Chapter 2

Review of Literature

2.1 Cancer an overview

Cancer is a general term applied to malignant diseases characterized by rapid and uncontrolled abnormal cells formation which may mass together to form a growth or proliferate throughout the body, and it may progress until it causes death. Among various diseases attributed to mortality in humans all over the world, cancer is a leading cause and it is one of the most dreaded diseases of the 20th century and spreading further with continuance and increasing incidence in 21st century. Between 2000 and 2020, the total number of cases of cancer is predicted to increase by 73% in the developing world and by 29% in the developed world (Parkin, 2001). 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. Malignant neoplasm was the leading cause of death in Hong Kong during 1996 to 2001 (Stanley, 2001). In Thailand the rate of people dying from cancer is still increasing every year and it is the first leading cause of death (National Statistical Office, 2003).

Mathers et al. (2001) reported that the malignant neoplasm is the third (12.4%) leading cause of death worldwide, the first (30%) being cardiovascular disease, and the second (18.8%) being infectious diseases, which include HIV/AIDS. Multidisciplinary scientific investigations are making best efforts to
combat this disease, but the sure-shot, perfect cure is yet to be brought into world medicine (Premalatha and Govindarajan, 2005). Nowadays, cancer is widely recognized as one of the most formidable human afflictions. It exists in >100 forms and has many causes, from genetic factors to infections. Probably, more than any other single disease, cancer provokes fearful images of pain, disfigurement and inevitable death (Nishma et al., 2006). Thus, research and development on anticancer agents has become a worldwide scientific effort in both private and public institutions.

Cancer chemotherapy now plays a significant role in the treatment of many malignancies, either curative (by itself or as an adjuvant to surgery and/or radiation) or palliative care, depending upon the specific tumor situation (Carter 1982). The objective of cancer chemotherapy is to kill cancer cells with as little damage as possible to normal cells (Halliwell and Gutteridge, 1988). Therefore, any discovery of anticancer agents must be related to novel molecular targets; \( i.e. \) they should be effective against specific types of cancer cells but less toxic to normal cells, or have a unique mechanism of action for specific types of cancer (Pezzuto, 1997).

Sugimura (2002) reported that, apart from cigarette smoking and chronic inflammation and infection, nutrition accounts for up to one third of the total cause of cancer. Dietary factors continue to play a complex and multifaceted role in the etiology of cancer. Cancer most commonly associated with diet include esophageal, stomach, colon, liver and the prostate. Cancer remains the major cause of morbidity and mortality through the world and still the management of cancer is not up to the mark. However, in view of the side effects due to drugs used in chemotherapy of different cancers, herbal medicines is being very popular among cancer patients (Yates et al., 2005).
2.2. Hepatocellular carcinoma (HCC)

Hepatocellular carcinoma (HCC) is the cancer that arises from hepatocytes, the major cell type of the liver, and usually relates to chronic liver diseases (Motola-Kuba et al., 2006 and Colombo, 2008). Hepatocellular Carcinoma is one of the most common malignancies in the world. Due to the global pandemic of hepatitis B and C viral infections, the incidence of HCC is rapidly increasing in Asian and Western countries (Keiichi et al., 1995; Seeff and Hoofnagle, 2006). Hepatocellular carcinoma (HCC) is the most common cancer found in Southeast Asia and Southern Africa, but it is rare in Europe and North America (Okuda, 1992). Several risk factors, such as exposure to aflatoxins and infection with hepatitis B (HBV) or C (HCV), are known to be associated with HCC (Tong et al., 1979). The prognosis of advanced HCC remains poor and novel treatment and diagnosis strategies are urgently needed (Bruix et al., 2006). Current curative options for HCC mainly include surgical resection, liver transplantation, radiotherapy and chemotherapy, however, no effective systemic treatment is available (Gish et al., 2008; Ye, 2008). Despite the existence of recently used liver cancer treatment, which include percutaneous ethanol injection, transarterial chemoembolisation, radiofrequency thermal ablation, liver resection and liver transplantation (Majno et al., 2005).

El-Serag (2002) and Marrero (2006) suggested, the incidence of HCC is predicted to increase over the next several decades as survival in patients with predisposing diseases, such as cirrhosis, is expected to increase over time. Hepatitis viral infection, food additives, alcohol, fungal toxins (aflatoxins), toxic industrial chemicals, air and water pollutants are the major risk factors of liver toxicity (Farazi and De Pinho, 2006 and Jemal et al., 2007).
Chronic alcohol exposure is also known to elicit hepatocyte hyper-regeneration due to the activation of survival factors and interference with retinoid metabolism (Seitz and Stickel, 2006). Wild and Gong (2010) noted that, aflatoxin exposure in food is a significant risk factor for HCC. Aflatoxins are primarily produced by the food-borne fungi *Aspergillus flavus* and *Aspergillus parasiticus*, which colonize a variety of food commodities, including maize, oilseeds, spices, groundnuts, and tree nuts in tropical and subtropical regions of the world.

Groopman *et al.* (2008) observed that, specific P450 enzymes in the liver metabolize aflatoxin into a reactive oxygen species (aflatoxin-8, 9-epoxide), which may then bind to proteins and cause acute toxicity (aflatoxicosis) or to DNA to cause lesions that over time increase the risk of HCC. Alcoholic cirrhosis has been identified as an important contributor to the worldwide burden of HCC (Xavier Bosch *et al.*, 2004; El-serag *et al.*, 2004 and El-serag and Mason, 2000).

Strauss (1995) described that, liver cancers have different growth patterns; the first type begins as a single tumor that grows larger in hepatic tissue. The second type is spread through the liver almost from the beginning and is not confined to a single tumor. This is seen most often in people with liver cirrhosis. In the third type, the cancer develops as nodules in several parts of the liver.

### 2.3 Carcinogen

A carcinogen is mostly simply viewed as an agent which causes cancers to develop that would not otherwise have done so. Carcinogen may, therefore be defined as an agent which increases the risk of development of one or more
particular forms of cancer compared with a risk where there is no exposure to the agent (Symington, 1976).

2.4 Chemical carcinogen

Dipple et al. (1985) reported that a wide range of different chemical structures has been associated with cancer induction in animals apart from their carcinogenic properties, many of these structures bear no particular obvious relationship to one another. They are widely distributed in the environment because of their origin in the incomplete combustion of fuels and organic matter and they also represent a very large group of carcinogens. Another substantial group of carcinogen is the aromatic amines and azo dyes which are principle products of the synthetic organic chemists.

2.5 Nitrosamines

Nitrosamines are compounds formed by the combination of amines and nitrates or nitrites. Studies have shown that nitrosamines can be formed in the gastric juice of the human stomach by a process commonly referred to as endogenous nitrosation. The bacteria in the mouth chemically reduce nitrate found in many vegetables to nitrite, which in turn can form nitrosating agents. Many foods that contain amines can react with these nitrosating agents in the acidic environment of the stomach to form nitrosamines (Jakszyn and Gonzalez, 2006). Nitrosamines seem to be a major candidate class of carcinogens that are likely to be casually related to human cancer in industrialized society. Nitrosamines comprised a large group of compounds exhibiting potent hepatotoxic and carcinogenic properties in a wide range of organs in a variety of animal species. They needed metabolic activation to generate the ultimate carcinogen to manifest their toxicity.
Williams *et al.* (1999) reported that the active metabolites reacted directly with DNA and other cellular molecules to initiate the process of carcinogenesis. The propyl diazohydroxide readily decomposes to the propyl diazohydroxide ion, a highly electrophilic species that prophylates DNA inducing various types of damage (Teiber *et al*., 2000).

### 2.6 Occurrence of nitrosamines

N-nirosodi-n-propylamine (NDPA) a dialkyl nitrosamines has been detected at low levels in various food such as cheeses, milk products (Scanlan 1983), cured meats, colored alcoholic beverages (Tricker *et al*., 1991) and at high levels in the air of factories that process molded rubber (ATSDR, 1989). Flavouring agents including meat flavorings, bread flavorings and some tooth paste contain nitrososarcosine (Lijinsky and Epstein, 1970). Further, cigarette smoke contains secondary amines including pyrolidine and Pepperdine and these could dissolve in saline and get converted into nitrosamines (Neurath *et al*., 1966).

The World Health Organisation (1997) states that the nitrate levels in surface water and ground water markedly increase due to an increased use of fertilizers, changes in land use and disposal of water from intensive animal farming which lead to increased risk of human exposure (Ingried *et al*., 1998).

