CHAPTER 2

REVIEW OF LITERATURE
2. REVIEW OF LITERATURE

Ethno-botanical study has immense importance in medical science. It is now a well established branch of science with much attention. Globally, about 85% of the traditional medicines used for primary health care are derived from plants. Traditional medicine and ethnobotanical information play an important role in scientific research; India is one of the twelve mega-biodiversity countries of the World having rich vegetation with a wide variety of plants with medicinal value. In many countries, scientific investigations of medicinal plants have been initiated because of their contribution to healthcare. Herbal medicines have good values in treating many diseases including infectious diseases, hypertension, etc. That can save lives of many, particularly in the developing countries, is undisputable. Rural people not only depend on wild plants as sources of food, medicine, fodder and fuel, but have also developed methods of resource management, which may be fundamental to the conservation of some of the world's important habitats.

Earlier studies showed that nearly one third of about 15000 higher plants species are used by tribals. India possesses a total of 427 tribal communities. Recently various ethnobotanical studies have been reported to expose the knowledge from the various tribals.
of India. Each and every tribal uses certain plants as medicine. Documenting the indigenous knowledge through ethnobotanical studies is important for the conservation of biological resources as well as their sustainable utilization. It is also necessary to collect the information about the knowledge of traditional medicines, preserved in tribal and rural communities of various parts of India.

Plants are a valuable source of new natural products. Despite the availability of different approaches for the discovery of therapeutics, natural products still remain as one of the best reservoirs of new structural types. Furthermore, Farnsworth claims that 119 characterized drugs are still obtained commercially from higher plants and that 74% were found from ethnobotanical information.

Of the several hundred thousand plant species around the globe, only a small proportion has been investigated both phytochemically and pharmacologically. When one considers that a single plant may contain up to thousands of constituents, the possibilities of making new discoveries become evident. The crucial factor for the ultimate success of an investigation into bioactive plant constituents is thus the selection of plant material. In view of the large number of plant species potentially available for study, it is essential to have
efficient systems available for the rapid chemical and biological screening of the plant extracts selected for investigation.

Natural Products, especially plants, have been used for the treatment of various diseases for thousands of years. Terrestrial plants have been used as medicines in Egypt, China, India and Greece from ancient time and an impressive number of modern drugs have been developed from them. The first written records on the medicinal uses of plants appeared in about 2600 BC from the Sumerians and Akkaidians (Samuelsson, 1999). The “Ebers Papyrus”, the best known Egyptian pharmaceutical record, which documented over 700 drugs, represents the history of Egyptian medicine dated from 1500 BC. The Chinese Materia Medica, which describes more than 600 medicinal plants, has been well documented with the first record dating from about 1100 BC (Cragg et al., 1997). Documentation of the Ayurvedic system recorded in Susruta and Charaka dates from about 1000 BC (Kappor, 1990). The Greeks also contributed substantially to the rational development of the herbal drugs. Dioscorides, the Greek physician (100 A.D.), described in his work “De Materia Medica” more than 600 medicinal plants (Samuelsson, 1999). The World Health Organization estimates that approximately 80% of the world’s inhabitants rely on traditional medicine for their primary health care (Farnsworth et al., 1985).
More than 50% of all modern drugs in clinical use are of natural products, many of which have been recognized to have the ability to induce apoptosis in various tumour cells.

According to the World Health Organization (WHO) estimates, more than 80% of the people in developing countries depend on traditional medicine for their primary health needs. Some medicinal plants and their products including vegetables, fruits and crops play an important role in cancer prevention. Consumption of large amounts of vegetables and fruits can prevent the development of cancer. Doctors recommend that people wishing to reduce their risk of cancer should eat several pieces of fruits and several portions of vegetables every day. Many plant-derived products exhibit potent antitumour activity against several cancer cell lines.

Natural products discovered from medicinal plants have played an important role in the treatment of cancer. Natural products or natural product derivatives comprised 14 of the top 35 drugs in 2000 based on worldwide sales (Butlet, 2004). Two plant derived natural products, paclitaxel and camptothecin were estimated to account for nearly one-third of the global anticancer market or about $3 billion of $9 billion in total annually in 2002 (Oberlines and Kroll, 2004). There are more than 270,000 higher plants existing on this planet. But only a small portion has been explored phytochemically. So, it is anticipated
that plants can provide potential bioactive compounds for the development of new ‘leads’ to combat cancer diseases.

