Program (UNEP) to convene a working group of experts in 1988, and this eventually led to the adoption of the agreed text of the Convention on Biological Diversity in May 1992. The CBD was opened for signature in June 1992 at the United Nations Conference on Environment and Development (the Rio “Earth Summit”) and went into force in December 1993 after ratification by more than 30 countries.

1.12 Why natural products for drug discovery?

Terrestrial plants, especially higher plants, have a long history of use in the treatment of human diseases. Historical experiences with plants as therapeutic tools have helped to introduce effective, safe and novel chemical entities in modern medicine. Plants, especially
those with ethanopharmacological uses have been the primary sources of drug discovery (Pezzuto, 1997). Biologically active plant-derived chemicals can be expected to play an increasingly significant role in the commercial development of new drugs, especially when we consider that most species of higher plants have never been described, much less surveyed for chemical or biologically active constituents, and new sources of commercially valuable materials remain to be discovered (Balandrin et al., 1985). In spite of the current preoccupation with synthetic chemistry as a vehicle to discover and manufacture drugs, the potential of plants to disease treatment and prevention is still enormous (Raskin et al., 2002).

Over 120 pharmaceutical products in use today are obtained from the plants (Soejarto and Farnsworth, 1989). A large number of therapeutic activities are mediated by these drugs, and a host of drugs in use are still obtained from plants. Examples include, cardiotonic glycosides (*Digitalis* glycosides), anticholinergics (belladonna type tropane alkaloids), analgesics and antitussives (opium alkaloids), antihypertensives (reserpine), cholinergics (physostigmine, pilocarpine), antimalarials (cinchona alkaloids), antigout (colchicines), anesthetic (cocaïne), skeletal muscle relaxant (tubocurarine), and anticancer agents (paclitaxel, vincristine, vinorelbine, teniposide, and analogs of camptothecin) (Balandrin et al., 1993).

Analysis of the number and sources of anticancer and anti-infective agents, reviewed mainly in *Annual Reports of Medicinal Chemistry* from 1984 to 1995, indicates that over 60% of the approved drugs and pre-NDA candidates (for the period 1989-1995), excluding biologics, developed in these disease areas are of natural origin. According to Newman et al., (2003) during the period 1981-2002 a vast majority of New Chemical Entities (10% are unmodified natural products, 68% are derived from natural products
source i.e semisynthetic and 1% is by total synthesis, but originally modeled on natural products parent and natural product mimic) is from the natural products source. Thus natural products have been playing an invaluable role in the drug discovery process, particularly in the areas of cancer and infectious diseases.

Plants have thus been a prime source of highly effective conventional drugs for the treatment of many forms of cancer. While the actual compounds isolated from the plants frequently may not serve as drugs, they provide leads for the development of potential novel agents. As new technologies are developed, some of the agents which failed earlier in clinical studies are now stimulating renewed interest.

The appreciation of the significance of natural products as sources for structurally novel and mechanistically unique drugs and the presence of an enormous biodiversity of India, prompted the writer’s interest in evaluating a medicinal plant for its antioxidant and anticancer properties.

1.13 Ipomoea plant species

Ipomoea is a group of annual vines as well as ever green shrubs and perennials. Ipomoea is large genus of twining, creeping, floating or erect herbs, shrubs or tree, widely distributed throughout the tropical and warm temperate regions of the world. About 50 species are found in India, most important being Ipomoea batatas (sweet potato) extensively cultivated for its edible root tubers. A number species have been introduced in to India and many species are grown in gardens for ornamental purposes; some are of medicinal value.

Several members of the genus Ipomoea are widely used in folk medicine all over the world specially as powerful cathartics (Shellard et al., 1961) Pharmacological studies have reported antimicrobial (Vallette, et al.,1938; Carlson, et al., 1948), analgesic (Bhargav, et al., 1978), spasmogenic (Matin, et al.,1969), spasmolytic (Gupta, et al.,
1967; Iyer, et al., 1974), hypotensive (Matin, et al., 1969; Rakhit, et al., 1958), insecticidal (Canonica, et al., 1972) and psychotomimetic effects (Heacock, et al., 1975). 4-Ipomeanol is a pneumotoxic furan derivative isolated from the sweet potato Ipomoea batatas (Convolvulaceae) that has been under clinical evaluation as a lung-cancer-specific antineoplastic agent, Chemical investigations indicate indole alkaloids (Chao, et al., 1973) and resin glycosides (Wagner et al., 1974) are the most common constituents in the family convolvulaceae. Ipomoea obscura plant leaves are used as an application to apthous affections after toasting, powdering and boiling with ghee and in admixture with the leaves of Argyreia mollis used for sores. Some reports (Van Jaarsveld et al., 2006) indicate that Ipomoea batatas contains abundant beta carotene with orange flesh and can reduce the incidence of certain cancer and coronary heart disease in humans (Gester, H., 1993; Ziegler, R. G. 1989). Some resin glycosides from ipomoea batatas, have been claimed to show cytotoxic activity (Bah, et al., 1997). The plant has also been reported to be helpful in metastasis inhibition (Cardenas, et al., 2004). Ipomoea genus thus seems to have the potential for isolating newer leads/ drugs for anticancer activity. The plant, belonging to convolvulaceae family Ipomoea leari Paxt known as blue dawn flower, morning glory and purple winder, is the plant proposed to the investigated by the writer for its anticancer activity.