### 2.7 Diethylnitrosamine (DEN)

Diethylnitrosamine (DEN) exposure occurs through diet, that is detectable in edible vegetable oil, alcoholic drinks, streamed and fried fish, formed endogenous in the body also from the use of tobacco products, cosmetics, pharamaceutical products and agricultural chemicals. DEN is one of the most important environmental carcinogens in its class that primarily induces tumours
of the liver because of its relatively simple metabolic pathway and potent carcinogenic activity (Leoppky, 1994). Hepatocarcinogenesis induced by DEN is a favourite model in rats as it facilitates the study of mechanism of chemical carcinogenesis and response of HCC of anticancer drug therapy. A compound often used for the chemical induction of HCC is N-nitrosodimethylamine (DEN). DEN is metabolised by cytochrome P450 enzymes, which are abundantly present in the liver, leading to the formation of the reactive ethyl diazonium ion (Swenberg et al., 1991). The latter holds the potential to alkylate DNA structures, causing alterations in the expression levels of tumour promoting and/or suppressing genes (Everhart and Ruhl, 2009). Single injections of DEN, sometimes in combination with phenobarbital treatment, are frequently used for the induction of HCC in mice and rats and have been validated as a genetically representative model for human HCC (Lee et al., 2004). However, it does not induce fibrosis.

Diethylnitrosamine (DEN), a representative compound of the nitrosamine family, is a well-known hepatocarcinogen, forming DNA-carcinogen adducts in the liver and inducing hepatocellular carcinomas without cirrhosis through the development of putative preneoplastic enzyme-altered hepatocellular focal lesions (Singer and Crusarderger, 1984). N-Nitrosodiethylamine (NDEA) is an N-nitroso alkyl compound described as an effective hepatotoxin and hepatotoxic in experimental animals, producing toxicity after repeated administration (Jose et al., 1998). NDEA is found in a wide variety of foods such as cheese, soybeans, smoked, salted and dried fish, cured meat and alcoholic beverages (Liao et al., 2001). Akintonwa, 1985 reported that, metabolism of certain therapeutic drugs is also reported to produce N-nitrosodiethylamine. Oxidative stress is considered as critical mechanism contributing to NDEA-induced
hepatotoxicity, and the use of antioxidant agents reduced liver damage (Vitaglione et al., 2004)

Lewis et al. (1997) noted, that DEN is considered to be a genotoxic carcinogen. It is often assumed that DEN initiates and propagates tumor development primary by inducing DNA alterations that lead to mutations (Peto et al., 1991).

Stowers et al. (1988) reported that, indicative mutations in the ras gene have been observed in mouse liver tumors arising in response to DEN treatment. DEN is widely reported to be found in the environment, in tobacco smoke and is also synthesized endogenously (Sander, 1967; Tricker and Preussmann, 1991). DEN induces oxidative stress possibly due to the generation of reactive oxygen species (ROS), which are capable of initiating peroxidative damage to the cell (Bansal et al., 2005). DEN is biotransformed by mixed-function cytochrome P-450 dependent monooxidase systems and its metabolic activation is reported to be responsible for the onset of the toxic effects (Zimmerman, 1993). Intermediate reactive compounds originating from the bioactivation of DEN are known to form covalent bonds with important cell constituents, thus inducing the onset of mutations, cancer, and necrosis (Schmitt et al., 1993).

Microsomal activation of DEN involves cytochrome P450 2E1 and hence compounds that selectively activate cytochrome P450 systems are being widely used by several investigators to induce hepatocellular carcinoma in experimental animals. Ethanol is a well known hepatotoxicant and induction of cytochrome P450 2E1 is believed to be the central pathway by which ethanol generates a state of oxidative stress thereby causing hepatotoxicity (Kim et al., 2006). Nitroso compounds are readily formed in the human body by the reaction of
nitrite with amines and amides (Ohkawa et al., 1979). NDEA has been suggested to cause oxidative stress and cellular injury due to involvement of free radicals (Ames et al., 1993; Beckman and Ames, 1998 and Noguchi et al., 2000). There are reports that oxygen free radicals and related lipid peroxides play a key role in the pathogenesis of age-related chronic degenerative diseases (Marklund and Marklund, 1974).

Studies have shown that ethanol strengthens the toxicity of DEN by augmenting cytochrome P450 2E1 mediated metabolism of DEN (Yang et al., 1990). Studies have suggested that dietary intake of phytochemicals could be a useful strategy to prevent the deleterious effects of carcinogens and mutagens (Spron and Suh, 2000), as plant based compounds are easily available, can be synthesized in large quantities and can be administered without any deleterious side effects.

2.8 Liver tumor promoters

2.8.1 Barbiturates

Phenobarbital (PB) is a non genotoxic barbiturate, used for many years as a liver tumor promoter in rodents (Sanders and Thorgerisson, 1999). The biochemical effects of PB are exceedingly pleiotropic, being both context and strain dependent. The main effects are pericentral hypertrophy and transient hyperplasia (Jime et al., 1991). PB treatment of normal hepatocytes causes a reduction in the number of epidermal growth factor receptors by a protein kinase C independent pathway (Eckl et al., 1988 and Meyer et al., 1989). PB selectively induces the growth of subset of initiated hepatocytes, increasing the number and relative volume of focal lesions which are predominantly eosinophilic (Pereira, 1993) and stain positive for gamma glutamyl transferase and the placental form of glutathione S transferase (Dragan and Pitot, 1992).
Kolaja *et al.* (1996) studied that, PB increases DNA synthesis, a decrease in the number of epidermal growth factor receptors (EGFR), increased levels of cytochrome P450 (Anoredht *et al.*, 1995). A decrease in gap junction communication and changes in thyroid hormones (Tsai and Deangelo, 1996). PB may also have cumulative effect by increasing reactive oxygen species and inducing oxidative stress (Klaunig *et al.* 1998) in bringing about such DNA damage. The advantage of the rodent liver model is the ability to identify and follow the development of very early preneoplastic lesions and to study the biochemical changes that accompany hepatocellular carcinogenesis with different initiating carcinogens and different classes of tumor promoter. However, of all the liver tumor promoters tested till date, Phenobarbital is the most widely studied compound (Diwan *et al.*, 1985).

### 2.9 Free radicals and Cancer

A free radical and is defined as a molecular species capable of independent existence and which contains one more unpaired electrons. Free radicals that are formed as byproducts of normal metabolism include superoxide anion (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), singlet oxygen (O$_2^+$), hypochlorous acid (HOCL) and hydroxyl radical (OH). All these derivatives are collectively referred to as reactive oxygen species (ROS), oxy radicals or oxygen free radicals (OFR) (Cheeseman and Stater 1993). Epidermiological and experimental studies have implicated the role of free radicals in the etiopathogenesis of cancer (Gonalez 1999).

Plants with antioxidant activities have been reported to possess free radical scavenging activity (Das and Pereira, 1990). Free radicals are known as major contributors to several clinical disorders such as diabetes mellitus, cancer,
living cells, renal failure and degenerative diseases as a result of deficient natural antioxidant defense mechanism (Parr and Bolwell, 2000).

Living cells may generate free radicals and other reactive oxygen species by-products as a result of physiological and biochemical processes. Free radicals can cause oxidative damage to lipids, proteins and DNA, eventually leading to many chronic diseases, such as cancer, diabetes, aging, and other degenerative diseases in humans (Harman, 1998).

Free radicals are highly reactive compounds with uneven number of electron in their outermost orbit. These can react with cellular compounds like unsaturated fatty acids and can generate new free radicals which results in irreversible biochemical injury like membrane damage, apoptosis and cell necrosis. Antioxidants scavenge free radicals and quench the subsequent reactions, hence protecting the macromolecules and cellular environment from toxicity and degeneration (Hong-Bo Shao et al., 2008). The oxygen consumption inherent in cell growth leads to the generation of series of reactive oxygen species (ROS). These ROS are molecules such as superoxide anion radicals (O$_2^-$) and hydroxyl radicals (OH). However, non-free radical species such as hydrogen peroxide (H$_2$O$_2$) and singlet oxygen (O$_2$) are formed in vivo also. Both the oxygen species play a positive role in energy production, phagocytes, regulation of cell growth, intercellular signaling and synthesis of biologically important compounds. However, ROS may also be very damaging; they can attack the lipids of cell membranes and DNA. The oxidation induced by ROS can result in cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate or propagate the development of many diseases (Valentao et al., 2002 and Gulcin et al., 2003). ROS are continuously produced during normal physiologic events and are removed by antioxidant
defense mechanisms (Buyukkuroglu et al., 2001; Chang et al., 2001 and Gulcin et al., 2002a, 2002b). Living cells may generate free radicals and other reactive oxygen species by-products as a result of physiological and biochemical processes. Free radicals can cause oxidative damage to lipids, proteins and DNA, eventually leading to many chronic diseases, such as cancer, diabetes, aging, and other degenerative diseases in humans. Plants are endowed with free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity (Zheng and Wang, 2001 and Cai et al., 2003). Studies have shown that many of these antioxidant compounds possess anti-inflammatory, anti-atherosclerotic, antitumor, anti-mutagenic, anti-carcinogenic, antibacterial, and antiviral activity (Sala et al., 2002 and Rice-Evans et al., 1995). The ingestion of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with ageing, (Veerapur et al., 2009 and Muselik et al., 2007) and in recent years, there has been a worldwide trend towards the use of the natural phytochemicals present in berry crops, teas, herbs, oilseeds, beans, fruits and vegetables (Kitts and Yuan, 2000).