Cancer is a major public health burden in both developed and developing countries. It was estimated that there were 10.9 million new cases, 6.7 million deaths, and 24.6 million persons living with cancer around the world in 2002 (Parkin et al., 2005). Cancer is the second leading cause of death in the United States (Hoyert et al., 2005), where one in four deaths is due to cancer. Plants have long been used in the treatment of cancer (Hartwell, 1982). The National Cancer Institute collected about 35,000 plant samples from 20 countries and has screened around 114,000 extracts for anticancer activity (Shoeb, 2005). Of the 92 anticancer drugs commercially available prior to 1983 in the US and among worldwide approved anticancer drugs between 1983 and 1994, 60% are of natural origin (Cragg et al., 1997). In this instance, natural origin is defined as natural products, derivatives of natural products or synthetic pharmaceuticals based on natural product models (Jaspars and Lawton, 1998).

2.1 PLANT DERIVED ANTICANCER AGENTS IN CLINICAL USE

The isolation of the Vinca alkaloids, vinblastine and vincristine from the Madagascar periwinkle, *Catharanthus roseus* G. Don. (Apocynaceae) introduced a new era
of the use of plant material as anticancer agents. They were the first agents to advance into clinical use for the treatment of cancer. Vinblastine and vincristine 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 (Cragg and Newman, 2005).

The discovery of paclitaxel (Taxol®) from the bark of the Pacific Yew, Taxus brevifolia Nutt. (Taxaceae) is another evidence of the success in natural product drug discovery. Various parts of Taxus brevifolia and other Taxus species (e.g., Taxus Canadensis Marshall, Taxus baccata L.) have been used by several Native American Tribes for the treatment of some non-cancerous cases (Cragg and Newman, 2005) while Taxus baccata was reported to use in the Indian Ayurvedic medicine for the treatment of cancer. The structure of paclitaxel was elucidated in 1971 and was clinically introduced to the US market in the early 1990s (Wani et al., 1971).

Paclitaxel is significantly active against ovarian cancer, advanced breast cancer, small and non-small cell lung cancer (Rowinsky et al., 1992). Camptothecin, isolated from the Chinese ornamental tree Camptotheca acuminata Decne (Nyssaceae), was advanced to clinical trials by NCI in the 1970s but was dropped because of severe bladder toxicity.
(Potmeisel, 1995). Topotecan and irinotecan are semi-synthetic derivatives of camptothecin and are used for the treatment of ovarian and small cell lung cancers, and colorectal cancers, respectively (Creemers et al., 1996; Bertino, 1997). Epipodophyllotoxin is an isomer of podophyllotoxin which was isolated as the active anti-tumor agent from the roots of Podophyllum species, Podophyllum peltatum Linnaeus and Podophyllum emodi Wallich (Berberidaceae) (Stahelin, 1973). Etoposide and teniposide are two semi-synthetic derivatives of epipodophyllotoxin and are used in the treatment of lymphomas and bronchial and testicular cancers (Harvey, 1997).

Homoharringtonine isolated from the Chinese tree Cephalotaxus harringtonia var. drupacea (Sieb and Zucc.) (Cephalotaxaceae), is another plant-derived agent in clinical use (Itokawa et al., 2005).

Teniposide and etoposide isolated from Podophyllum species are used for testicular and lung cancer. Taxol isolated from Taxus brevifolius is used for the treatment of metastatic ovarian cancer and lung cancer. The above drugs came into use through the screening study of medicinal plants because they showed fewer side effects, were cost effective and possessed better compatibility.
TABLE 1: Some of the important medicinal plants used for major modern drugs for cancer.

<table>
<thead>
<tr>
<th>Plant name/family</th>
<th>Drugs</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Podophyllum emodi Wall. (Beriberidaceae)</td>
<td>Podophyllotaxin,</td>
<td>Testicular cancer, small cell lung cancer and lymphomas.</td>
</tr>
<tr>
<td>Taxus brevifolius (Taxaceae)</td>
<td>Paciltaxel, taxotere</td>
<td>Ovarian cancer, lung cancer and malignant melanoma.</td>
</tr>
<tr>
<td>Mappia foetida Miers.</td>
<td>Comptothecin, lretenoteccan and topotecan</td>
<td>Lung, ovarian and cervical cancer.</td>
</tr>
<tr>
<td>Comptotheca acuminata</td>
<td>Quinoline and comptothecin alkaloids</td>
<td>used in Japan for the treatment of cervical cancer</td>
</tr>
<tr>
<td>Juniperus communis L. (Cupressaceae)</td>
<td>Teniposide and etoposie</td>
<td>Lung cancer</td>
</tr>
</tbody>
</table>

A racemic mixture of harringtonine and homoharringtonine has been used successfully in China for the treatment of acute myelogenous leukemia and chronic myelogenous leukemia (Kantarjian et al., 1996). Elliptinium, a derivative of ellipticine, isolated from a Fijian medicinal plant Bleekeria vitensis A.C. Sm., is marketed in France for the treatment of breast cancer (Cragg and Newman, 2005).