2.10. **Lipid peroxidation (LPO)**

Lipids, particularly polyunsaturated fatty acids (PUFA) are the major class of biomolecules susceptible to oxidative damage by free radicals (Datta et al., 2000). Lipid peroxidation is a chain reaction, which is initiated by the attack of free radicals on the membrane lipids that are capable of abstracting a hydrogen atom from the methylene group is known as initiation phase (Halliwell and Gutteridge, 1985). The carbon radical thus formed is stabilized by molecular rearrangement to produce conjugated diene, which easily reacts with an oxygen
molecule to give a peroxyl radical (Eslerbauer et al., 1991 and Mailard et al., 1983).

Alternatively, the peroxyl radical can form cyclic peroxide and endoperoxide which undergo fragmentation leading to the formation of cytotoxide aldehydes like malondialdehyde (Pryor et al., 1976; Gutteridge et al., 1984; Wills 1987 and Wade et al., 1987). Once started, LPO proceeds as a chain reaction until the poly unsaturated fatty acids is consumed of until the radical self annihilates is referred to be termination phase of LPO.

2.11 Oxidative damages in DNA, lipids and proteins

Experimental studies revealed that oxygen free radicals are involved in initiation and promotion of carcinogenesis where inactivation or loss of certain tumor suppressor genes occurs (Cerruti 1994). Cellular genes are usually converted to oncogenes, particularly ras family oncogenes, in codons 12 and 13. These G-C sites have been demonstrated as the main targets of oxidative damage thereby resulting in the formation of 8-oxoguanosine, which is a mediator of mutagenesis (Guyton and Kensler 1993). The DNA damage by Oxygen Free Radical has been demonstrated in the form of base damage (Floyd 1982), single and double strand breaks (Brawn and Fridrich 1981 and Cacciuttolo et al., 1993), cross linking between DNA (Begleiter and Flair 1984). Several different pathways by which oxidative DNA damages leading to mutations have been proposed, including chemical modification of nucleotides in DNA causing alteration in their hydrogen bonding, exacerbation of polymerase specific hot spots, conformational change in the DNA templates, and the induction of DNA polymerase conformation (Hemnani and Parivar, 1998).
Cellular fatty acids are readily oxidized by ROS to produce lipid peroxyl radical propagating into malondialdehyde. These lipid radicals can diffuse through membranes, thus modifying the structure and function of the membrane and resulting in a loss of cell homeostasis. In addition, lipid peroxides may interact with cellular DNA and cause the formation of DNA-MDA adducts (Chaudhary et al., 1994).

Proteins are also attacked by ROS directly or indirectly by lipid peroxidation. Protein radicals rapidly transferred to other sites within the protein infrastructure can result in either stimulation or inhibition of enzyme activity (White et al., 1976 and Belloma et al., 1983). In addition, damage to membrane in intracellular calcium and potassium that will trigger a series of changes in cells (Khan et al., 1992).

2.12 Oxidative stress in cancer processes

All aerobic organisms are continually exposed to oxidative stress; normally there is equilibrium between free radical and antioxidant defense mechanism. An imbalance between ROS and antioxidant defense formation of ROS and antioxidant defense mechanisms could lead to oxidative stress (Abdi and Ali 1999).

Hydroxy radical is a highly reactive radical formed in biological systems and capable of damaging almost every molecule found in living cells (Halliwell, 1991). This radical has the capacity to induce carcinogenesis, mutagenesis and rapidly initiates lipid peroxidation (Rajesh Manian et al., 2008).

Seis (1993) suggested that the oxygen free radicals are known to be involved in various processes such as carbohydrate damage, damage, membrane
damage, lipid peroxidation, mutagenesis and carcinogenesis. Free radical induced oxidative stress has been implicated in all stages of cancer development (Ather, 2002). About 10,000 to 20,000 oxidative hits to DNA/cell/day are estimated to occur in humans (May, 1999).

2.13 Adriamycin

Adriamycin is an anthracyclin anti-neoplastic agent used in the treatment of a variety of human neoplasms. Adriamycin is a commonly used chemotherapeutic agent for cancer. It has proven efficacy in acute leukemia, lymphoma and a number of solid human tumors. Koukovrakis et al. (2000) have reported that Adriamycin is considered to be one of the most effective drugs for the treatment of sarcomas. The mechanism of action of Adriamycin (ADR), an anthracyclin antitumor antibiotic has been extensively studied. This drug was found to have a high affinity for DNA. ADR-DNA interactions have been reported to result in inhibition of DNA replication, transcription and repair possibly by interfering with the template function of DNA.

Structure of Adriamycin
2.14 Adriamycin induced cardiotoxicity

Cardiac oxidative injury is a major limiting factor for clinical application of adriamycin in cancer chemotherapy. ADR-induced cardiotoxicity is mediated through different mechanisms including membrane lipid peroxidation, free radical formation, mitochondrial damage and iron dependent oxidative to biological macromolecules (Saad et al., 2001). Recent studies have been demonstrated that ADR metabolically generates oxygen free radicals that complex with DNA and RNA synthesis. Increased lipid peroxidation and enhanced free radical generation in the heart have been documented after ADR administration and subsequently implicated in cardiac toxicity. Moreover inhibition of oxidative phosphorylation, decreased ATP synthesis, a characteristic features of ADR induced cardiotoxicity (Mohamed et al., 2000).

The clinical use of adriamycin is associated with nephritic syndrome characterized by heavy proteinuria, albuminuria, hypoalbuminaemia and hyperlipidaemia. Several lines of evidence suggest that reactive oxygen species (ROS) is the principal mediator in the development of nephrosis caused by ADR (Venkatesan et al., 2000).

2.15 Chemopreventive agents

The chemopreventive agents also known as anti-carcinogens should ideally possess little or no adverse effects, high efficacy against multiple sites, effectiveness at achievable dose level, a known mechanism of action, low cost and general human acceptance (Kelloff et al., 1996). A large number of potential chemopreventive agents have been identified from epidemiological studies, preclinical systems, clinical observations and laboratory evidences from animal models (Sharma et al., 1994). Most of the chemopreventive agents can act at more than one carcinogenic site and can have more than one mechanism of
action (Lipkin, 1996). Based on the mechanism of action, Wattenberg (1996) has classified chemopreventive agents into three major categories: inhibitors of carcinogen formation, blocking agents, and suppressing agents.

### 2.16 Dietary Chemoprevention

Epidemiological studies have documented that diets, high in vegetables and fruits, are associated with reduced risk of cancer in a variety of organs (Polusa, 2000). The effects of plant-derived human nutrients have been confirmed in different animal tumor models. Numerous diet-derived agents are included among the ≤ 40 promising agents that are being evaluated clinically as chemopreventive agents for major cancer targets.

Numerous compounds in the human diet have chemoprotective properties against chemical carcinogen (Wattenberg, 1990). Intake of food rich in fruits and vegetables is associated with a lowering risk of certain types of cancer (Steinmetz and Potter, 1991). Certain leafy vegetables of all varieties and cruciferous plants such as cabbage, Brussels sprouts, cauliflower, and broccoli are rich in anticarcinogens (Block et al., 1992 and Wargovich, 1999). Cruciferous vegetables contain a number of bioactive components such as folate, vitamin C, tocopherols, carotenoids, flavonoids, and polyphenols (Price et al., 1998 and Kurilich et al., 1999).

An advantage of diet-derived products in cancer prevention is that they also have apparent benefits in other chronic diseases (e.g., Prevention from heart diseases); green and black tea polyphenols, lycopene, soy isoflavone, curcumin, and allylsulphides have shown preventive potential against several chronic diseases. Fruits and vegetables abound in potential anticarcinogenic compounds.
such as carotenoids, ascorbate, tocopherol, selenium, dietary fiber plant sterol, Allium compounds and limonone (Kelloff et al., 2000).

Review of the epidemiological data, including both cohort and case-control studies, of all cancer sites strongly suggests that plant foods also have preventive potential and that consumption of the following groups and types of vegetables and fruits is lower in those who subsequently develop cancer, raw and fresh vegetables (Yun et al., 2008), leafy green vegetables (Shao et al., 2001), Cruciferae (Morimitsu et al., 2000), carrots (Galeone et al., 2007), broccoli and cabbage (Hara et al., 2003), lettuce and raw and fresh fruit (including tomatoes and citrus fruit) (Do et al., 2007 and Huang et al., 2004).

Asians have a long history of medicinal use of plants, some of which have proved useful as pharmaceuticals. Besides, traditional Asian diet contains less animal fats and higher plant-based foods as it is compared to western diet. Such a higher consumption of plant foods in Asian countries as a result of their tropical climates, results in a wider choice of edible plants (Runnie et al., 2004). The practice of medicine—both in the past and present, of ten involves the prescription of specific foods (almost always plants) or their potent derivatives, to treat a wide spectrum of illnesses (Rigas et al., 2008).

A good example of such Asian foods are Indian food ingredients which can be used in preventive strategies aimed at reducing the incidence and mortality of different types of cancers because of their anti oxidative (Devasagayam and Sainis, 2002), anti mutagenic and anti carcinogenic properties (Arora et al., 2003).
2.17 Natural antioxidants

Herbal plants are an important source of new chemical substances with potential therapeutic uses (Alam et al., 2010). In recent years attention has been directed in utilizing natural antioxidants. Secondary metabolites of plants such as flavanoids, anthocyanins, carotenoids and vitamins have been reported as promising antioxidants (Kivits et al., 1997) plant derived antioxidants function as singlet and triplet oxygen quenchens, peroxide decomposes and/or chelators of the catalytic metal ions (Roback and Marcinkiewiez, 1995).

Dreosti (2000) reported that the beneficial role of flavonoids in variety of cellular processes. Numerous investigators have shown that foods containing phytochemicals with antioxidant potential have strong protective effects against the risk of cancer and cardiovascular disease (Osman et al., 2004).

Medicinal plants have been used as remedies for human diseases for centuries. The reason for using them as medicine lies in the fact that they contain chemical components of therapeutic value (Nostro et al., 2000). The medicinal value of plants lies in some chemical substances (usually secondary metabolites) that produce a definite physiological action as the human body. The most important of these bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolics (Edeoga et al., 2005). Oxidative stress is a crucial etiological factor to the pathophysiology of a variety of degenerative or pathological conditions such as aging, cancer, coronary heart disease and inflammation (Pong 2003 and Sandhya et al., 2010).

Human body has multiple mechanisms especially enzymatic and non enzymatic antioxidant systems to protect the cellular molecules against reactive oxygen species (ROS) induced damage (Anderson, 1999). However due to the
overproduction of reactive species and/or inadequate antioxidant defense, it culminates in severe or continued oxidative stress. The harmful action of the free radicals can, however, blocked by antioxidant substances, which scavenge the free radicals and detoxify the organism (Balakumar et al., 2010). Many synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) are very effective and have been added to food stuffs during food processing but they may possess toxic side effects and also acts as carcinogens (Anagnostopoulou et al., 2006). The safety and toxicity of synthetic antioxidants have been important concerns; therefore, attention has been focussed on the use of natural antioxidants for inhibition or protection from oxidative damage (Howell, 1986). In recent years attention has been directed in utilizing natural antioxidants substantially (Shahidi et al., 2006 and Vetrivel Rajan and Shanmugavalli, 2009). A special feature of higher plants is their capacity to produce a large number of organic chemicals of high structural diversity, the so called secondary metabolites (Castello et al., 2002).

Flavanoid is of natural compound which exhibits activity as reductor to hydroxyl, superoxide and peroxyl radicals. In addition, many polyphenols and flavanoids have been proved to inhibit proliferation and angiogenesis of tumor cells invitro (Fotsis et al., 1997) as well as in experimental animals (Hertog et al., 1993). Phenols compounds are very important constituents of plants because of their radical scavenging ability is due to their hydroxyl groups (Hatano et al., 1989).

The anticancer activity are ascorbate and phenols which block the formation of carcinogens such as nitrosamines (Mitacek et al., 2008; Qiu et al., 2005 and Takeyama, 2005) ; flavonoids and carotenoids act as antioxidants, essentially disabling the carcinogenic potential of specific compounds by having
cytoprotective effects against ONOO- and HOCl mediated cytotoxicity (Persson et al., 2008 and Rose et al., 2005); lipid-soluble compounds such as carotenoids and sterols may alter membrane structure or integrity and show significant growth inhibition activity against various human cancer cell line (Damu et al., 2007 and Iwashima et al., 2002); carotenoids can suppress DNA synthesis and enhance differentiation (Kawashima et al., 2007). The potent chemopreventive effects have been demonstrated in various in vivo and in vitro models for sulfur-containing compounds found in naturally occurring products (Lee et al., 2008).

2.18 Medicinal plants in human health care

Plants have formed a basis for traditional medicine systems that have been used for thousands of years in countries with ancient civilizations such as China (Chang and But 1986), India (Kapoor 1990) and Thailand (Subchareon 1998a). It has been reported that there has been an alarming increase in number of diseases and disorders caused by synthetic drugs prompting a switch over to traditional herbal medicine (Ghule and Patil, 2001). The use of plants in traditional medicine systems of many other cultures has been extensively documented (Schultes and Raffauf 1990; Arvigo and Balick 1993; Gupta 1995; Ayensu 1981; Iwu 1993 and Jain 1991). Plant-based systems continue to play an essential role in healthcare and it has been estimated by the WHO that approximately 80% of the world’s inhabitants rely mainly on traditional medicine for their primary healthcare (Farnsworth et al. 1985).

Plant products also play an important role in the healthcare systems of the remaining 20% of the population who reside mainly in developed countries. Analysis of data on prescriptions dispensed from community pharmacies in the United States from 1959 to 1980 indicates that about 25% contained plant extracts or active principles derived from higher plants. About 74% of these 119
drugs were discovered as a result of chemical studies directed at isolation of the active substances from plants used in traditional medicine (Cragg et al., 1997). Ethnopharmacological or traditional use of plants often results in the discovery of new biologically active molecules (Houghton 1995). However, it is important that the investigators understand the principles of folk medicine or mode of action of folk herbs (Nakanishi, 1999). Plants have a long history of use in the treatment of cancer (Hartwell, 1982). However, many of the claims for the efficacy of such treatments should be viewed with some skepticism because cancer, as a specific disease entity, is likely to be poorly defined in terms of folklore and traditional medicine (Cragg et al., 1994). Scientists are interested in investigating medicinal plants which are commonly used by public and derived from folklore or anecdotal information (Helton, 1996; Mail et al., 1989).

Dubick (1986) reported that the medical use of herbs is deeply rooted in human history and folklore, and incorporated into the historical medicine of virtually all human cultures. The medicinal plants derived from folklore are huge, *Vinca rosea* (Sun and Zeng, 2005), *Podophyllum peltatum* (Gordaliza et al., 1994) and *Taxus spp* (Wani et al., 1980) are selected examples. These plants and many others lead to discover important drugs including vincristine, vinblastine, podophyllotoxin, 10-hydroxycamptothecin and Taxol (Coker et al., 2003).

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds (Hill, 1952). Many of these indigenous medicinal plants are used as spices and food plants. They are also sometimes
Traditional medicine has a long history of serving peoples all over the world. Documentation of the Ayurvedic system recorded in Susruta and Charaka dates from about 1000 BC. The first written records on the medicinal uses of plants appeared in about 2600 BC from the Sumerians and Akkaidians (Samuelsson, 1999). 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 “Ebers Papyrus”, the best known Egyptian pharmaceutical record, which documented over 700 drugs, represents the history of Egyptian medicine dated from 1500 BC. Herbal drugs constitute a major part in all the traditional system of medicines (Higa et al., 1994). The use of drugs derived from plants has been in practice for a very long time (Lewis and Elvin-Lewis, 1977). Plants have a limitless ability to synthesize aromatic substances mainly secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total (Mallikharjuna, et al., 2007). The synthesized aromatic substances (Metabolites) are used by plants as defensive molecules against predation by microorganisms, insects and herbivores. However, some of which may involve in plant odour (terpenoides), pigmentation (tannins and quinines), and flavour (Capsacin). However, these defensive molecules give plants their medicinal value which is appreciated by human beings because of their great importance in health care of individuals and communities.

Natural products and related drugs are used to treat 80% of all categorized human diseases including bacterial infection, cancer and immunological
disorders (Newman and Cragg, 2007) About 25% of the prescribed drugs in the world originate from plants (Rates, 2001). Many of the developing countries practice traditional medicine as its main source of healthcare, which is usually of plant origin (Rehan Ahmad et al., 2008 and Stephen Bent, 2008). Today, nearly 88% of the global populations switch to plant derived medicines as their first line of defense for maintaining health and combating diseases (Kintzios et al., 2006). At present there are about 60 types of medicinal plants that have already been promoted to use in primary healthcare and classified according to their pharmacological actions such as peptic ulcers, anti-flatulence, laxative, antidiarrhoea and anti-herpetic (Viomolos et al., 2003). In future, the discovery of novel therapeutic agents will be only dependent on plant origin (Perumalsamy et al., 1999).

Phytochemicals from medicinal plants serve as lead compounds in drug discovery and design. Medicinal plants are rich source of novel drugs that forms the ingredients in traditional system of medicine, modern medicines, pharmaceutical intermediates, bioactive principles and lead compounds in synthetic drugs (Ncube, 2008). In recent times focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems (Uma maheswari and Niveditha., 2007). Various medicinal plants have been studied using modern scientific approaches. The results from these plants have released the potential of medicinal plants in the area of pharmacology. Pharmacological studies have acknowledged the value of medicinal plants as potential source of bioactive compounds (Prusti et al., 2008). Herbal medicines are being used by nearly about 80% of the world population, primarily in developing countries for primary health care (Kamboj, 2000). Assessing the current status of health care
system, inadequacies of synthetic drugs are likely to be more glaring in the coming years.

2.19 Plant Components as Promising Anticancer Agents

The uses of plant and plant materials for curing various ailments have been known to the world since time immemorial (Suffiness and Douros, 1982) and gaining wide acceptance by scientific community, particularly in view of the toxic side-effects of most synthetic drugs.