2.2 PLANT-DERIVED ANTICANCER AGENTS FOR FUTURE PERSPECTIVES

Numerous types of bioactive compounds have been isolated from plant sources. Several of them are currently in clinical trials or preclinical trials or undergoing further
investigation. Flavopiridol is a synthetic flavone, derived from the plant alkaloid rohitukine, which was isolated from *Dysoxylum binectariferum* Hook. f. (Meliaceae) (Kelland et al., 2000). It is currently in phase I and phase II clinical trials against a broad range of tumours, including leukemia, lymphomas and solid tumours (Christian et al., 1997). Synthetic agent roscovitine which is derived from natural product olomucine, originally isolated from *Raphanus sativus* L. (Brassicaceae), is in Phase II clinical trials in Europe (Meijer et al., 2003). Combretastatins were isolated from the bark of the South African tree *Combretum caffrum* (Eckl. & Zeyh.) Kuntze (Combretaceae) (Pettit et al., 1987). Combretastatin A-4 is active against colon, lung and leukemia cancers and it is expected that this molecule is the most cytotoxic phyto-molecule isolated so far (Ohsumi et al., 1998).

### 2.3 INTERNATIONAL SCENARIO

In the United States in 1999, it is estimated that over 1.2 million persons will be diagnosed with invasive forms of cancer, and over 1,500 people will die as a result of cancer each day (Landis, 1999). Among many recent advances in cancer chemotherapy, plant natural products have played an important role in contributing to the arsenal of the approximately 60 cancer chemotherapeutic drugs on the market. For instance, in the United States, there
are now four structural classes of plant anticancer agents available, constituted by the 
*Catharanthus* (Vinca) alkaloids (vinblastine, vincristine, vinorelbine), the 
epipodophyllotoxins (etoposide, etoposide phosphate, teniposide), the taxanes (paclitaxel 
and docetaxel), and the camptothecin derivatives (irinotecan and topotecan) (Cragg and 
Newman, 1997). Several other plant-derived compounds are currently in preclinical and 
clinical trials (Cragg and Newman, 2005).

As part of a National Cooperative Natural Products Drug Discovery Group 
(NCNPDDG) research project funded by the United States National Cancer Institute (1995-
2000), our collaborative team at the College of Pharmacy, University of Illinois at Chicago 
(Chicago, Illinois), and Research Triangle Institute (Research Triangle Park, North 
Carolina), and Bristol-Myers Squibb (Princeton, New Jersey) is evaluating about 400 plant 
samples per year, with the aim of discovering and evaluating novel plant derived anticancer 
agents. During the funding period 1990-1995, the industrial partner was Glaxo Wellcome 
Medicines Research Centre (Sevenage, U.K.), and past progress made in the project has 
been reviewed (Kinghorn, 1995). Since 1995, the primary plant samples have been 
collected in the Dominican Republic, Peru, and Indonesia. Plant recollections have taken 
place mainly in Thailand and Zimbabwe in *Molecules* **2000**, 5286 recent years. Our funding
agency requires that we obtain permission through formal written agreements to acquire plants for research. For each plant acquisition, a non-polar extract is prepared and screened against batteries of cultured human cancer cells and panels of mechanism-based assays. An LC-MS de replication procedure has been developed to attempt to avoid the re-isolation of common classes of known cytotoxic compounds (Constant, 1995).

As a result of bioactivity-guided fractionation on selected plant leads, well over 100 active compounds have been isolated and structurally characterized in the project to date. Many of these of novel structure and several have been further evaluated in secondary in vitro bioassays and in vivo assays.

Plant secondary metabolites also show promise for the cancer chemoprevention, which has been defined as “the use of non-cytotoxic nutrients or pharmacological agents to enhance physiological mechanisms that protect the organism against mutant clones of malignant cells” (Morse, 1993). There has been considerable prior work on the cancer chemopreventive effects of extracts and purified constituents of certain culinary herbs, fruits, spices, teas, and vegetables, which have shown the ability to inhibit the development of cancer in laboratory animal models (Huang, 1994). Clinical trials as cancer chemopreventive agents under the auspices of the United States National Cancer Institute...
are planned for plant products such as curcumin, ellagic acid, and phenethyl isothiocyanate (Kelloff, 1994)