There is currently a large and ever-expanding global population that prefers the use of natural products in treating and preventing medical complications (Gautam et al., 2007 and Jassim and Naji, 2003). The worldwide upsurge in the use of herbal preparations and active ingredients isolated from medicinal plants have provided the pharmaceutical industry with one of its most important sources of lead compounds, as up to 40% of modern drugs are derived from natural sources, using either the natural substance or a synthesized version. Furthermore, over a 100 new products are in clinical development, particularly as anti-cancer agents and anti-infectives (Harvey, 2008).

In the absence of reliable liver protective drugs in allopathic medical practices, herbs play a role in the management of various liver disorders. A number of plants have shown hepatoprotective properties (Suresh Kumar and Mishra, 2008). There is growing interest in identifying new chemopreventive agent from dietary sources (Hae-Jeung et al., 2005). Since the increase in the use of synthetic chemicals in cancer therapy has led to many side effects and undesirable hazards, there is a worldwide trend to go back to natural resources (medicinal plants) which are therapeutically effective, culturally acceptable and economically within the research the poor people (Fauziah et al., 2005).
Medicinal plants have become a major component of human health care as they have no or less side effects. Surveys conducted in Australia and US indicate that almost 48.5 and 34% of respondents had used at least one form of unconventional therapy including herbal medicine (Edease, 2000).

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 (Cragg and Newman, 2005). Active compounds obtained from several plants such as Angelica gigas, Catharanthus roseus, Taxus brevifolia, Podophyllum peltatum, Podophyllum emodii, Ocrosia elliptica and Campototheca acuminata have been used as anticancer medicines (Park and Pezzuto, 2002). Moreover, many plant-natural compounds such as flavones, flavanols, isoflavones, catechin and taxanes exhibit preventive and curative activities to cure cancer (Lopez-Lazaro, 2002 and Philip, 2005). Although, many anticancer compounds such as alkilating compounds, antimetabolites, radiomimetics, hormones and antagonist have been developed (Calabresi and Chabner, 1991; Hoppe et al., 1982). National Cancer Institute collected about 35,000 plant samples from 20 countries and has screened around 114,000 extracts for anticancer activity (Shoeb, 2005).

Various medicinal plants have been studied using modern scientific approaches. The results from these plants have revealed the potential of medicinal plants in the area of pharmacology (Dahanukar et al., 2000). Recent research has identified food components (phytochemicals) that may have important anticarcinogenic activities (Mazur and Adlercreutz, 2000). These
phytochemicals can suppress the initiation or reverse the promotion stage in multistep carcinogenesis.

2.20 Phytochemicals in Cancer Prevention

Several hundred scientific studies focused on the activity of non-nutritional compounds present in the diet, preventing the occurrence of degenerative diseases, such as cancer. This heterogeneous class of molecules, generally known as phytochemicals includes vitamins (carotenoids) and food polyphenols, such as flavonoids, phytoalexins, phenolic acids indoles and sulfur rich compounds (Russo, 2007). More than 10,000 phytochemicals have been described, and among them more than 6,000 compounds are included in the class of flavonoids (Hairborne, 1993) 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).

Phytochemicals have been associated to pleiotropic effects in animal cells. Phytochemicals attracted scientists’ interests since the demonstration that their biological targets in mammalian cells were the same involved in inflammatory processes and oncogenic transformation, such alterations of cell cycle control, apoptosis evasion, angiogenesis and metastases. In addition, a large number of epidemiological studies suggest that a daily intake of phytochemicals can reduce the incidence of several types of cancers (Sporn et al., 2002; Surh, 2003 and Russo, et al., 2005)

A number of plant products include polyphenolic substances such as, flavanoid and tannins are antioxidative substances usually have a phenolic moiety in their molecular structure (Shanmugavalli et al., 2009). They have been found among flavanoid, tocopherol and catechin. Organic acids,
carotenoids, protein hydrolysates and tannins can also act as antioxidants or have synergistic effects (Dugan, 1980 and Langseth, 1995).

Plants lies in some chemical substances that produce a definite physiologic action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds. The phytochemical research based on ethno-pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants (Duraipandiyan et al., 2006).

Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies (Mojab et al., 2003). Chemically constituents may be therapeutically active or inactive. The ones which are active are called active constituents and the inactive ones are called inert chemical constituents (Iyengar, 1995).

Recent research has shown that plant-derived polyphenolic compounds are promising nutraceuticals for control of various disorders such as cardiovascular, neurological and neoplastic disease. The richness of the polyphenolic contents of green tea and red wine has made them popular choices for associated anticancer and cardiovascular health benefits (Ullah and Khan, 2008).
The flavonoids are polyphenolic compounds found as integral components of the human diet. They are universally present as constituents of flowering plants, particularly of food plants (Miean and Mohamed, 2001). Several plants and spices containing flavonoid derivatives have found application as disease preventive and therapeutic agents in traditional medicine in Asia for thousands of years (Nakatani, 2000). Many studies around the world proved that the selection of a particular food plant, plant tissue or herb for its potential health benefits appears to mirror its flavonoid composition. The ability of flavonoids to scavenge free-radicals and block lipid peroxidation raises the possibility that they may act as protective factors against carcinogenesis (Tseng and Lee, 2006 and Zhou et al., 2003). An impressive body of information exists on the antitumor action of plant flavonoids. In vitro work has concentrated on the direct and indirect actions of flavonoids on tumor cells and has found a variety of anticancer effects such as cell growth (Weng et al., 2007), kinase activity inhibition (Yagura et al., 2008), apoptosis induction (Lee et al., 2005), suppression of the secretion of matrix metalloproteinases and of tumor invasive behavior (Ha et al., 2004). Many of experimental animal studies indicate that certain dietary flavonoids possess antitumor activity. The hydroxylation pattern of the bring of the flavones and flavonols, such as luteolin and quercetin, seems to critically influence their activities, especially the inhibition of protein kinase activity and antiproliferation (Ong et al., 2004 and Steffan et al., 2005). The different mechanisms underlying the potential anticancer action of plant flavonoids await further elucidation. Certain dietary flavonols and flavones targeting cell surface signal transduction enzymes, such as protein tyrosine and focal adhesion kinases and the processes of angiogenesis appear to be promising candidates as anticancer agents (Kandaswami et al., 2005). In present opinion further in vitro and in vivo studies of these bioactive constituents are deemed necessary in order to develop flavonoid-based anticancer strategies. Other data
suggest that foods high in phytoestrogens, particularly soy (which contains isoflavones (Dos Santos Silva et al., 2004; Lu et al., 2000; Sakauchi et al., 2007 and Wu et al., 2008) and also phytoestrogens derived from some vegetables and berries as well as grains and seeds (Ozasa et al., 2005), or high in precursor compounds that can be metabolized by gut bacteria into active agents, particularly some grains and vegetables with woody stems (which contain precursors to lignans) (Cai et al., 2005, Kumar et al., 2004 and Penalvo et al., 2008) are plausibly associated with a lower risk of sex-hormone-related cancers. Phytoestrogens compete with estradiol for estrogen receptors in a way that is generally antiproliferative. The lower incidence of hormone-dependent tumors in Asian population compared to Europeans is believed to be related their rich phytoestrogen diet (Vij and Kumar, 2004 and Waldschlager et al., 2005). Consumption of diets low in plant foods results in a reduced intake of a wide variety of these substances that can plausibly lower cancer risk. There are many biologically plausible reasons why consumption of plant foods might slow or prevent the appearance of cancer. These include the presence in plant foods of such potentially anticarcinogenic substances as carotenoids and vitamin C (Huang et al., 2007 and Kapil et al., 2003), vitamin E (Xu et al., 2007), selenium (Cai et al., 2006 and Pourmand et al., 2008), dietary fibre and its components (Bolin, 2008 and Dos Santos Silva et al., 2002), isothiocyanates (Moy et al., 2008), indoles (Hecht et al., 2004), phenols (Saxena et al., 2007), protease inhibitors (Seo et al., 2005), allium compounds (Setiawan et al., 2005), plant sterols (Iwashima et al., 2002) and limonene (Tsuda et al., 2004). Most of the data for the observations on the anticarcinogenic potential of all of these compounds have come from animal and in vitro studies.
2.21 Anticancer Bioactivity of Some of Asian Plants

Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals we use today for our various ailments (Abraham, 1981). The discovery of medicinal plants has usually depended on the experience of the populace based on long and dangerous self experiment. Progress over the centuries towards a better understanding of a plant derived medicine has depended on two factors that have gone hand in hand.

One has been the development of increasingly strict criteria of proof that a medicine really does what it is claimed to do and the other has been the identification by chemical analysis of the active compound in the plant (Holiman, 1989).

Traditional Chinese Medicines (TCM) have long been consumed to prevent and treat various kinds of cancers prevalent in Taiwan, mainland China and Japan (Lau et al., 1994). Lin et al. (2004) revealed the potential of Coptis chinensis root extract, berberine and coptisine to treat hepatoma and leukaemia cancers and the Epimedium sagittatum extract to treat leukaemia. The studies are conducted by Kummalue et al. (2007), on a Thai traditional medicine, found that a fraction from methanolic stem extract of Erycibe elliptilimba Merr. And Chun. (Convolvulaceae), which is widely used in the treatment of various infectious and malignant diseases, has antiproliferative effect on SKBR3 and MDA-MB435 human breast cancer cells. These results indicated that the extract fraction could induce cell cycle arrest in some way. In addition, Pudhom et al. (2007) isolated four novel furanocembranoids from the stem bark of Croton oblongifolius Roxb. (Nagdanti, Euphorbiaceae), which exhibited good cytotoxicity against several human tumor cell lines. A similar study on the Thai medicinal plants found that rhinacanthins-C, -N and -Q, three main
naphthoquinone esters, which were isolated from the roots of Thai medicinal plant; *Rhinacanthus nasutus* (Linn.) Kurz. (*Rhinacanthus nasutus* root, Acanthaceae), induced apoptosis of human cervical carcinoma HeLaS3 cells. Based on these results, their findings demonstrated that rhinacanthin-N suppresses tumor growth *in vivo* (Siripong *et al.*, 2006a, 2006b).

Moreover, a study on the direct relationship between the diet habit and cancer risk by a Taiwanese group in 2001 revealed that the mung bean aqueous extract showed the best hepatoprotective effect on hepatotoxicity. The pathological changes of liver injury were improved by the treatment with all of the legume extracts belonging to Fabaceae family: *Phaseolus aureus* or *Vigna radiate* (L.) R. Wilczek (mung bean), *Vigna angularis* (Willd.) Ohwi and H. Ohashi (adzuki bean), *Castanospermum australe* A. Cunn and C. Fraser ex Hook (black bean) and *Vigna umbellate* (Thunb.) Ohwi and H. Ohashi (rice bean). When compared to silymarin as a standardized drug, these beans are used as foods and folk medicines in Taiwan. In addition, the extract of mung bean acted as a potential hepatoprotective agent in dietary supply (Wu *et al.*, 2001). In view of the vast data available online regarding the bioactive principles from plants against different human cancer cells with proved *in vivo* and *in vitro* studies, it is suggested that dietary prevention coupled with other life-style changes is perhaps the right answer for prevention of cancer and other chronic diseases.

Other commonly used plants by Asian countries people are *Boesenbergia pandurata* (Roxb.) Schltr. (Chinese ginger, Zingiberaceae), *Languas galanga* or *Alpinia galanga* (L.) Willd. (Siamese ginger, Zingiberaceae) and *Citrus hystrix* DC. (kaffir lime, Rutaceae) which are edible plants that are commonly used as flavors or condiments in various Thai food dishes. They are known to exert
strong anti-promoting activity in a test of tumor promoter-induced Epstein-Barr Virus (EBV) activation (Tiwawech et al., 2000).

In Asian countries, herbal formulations prepared from a mixture of plants are often used by traditional medical practitioners for the treatment of cancer (Eum et al., 2005 and Sliva et al., 2003). In herbal medicines containing a mixture of plants, the total herb extract often has better effects than an equivalent dose of an individual plant in the mixture or chemical compounds isolated from the plant material (Thabrew et al., 2005; Wang et al., 2008). One such remedy used by a family of indigenous medical practitioners in Sri Lanka, is a decoction prepared from *Nigella sativa* L. (black-cumin, Ranunculaceae), *Hemidesmus indicus* (L.) W. T. Aiton (East Indian-sarsaparilla, Apocynaceae) and *Smilax glabra* Roxb. (Chinese smilax, Simlacaceae). In Sri Lanka, all three of the plant species in this decoction are used in the preparation of medications for the treatment of boils and other skin conditions. The *in vitro* assay demonstrated that the decoction prepared from a mixture of *N. sativa* seeds, *H. indicus* roots and *S. glabra* rhizome has powerful cytotoxic properties towards human liver cancer cells as assessed by the resulting inhibitory effects. The aqueous extracts of each of the three individual plants used for the preparation of the decoction were shown to be cytotoxic to HepG2 cells (Perera and De Silva, 2002).

In Taiwan, medicinal plants have been historically used as treatment for different kinds of human diseases. Chiang et al. (2004) used the hot water extract of Taiwanese traditionally used medicinal plants to evaluate their *in vitro* anti-leukemic properties. Results showed that *Blumea lacera* (Burm. f.) DC. (Malay Blumea, Asteraceae) exhibited broad anti-leukemic activity at magnitudes ranging from moderate to mild and *Ixeris chinensis* Thunb. Nakai (Asteraceae) is effective at inhibiting the proliferation of K562 cells. Another
study done on the shoot extracts of *Sauropus androgynus* (L.) Merr. (Star gooseberry, Euphorbiaceae) and *Manihot utilissima* Pohl. (Cassava, Euphorbiaceae) suggested that *S. androgynus* shoots and *M. utilissima* shoots have potential as an anticancer agent against breast cancer cell lines (Rahmat *et al.*, 2004).

*In vivo study* in India on *Tinospora cordifolia* (Willd.) Hook. f. and Thomson (*Gulancha tinospora*, Menispermaceae) extract, an Indian medicinal plant, was conducted to explore antitumor promoting activity in a two-stage skin carcinogenesis model. The results strongly suggest that the *T. cordifolia* extract has anti-tumor potential in a two-stage skin carcinogenesis mouse model by recording significant reduction in tumor weight, tumor incidence in comparison to control. Furthermore, cumulative number of papillomas, tumor yield, tumor burden and tumor weight showed significant reduction along with significant elevation of phase II detoxifying enzymes and inhibition of lipid peroxidation in liver and skin in the animals administered with such plant extract was concomitant to carcinogen exposure (Chaudhary *et al.*, 2008).

Turmeric, derived from the plant *Curcuma longa* L. (Zingiberaceae), is a gold-colored spice commonly used in the Indian subcontinent, curcumin, which gives the yellow color to turmeric, was first isolated almost two centuries ago and its structure as diferuloylmethane was determined in 1910 (Jagetia and Aggarwal, 2007). Both turmeric and curcumin were found to increase detoxifying enzymes (Nishinaka *et al.*, 2007), prevent DNA damage and improve DNA repair (Krishnaswamy, 2008), decrease mutations and tumor formation (Ragunathan and Panneerselvam, 2007) and exhibit antioxidative potential in animals (Surh and Chun, 2007). Recently, several molecular targets have been identified for therapeutic/preventive effects of turmeric
These effects are mediated through the regulation of various transcription factors (Shishodia et al., 2007), growth factors (Lin and Chen, 2008), inflammatory cytokines (Bachmeier et al., 2008), protein kinases and other enzymes (Chen et al., 2001). Considering the recent scientific bandwagon, multitargeted therapy is better than monotonetargeted therapy for most diseases, curcumin can be considered an ideal spice for life (Aggarwal et al., 2007).

2.22 *Sphaeranthus indicus* Linn

*Sphaeranthus indicus* Linn. is a medicinal plant widely used in Indian traditional system of medicine for curing various ailments. It grows in rice fields, dry waste places and cultivated lands in tropical parts of India. It is distributed throughout India, Sri Lanka, Africa and Australia from sea level to 1200 m altitude. (Chatterjee and Pakrashi, 2003). All the parts of the *S. indicus* have medicinal uses. In Ayurvedic system of medicine, the whole herb is used in insanity, tuberculosis, indigestion, bronchitis, spleen diseases, elephantiasis, anaemia, pain in the uterus and vagina, piles, biliousness, epileptic convulsions, asthma, leukoderma, dysentery (Chopra et al. 1956) vomiting, urinary discharges, pain in the rectum, looseness of the breasts, hemicrania. (Kirtikar and Basu, 1981). The whole herb is used in Ayurvedic preparations to treat epilepsy, mental disorders and hemicranins (Ambavade et al., 2006 and Jha et al., 2010). Leaves dried in the shade and powdered are used in doses of 20 grains twice a day in chronic skin diseases as an antisyphilitic and a nervine tonic. (Prajapati et al., 2003 and Nadkarni, 2007). Hot water extract of the herb is used as an anthelmintic, as a diuretic, as a fish poison (Paranjape, 2001) and as an aphrodisiac (Kapoor and Kapoor, 1980). Flowers are tonic, cooling, alterative and used in conjunctivitis (Chopra et al. 1996) and give strength to weak eyes (Agarwal, 1997). The oil prepared using the plant root is reportedly
useful in treating scrofula and as an aphrodisiac. The external application of a paste of this herb is beneficial in treating pruritus and edema, arthritis, filariasis, gout and cervical adenopathy (Sahu, 1984). Pulverized seeds have antimicrobial property. It is also stuffed into holes of crabs to kill them. Aqueous extract is poisonous to American cockroaches (Chopra et al., 1958). In unani, the herb is used as a tonic, laxative, emmenagogue, and also it increases the appetite, enriches the blood, lessens inflammation, cools the brain and gives luster to the eye, is good for sore eyes, jaundice, scalding of urine, gleet, biliousness, boils, scabies, ringworm in the waist, diseases of the chest. The plant is traditionally used for diarrhoea. (Girach et al., 1994) The entire plant is used as an emmenogogue (Saha et al. 1961). Hot water extract of the entire plant is used for glandular swelling of the neck and for jaundice. (Ikram, 1981).