2.4 INDIAN SCENARIO

Earlier studies showed that nearly one third of about 15000 higher plants species are used by tribals. India possesses a total of 427 tribal communities. Recently various ethnobotanical studies have been reported to expose the knowledge from the various tribals of India. Each and every tribal uses certain plants as medicine. Documenting the indigenous knowledge through ethnobotanical studies is important for the conservation of biological resources as well as their sustainable utilization. It is also necessary to collect the information about the knowledge of traditional medicines, preserved in tribal and rural communities of various parts of India. (Shanmugam, 2009)

India is the largest producer of medicinal plants and is rightly called the "Botanical garden of the World". Medical information referred in the old Indian literatures includes several medicinal herbs, which have been in the use for thousands of years, in one form or the other, under the indigenous system of medicine. In India, 45,000 plant species have been identified, out of which about 15-20 thousand plants are of good medicinal value. However, traditional communities use only about 7000-7500 plants for medicinal purposes.
The Siddha system of medicine uses about 600, Ayurveda 700, Unani 700 and modern medicine about 30 medicinal plants for treating a variety of diseases in man and animal. Only few medicinal plants have attracted the interest of scientists, to investigate them for a remedy for tumour (Prajapati, 2003).

2.5 ANTIOXIDANTS

Oxidation is essential in many living organisms for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen derived free radicals is involved in the onset of many diseases such as atherosclerosis, rheumatoid arthritis and cancer as well as in degenerative processes associated with aging (Halliwell and Gutteridge, 1984). Almost all organisms are well protected against free radical damage by enzymes such as superoxide dismutase and catalase, or compounds such as ascorbic acid, tocopherols and glutathione (Mau et al., 2002). When the mechanism of antioxidant protection becomes unbalanced by factors such as aging, deterioration of physiological functions may occur resulting in diseases and accelerated aging. However, the antioxidants present in human diet are of great interest as possible protective agents to help the human bodies reduce oxidative damage.

An antioxidant is a molecule capable of slowing or preventing the oxidation of
other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reaction can produce free radicals, which start chain reactions that damage cells. Antioxidant terminates this chain reaction by removing free radical intermediate, and inhibits other oxidation reaction by being oxidized themselves.

The antioxidant system in plants is very complex, with antioxidants having different targets, sizes and interactions with each other. Halliwell defined biological antioxidants as “molecules which, when present in small concentrations compared to the biomolecules they are supposed to protect, can prevent or reduce the extent of oxidative destruction of biomolecules”. They can be divided into enzymatic (e.g. superoxide dismutase, catalase, glutathione peroxidase) and nonenzymatic (e.g. glutathione, vitamin E, ascorbic acid).

Total Antioxidant Capacity (TAC) is a parameter that takes into account all the synergistic and cumulative interactions between the known and unknown antioxidants present in the sample. Several methods have been developed to measure the TAC of biological samples including plants. Some of the most used are “TRAP” (Total Radical-Trapping Antioxidant Parameter), “TEAC” (Trolox Equivalent Antioxidant Capacity), “ORAC” (Oxygen-Radical Absorbance Capacity), “FRAP” (Ferric/ Reducing Antioxidant Power). Sample
preparation and extraction solvents are vital but the information on how it affects the subsequent measurements is insufficient.

2.6 CANCER

Cancer is undoubtedly one of our most serious human health problems, with there being high mortality rates worldwide of more than seven million deaths per year (Weinstein, 1991). In 1999, in the United States alone, it was estimated that 1,221,800 persons will have been diagnosed with invasive cancer, and an additional one million people would also contract basal or squamous cancers of the skin. Over 1,500 persons per day (or over 563,100 Americans) will have died in 1999 from various manifestations of cancer (Landis et al., 1999). The relative five-year survival rate for cancer patients is about 56% in the United States. Among those organ sites with exceptionally low five-year survival rates are esophageal, lung, pancreatic, stomach (all at 2%), ovarian (39%), and breast cancers (20%) (Anonymous, 1997). Thus, the prevention rather than the treatment of cancer has become of increasing importance in recent years.

Cancer chemoprevention is a relatively new concept. The pioneering work to reduce cancer incidence by chemical intervention was initiated by the groups of Wattenberg and Sporn in the early 1960s and 1970s (Sporn, 1993). Later, scientists have embraced the
concept of cancer chemoprevention as a distinct new discipline of oncology (Stoner et al., 1997). As part of his work concerning retinoids and cancer prevention, Professor Michael B. Sporn has defined the term ‘cancer chemoprevention’ as ‘the prevention or delay of the process of carcinogenesis in humans by ingestion of dietary or pharmaceutical agents’ (Sporn et al., 1976).