2.23 Phytochemistry

A sesquiterpene lactone, 7-hydroxyeudesm-4-en-6, 12-olide, and a sesquiterpene acid, 2-hydroxycostic acid, along with the known compounds, β-eudesmol and ilicic acid, have been isolated from the acetone extract of S. indicus. (Sohoni et al., 1988). Three 7-hydroxyeudesmanolides and two sesquiterpenoids, cryptomeridiol and 4-epicryptomeridiol, have been isolated from this plant. (Rojatkar and Nagasampagi, 1992). Eudesmanoids such as 11α, 13-dihydro-3α, 7α-dihydroxy-4, 5-epoxy-6β, 7-eudesmanolide, 11α, 13-dihydro-7α-acetoxy-3β-hydroxy-6β, 7-eudesm-4-enolide and 3-keto-β-eudesmol have been isolated from S. indicus. (Pujar et al 2000). A bicyclic sesquiterpene lactone + has been isolated from petroleum ether extract of aerial parts of S. indicus. (Singh, 1988). Some other sesquiterpene lactones have also been reported to have been isolated from this plant. (Gogate, 1986). Isolation and characterization of sterol glycoside, the β-d-glucoside of (24S0)-24-ethylcholesta-4, 22-dien-3-β-ol, has also been reported. (Singh et al., 1989). A
flavanoid C-glycoside, namely, 5-hydroxy-7-methoxy-6-C-glycosylflavone has been isolated from the aerial part of *S. indicus* (Mishra *et al.*, 2007). The plant is reported to contain deep cherry colored essential oil having methyl chavicol, d-cadinene, α-ionone, *p*-methoxycinnamaldehyde, α-terpinene, citral, geraniol, geranyl acetate, β-ionone, oiscimene, eugenol, sphaeranthene, sphaeranthol, estragole, Indicusene (Baslas, 1959; Lodha, 2003). A novel isoflavone glycoside from leaves (Yadava and Kumar, 1999) and a new sesquiterpene glycoside and sphaeranthanolide were isolated from the flowers of *s. indicus* and it was found to be an immunostimulant (Shekhani *et al.*, 1990).

### 2.24 Pharmacological activity

#### 2.24.1 Anxiolytic activity

Petroleum ether, alcohol and water extracts (10, 30 and 100 mg/kg, p. o.) from the flowers of *S. indicus* were evaluated for anxiolytic activity, using elevated plus maze, open field test and foot-shock induced aggression test. Petroleum ether extract (10 mg/kg), alcoholic extract (10 mg/kg) and water extract (30 mg/kg) of *S. indicus* flowers produced prominent anxiolytic activity in mice. The study showed an increase in the time spent, percent entries and total entries in the open arm of the elevated plus maze; increased ambulation, activity at center and total locomotion in the open field test and decreased fighting bouts in the foot-shock induced aggression test suggesting anxiolytic activity. (Ambavade *et al.*, 2006). Another study also reported the anxiolytic activity of hydroalcoholic extract of whole herb of *S. indicus* (100 mg/kg, p.o.) in the elevated plus maze test and open field test (Galani and Patel, 2010).

#### 2.24.2 Neuroleptic activity

Neuroleptic activity of petroleum ether, alcohol and water extracts of flowers of *S. indicus* (30, 100 and 300 mg/kg, i.p.) were evaluated using
apomorphine induced cage climbing and catalepsy in mice model. Only the petroleum ether extract (300 mg/kg, i.p.) reduced total time spent in apomorphine induced cage climbing. Aqueous (300 mg/kg, i.p.) and alcoholic (300 mg/kg, i.p.) extracts showed catalepsy while petroleum ether extract was devoid of it (Mhetre et al., 2006).

### 2.24.3 Sedative effect

The sedative potential of hydroalcoholic extract of whole herb of *S. indicus* (100, 200 and 500 mg/kg, p.o.) has been reported using experiments in which it reduced locomotor activity of mice, exploratory activity and potentiated pentobarbital induced sleep in mice (Galani and Patel., 2009).

### 2.24.4 Immunomodulatory activity

The immunomodulatory activity of *S. indicus* was explored by evaluating its effect on antibody titre titer, delayed type hypersensitivity response, phagocytic function and cyclophosphamide-induced myelosuppression in mice. Administration of methanol extract and its fractions (100 and 200 mg/kg, p.o.) showed immunostimulating activity. Methanol extract, and petroleum ether, chloroform and remaining methanol fractions of flower heads of *S. indicus* Linn. were found to be effective in increasing the phagocytic activity, haemagglutination antibody titre and delayed type hypersensitivity, whereas only remaining methanol fraction was found active in normalizing total WBC levels in the case of cyclophosphamide-induced myelosuppression in mice. (Bafna and Mishra, 2004) Eudesmanolide type of sesquiterpene from *S. indicus* was reported to have immunostimulating activity. (Shekhani et al 1990).
2.24.5 Antioxidant activity

In an in vitro study, ethanolic extract of *S. indicus* (1000 µg/mL) showed maximum scavenging of the radical 2, 2-azinobis- (3-ethylbenzothiazoline-6-sulfonate) (ABTS), 1, 1-diphenyl, 2-picryl hydrazyl (DPPH), superoxide and nitric oxide radical. The extract also showed moderate scavenging activity of iron chelation. (Shirwaikar *et al.*, 2006). In an in vivo study, methanolic extract of *S. indicus* exhibited a significant antioxidant effect showing increasing levels of superoxide dismutase, catalase, and glutathione peroxides by reducing malondialdehyde levels in rats (Tiwari and Khosa, 2009).

2.24.6 Anti-inflammatory activity

The herb showed anti-inflammatory activity by suppressing the capacity of *Propionibacterium acnes* induced reactive oxygen species and pro-inflammatory cytokines, the two important inflammatory mediators in acne pathogenesis. To prove the anti-inflammatory effects of *S. indicus*, polymorphonuclear leukocytes and monocytes were treated with culture supernatant of *P. acnes* in the presence or absence of the herb *S. indicus* (5 and 50 µg/mL). This caused a smaller, still significant, suppression of reactive oxygen species. The aqueous extract obtained from the root of *S. indicus* was found to be moderately active in down-regulating *P. acnes* induced TNF-α and IL-8 production. (Jain and Basal, 2003). Another study has also reported its anti-inflammatory activity. (Heinrich *et al.*, 1998).

2.24.7 Analgesic and antipyretic activity

Petroleum ether, benzene, chloroform, ethanol and triple distilled water extracts of whole plant of *S. indicus*, obtained by successive solvent extraction, were screened for analgesic and antipyretic activity (200 and 400 mg/kg, p.o.) using Eddy's hot plate, tail immersion and brewer's yeast induced pyrexia
methods, respectively. The petroleum ether, chloroform and ethanol extracts showed significant analgesic activity at both the doses from 1 hour onward as compared to the standard drug diclofenac sodium. The chloroform and ethanol extracts showed potential significant antipyretic activity from 1 hour onward, whereas aqueous extracts exhibited this activity from 2 hours onward as compared to the standard drug paracetamol amongst various extracts. (Nanda et al., 2009).

2.24.8 Mast cell stabilizing action

Ethanol extract (150 and 300 mg/kg) and ethyl acetate extract (100, 150 and 300 mg/kg) of *S. indicus* showed better protective action of mast cell degranulation in sheep serum induced allergy test and compound 48/80 induced allergy (Mathew et al., 2009).

2.24.9 Antihyperglycemic activity

The 50% ethanolic extract of plant was reported to have hypoglycemic activity. Anti hyperglycemic effect of alcoholic extract of *S. indicus* was evaluated in the nicotinamide (120 mg/kg, i.p.) and streptozotocin (60 mg/kg, i.p.) induced diabetes in rats. Fasting plasma glucose levels, serum insulin levels, serum lipid profiles, magnesium levels, glycosylated hemoglobin, changes in body weight and liver glycogen levels were evaluated in normal and diabetic rats. Fasting normal rats treated with the alcoholic extract of *S. indicus* showed significant improvement in oral glucose tolerance test. Oral administration of *S. indicus* for 15 days resulted in a significant decrease in blood glucose levels and increase in hepatic glycogen and plasma insulin levels (Prabhu et al., 2008).
2.24.10 Hepatoprotective activity

The hepatoprotective effect of aqueous and methanolic extracts of flower heads of *S. indicus* on acetaminophen-induced hepatotoxicity was studied in rats. A significant decrease in liver function markers such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), acid phosphatase (ACP) and alkaline phosphatase (ALP), bilirubin and total protein, was observed while using methanolic extract of *S. indicus* (300 mg/kg, p.o.) in comparison with the same dose of aqueous extract. This fact was also confirmed by studying the liver histopathology of treated animals. (Tiwari and Khosa, 2009). Moreover, the methanolic extract of *S. indicus* enhanced the activities of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase and diminished the amount of lipid peroxides against acetaminophen-induced hepatotoxicity in these animals. (Nayak *et al.*, 2007).