Based on the results of animal studies and epidemiological data (Bjelke, 1975), various groups of compounds have been classified as cancer chemopreventive agents and clinical intervention studies are underway in human subjects at high risk of developing specific types of cancers (Kelloff et al., 1994a,b). Thus far, large intervention trials on two synthetic compounds [tamoxifen (Novaldex®) and finasteride (Proscar®)] are being performed (Thompson and Coltman, 1996).

In recent years, there have been intensive research efforts to investigate the cancer chemopreventive effects of dietary constituents such as beverages, culinary herbs, fruits, spices, and vegetables.

Carcinogenesis inhibition studies in animal models have been performed on dietary compounds known to act as either anti-initiating agents (e.g. diallyl sulfide, ellagic acid,
and certain isothiocyanates) or antipromotion/antiprogession agents (e.g. epigallocatechin gallate, limonene, and quercetin) (Huang et al., 1994).

Moreover, a number of plant-derived natural products are under development at various levels of preclinical and clinical trial as chemopreventive agents (such as curcumin, β-carotene, ellagic acid, and 18-glycyrrhetinic acid) (Steele et al., 1998). Thus, it seems likely that there will be a continued need for the discovery and development of novel plant-derived cancer chemopreventive agents.

Vahitha et al (2002) showed through their studies of the leaf extracts of Pavonia zeylanica and Acacia ferrugginea that 50% larval mortality (LC50) occurred at 2214.7 and 5362.6ppm respectively against the third instars larvae of Culex quinquefasciatus mosquitoes after 24 hours of treatment.

B. D. A.Ojo (2005) conducted a survey on Malaria in endemic area of Ogun state, Nigeria as an Investigation of perceptions and practices among the residents and health providers in Abeokuta, located in the tropical rain forest area of Nigeria and among the many herbs listed for malaria Plumbago zyelanica (Inabiri) is being used by native 5% doctors and by 4% herb sellers.
Rana Abu-Dahab et al., (2007) evaluated 76 ethanolic crude extracts of medicinal herbs from the Jordanian flora, belonging to 67 species and 34 families for their antiproliferative activity on a breast cancer cell line (MCF7). The cytotoxic effect of ethanolic plant extracts were characterized by conducting cell viability assay stained with sulphorohdamine.

Adeloye et al.,(2007) examined leaf extracts of Urena lobata for their antioxidant, antibacterial and antifungal activities. Preliminary evaluation of both the crude and the solvent fractions showed a broad spectrum of activity since the extracts inhibit the growth of both gram positive and gram negative bacterial isolates. The ethyl acetate and n-butanol fractions had a fast antioxidant reaction with DPPH solution, while the n-hexane and dichloromethane fractions gave no reaction.

Olaleye et al (2007) investigated the aqueous and methanolic extract of Hibiscus sabdariffa for its phytochemical constituents. The extract was found to contain cardiac glycosides, flavonoids, saponins and alkaloids.

Arulvasu et al., (2010) evaluated the anti-proliferative potential of aqueous and ethanolic extract from Vitex negundo against human breast cancer cell (MCF-7) and showed that these extracts possesses significant anti-cancer properties and causes selective
growth inhibition and apoptosis in cancer cells. They observed the maximum growth inhibitory effects (49.39%) on MCF-7 human breast cancer cells at 300 µg/ml of aqueous extract and 200 µg/ml in the case of ethanol extract.

Wamidh et al., (2010) conducted experiments on Forty four extracts from sixteen plants used traditionally as anticancer agents were evaluated in vitro for their antiproliferative activity against Hep-2, MCF-7, and Vero cell lines. They found that the extracts prepared from methanol *Ononis hirta* (aerial parts) and *Inula viscosa* (flowers) were the most active fractions against MCF-7 cells with IC50 of 27.96 and 15.78 µg/ml respectively and they were less toxic against other cell lines.
Justification for under taking the present study

The literature survey reveals about the importance of plant based medicines and their utilization for various aspects of health of mankind. The discovery of new medicines from plants continues to provide an important source of new drug leads, numerous challenges are encountered including the procurement of plant materials, selection and implementation of appropriate high-throughput screening bioassays, and scaling up active compounds. Further, the review also clearly indicates that the plants which are selected for the present investigation have not been explored for their potentiality because of their rare and scanty distribution. Based on the ethno-pharmacological approach, the plants are reported to have used for sedative, dyspepsia, asthma, eczema, leucoderma, and tumour. Thus, visualizing the importance and its scope, the present investigation was undertaken with the following objectives.

2.7 OBJECTIVES

1. To screen the physicochemical and phytochemical aspects of the selected medicinal plants.

2. To evaluate the antioxidant potentiality of the selected medicinal plants.

3. To evaluate the in-vitro anticancer activity of the selected medicinal plants.