2.24.11 Wound healing activity

Ethanolic extract of aerial part of *S. indicus* Linn. was evaluated for wound healing activity in guinea pigs. The cream containing the extract was applied *in vivo* on the paravertebral area of six excised wounded models once a day for 15 days. The cream significantly enhanced the rate of wound contraction and the period of epithelialization and this effect was comparable to neomycin. (Sadaf *et al.*, 2006). Various ointments of ethanolic extract of flower head of *S. indicus* in various proportions were screened for the assessment of wound healing activity in albino rats. Based on the comparison made of the wound healing activity of various formulations, the formulation comprising 2% (w/w) alcoholic extract of flower head of *S. indicus* was found to be superior to that of control and standard formulation. Hydroxyproline content was also found greater in healed wounds as compared to control and standard formulation (Jha *et al.*, 2009).
2.24.12 Antimicrobial activity

A bicyclic sesquiterpene lactone isolated from the petroleum ether extract of the aerial part of the *S. indicus* was reported to have antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Fusarium* sp., *Helminthosporium* sp. and other microorganisms (Singh, 1988). Antimicrobial activity of alkaloidal and nonalkaloidal fractions of alcoholic extract of flowers has also been reported (Shaikh et al., 1986).

2.24.13 Antibacterial and antifungal activities

Alcohol and water extracts of *S. indicus* were reported to have antibacterial activity against *Alternaria solani*, *Fusarium oxysporum* and *Penicillium pinophilum* (Dubey et al., 2000). Ethanol extract of *S. indicus* has antibacterial activity against enteropathogens (Vijaya and Anathan, 1997). Aerial parts of *S. indicus* show antibacterial activity against *Bacillus cereus* var. *mycoides*, *Bacillus pumilus*, *Bacillus subtilis*, *Bordetella bronchiseptica*, *Micrococcus luteus*, *S. aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae* and *Streptococcus faecalis*. Essential oil from the leaves of *S. indicus* has antibacterial activity against *Salmonella paratyphi* A, *Salmonella paratyphi* B, *Salmonella paratyphi* C, *Schigella Flexneri*, *Salmonella Enteritidis*, *Salmonella typhimurium*, *Shigella sonnei* and *Vibrio cholera*. (Garg and Kasera, 1983). The fruits of *S. indicus* exhibited excellent antibacterial activity against gram positive as well as gram negative bacteria (Naqvi et al., 1998). It also possesses antifungal property (Garg and Kasera, 1982). Hexane extracts of flowers aerial parts of *S. indicus* exhibited antibacterial and antifungal activity. The essential oil of *S. indicus* has been reported for its antifungal activity against plant pathogenic fungi (Rao et al., 1971). Petroleum ether, acetone, methanol (90%) and water extracts of flowers were tested for antibacterial and antifungal activities by diffusion method in bacterial and fungal test cultures. All the
extracts showed considerable antibacterial and strong antifungal activities. (Lalla et al., 2005). In another study, \textit{n}-hexane, benzene, chloroform, ethylacetate and acetone extracts of aerial parts and flowers of \textit{S. indicus} were tested for antibacterial and antifungal activities using \textit{in vitro} disk diffusion method at concentrations of 5, 2.5 and 1.25 mg/disk. The \textit{n}-hexane extract of flowers showed significant activity against \textit{S. aureus} and \textit{Candida albicans} (Duraipandiyan et al., 2009).

2.24.14 Antiviral activity

Methanol extract of \textit{S. indicus} showed inhibitory activity against mouse corona virus and herpes simplex virus at a concentration as low as 0. 4 \(\mu\text{g/mL}\) (Vimalanathan et al., 2009). The plant extract also exhibited antiviral activity against vaccinia and ranikhet viruses (Dhar et al., 1968).

2.24.15 Psychotropic activity

The hydroalcoholic extract of \textit{S. indicus} was evaluated for its neuropharmacological effects in rats and mice. The extract at doses of 100, 200 and 500 mg kg\(^{-1}\) when administered to mice by the oral route, showed significant reduction of spontaneous motor activity, exploratory behaviour and prolonged pentobarbital sleeping time in the mice. Neuroleptic potential of \textit{S. indicus} extract was observed by the results in which \textit{S. indicus} antagonized apomorphine-induced stereotypy in mice, produced catalepsy and potentiated haloperidol-induced catalepsy in rats. Furthermore \textit{S. indicus} had no effect on motor-coordination as determined by the rota-rod test. These results provide evidence that the hydroalcoholic extract of \textit{S. indicus} may contain psychoactive substances that are sedative in nature with possible neuroleptic properties (Galani and Patel, 2009).
2.24.16. Larvicidal action

Acetone extracts of root and leaves of the plant (at concentrations of 750 and 1000 ppm) were shown to cause more than 50% mortality in a predominant Indian mosquito species which acts as a vector of filarial worm. Larvicidal activity was found to be higher in root extract than leaves extract (Hameed and Shah, 2003). Purified fraction of acetone extract of S. indicus showed mosquito larvicidal effect. Methanolic extract of S. indicus showed repellent and feeding deterrent activities against Tribolium castaneum at 1% concentration. Complete feeding deterrent activity was observed at 5 mL dose, whereas repellent activity was noticed at 4 mL dose (Tiwari and Saxena, 2003).

2.24.17. Macrofilaricidal activity

The methanolic extract of S. indicus (1-10 mg/mL) was screened for in vitro macrofilaricidal activity by worm motility assay against adult Setaria digitata, the cattle filarial worm. It showed macrofilaricidal activity at concentrations below 4 mg/mL and an incubation period of 100 minutes (Nisha et al., 2007).

2.24.18 Nematocidal action

It produced toxic effects on the second and fourth instar larvae of Calex quinquefasciatar mosquito at 100-500 ppm concentration. The fourth instar larvae were more susceptible than the second instar larvae. (Sharma and Saxena, 1996) Methanolic extract of dried fruit of the plant is reported to have nematocidal activity (Ali et al., 1991).

2.24.19 Bronchodilatory effect

The methanolic extract of whole plant of S. indicus Linn. and its various fractions (87 and 174 mg/kg, p.o.) were tested for their bronchodilatory effect against histamine-induced acute bronchospasm in guinea pigs. The methanolic extract and its fractions, viz., petroleum ether, benzene, chloroform and ethyl
acetate exhibited significant protective action against bronchospasm induced by histamine in guinea pigs. (Sarpate et al., 2009).

2.24.20 Antihyperlipidemic activity

Antihyperlipidemic activity of alcoholic extract of S. indicus Linn. flower heads in atherogenic diet induced hyperlipidemia was studied in rats. S. indicus extract (500 mg/kg/day, p.o. for 8 days) caused a marked decrease in body weight, total cholesterol, triglyceride, and low density lipoprotein and very low density lipoprotein. A significant increase in the level of high-density lipoprotein was observed after treatment with S. indicus extract (Pande and Dubey, 2009).

2.24.21 Renoprotective effect

The ethanolic extract of S. indicus was evaluated for nephroprotective screening in gentamicin-induced acute renal injury in rats. Gentamicin-induced renal injury resulted in elevated biochemical markers, namely, blood urea and serum creatinine followed by a decrease in total protein and serum albumin. The histopathologic feature was that of acute tubular necrosis. The ethanolic extract of S. indicus at a dose level 300 mg/kg was found to normalize the above mentioned biochemical markers and bring about near to normal recovery in the kidneys as evidenced microscopically (Srinivasan et al., 2008).

2.24.22 Miscellaneous activity

Extract of S. indicus has been reported to inhibit hyaluronidase (Nanba et al., 2006). The alcoholic extract of flowers of S. indicus is reported to have hypotensive, peripheral vasodilatory and cathartic activities (Srivastav et al., 1971). The plant is also reported to have anticancer activity and antiprotozoal activity against Entamoeba histolytica (Dhar et al., 1968).
S. indicus Linn. is widely distributed throughout India. The plant appears to have a broad spectrum of activity on several ailments. Various parts of the plant have been explored for anxiolytic activity, neuroleptic activity, immunomodulatory activity, anti-inflammatory activity, mast cell stabilizing action, antihyperglycemic activity, hepatoprotective activity, larvicidal action, bronchodilatory effect, antihyperlipidemic activity, renoprotective effect and many other miscellaneous activities. It is reported to contain eudesmanoids, eudesmanolides, sesquiterpene lactone, sterol glycoside, flavanoids, and essential oil. The pharmacological studies reported in this review confirm the therapeutic value of S. indicus Linn. However, less information is available regarding the clinical, toxicity, and phytoanalytical properties of this plant. Several phytochemical studies have been reported but still it needs to progress. With the availability of primary information, further studies can be carried out like clinical evaluation, phytoanalytical studies and toxicity evaluation. The plant is preclinically evaluated to some extent; if these claims are scientifically evaluated clinically, then it can provide good remedies and help the mankind in various ailments.