INTRODUCTION

Cancer is a class of diseases characterized by uncontrolled proliferation of the cells. Most cancers are originated from a single cell that has experienced an initial mutation of which the progeny must undergo further changes, requiring numerous additional mutations. Normal cells become cancerous through a process called transformation, leading to the uncontrolled growth of the cancer cells, which produce tumors or neoplasms. As long as the tumor remains intact and the cells do not try to invade other parts of the body, the tumor is called benign. Tumors become dangerous, and potentially deadly, when some of the cells develop the ability to leave the main tumor mass and migrate to other parts of the body, where they form new tumors. Tumors like these are malignant, spreading the cancer by a process known as metastasis. The danger associated with all tumors is that they will switch from benign to malignant before being detected.

Progression of the cancer is divided into five stages according to the size of the tumor as well as their ability to migrate from the original site: Stage 0 to Stage IV. Stage 0 is non-invasive and the tumor at this stage remains localized at the original site. Stages I and II mark the period when the cancer becomes malignant and begins to spread. At stage I, the size of the tumor is not more than 1 inch in diameter and it does not spread beyond the organ or tissue in which it first appeared. Stage II tumors are still small, their size not more than 1 inch. But they have begun to spread to
A tumor that has increased its size to 2 inches but has not begun to spread from the original site is still at Stage II. A tumor at Stage III is locally advanced one. It has attained the diameter of at least 2 inches and has spread to the surrounding tissues. Stage IV tumor is fully metastatic cancer. The cancer has spread to many other tissues and organs of the body.

Technological advances in the recent past have drastically contained most of the infection related diseases but the opposite holds true in respect of cancer. The present day increase in lifespan coupled with modern lifestyle and pollution culminated in the spurt of this dreaded disease. In fact, there is gradual yet consistent increase in the incidence of cancer the world over. The World Health Organization reports that in 2000, 5.3 million men and 4.7 million women developed malignant tumor and altogether 6.2 million died from the disease. The disease was responsible for 12 percent of the nearly 56 million deaths worldwide from all causes. In 2008, 12.6 million new cancer cases were reported out of which 7.6 million patients died due to this disease and the death from cancer accounts for 13% of all deaths. Thus, the cancer burden the world over is quite alarming nowadays. What is more alarming is that this figure is going to increase by 50% in 2020 to 15 million cancer patients as predicted by World Health Organization (Jemal et al. 2011). In India, there are 9.7 lakh new cases of cancer and this number is projected to increase to 11.4 lakh by 2020 (Rammnath et al. 2010). The National Cancer Registry Programme taken up under Indian Council of Medical Research reported that there are 3969 cancer incidence in
Manipur during the period 2006-2008 out which 742 patients have died with lung cancer contributing maximum number of death in both males and females, followed by stomach and nasopharyngeal cancers in males and cervix-uteri and breast cancers in females.

Cancers are classified according to the tissue and cell type from which they arise. Cancers that develop from epithelial cells are called carcinomas, those arising from connective tissues or muscles are called sarcomas. Leukemia and lymphoma are the cancers of blood and lymph gland respectively (Souhami & Moxham 1990). More than 90 percent of all human cancers are carcinomas. Among the different types of cancers, breast cancer is the most common diagnosed as well as leading cause of cancer death in the world for women. In 2008, 13.83 lakh new cases were diagnosed with breast cancer and out of this 4.5 lakh patients died. The disease accounted for 23% of all new cancer cases in females with a dead toll accounting for 14%. Colon and cervical cancers followed the breast cancer in the most widely diagnosed cancer cases in women with a total case of 5.7 lakh and 5.29 lakh respectively while the lung and colon cancers are the leading causes of cancer death in women after breast cancer with a death toll of 4.27 lakh and 2.88 lakh respectively. In males, lung cancer is the most widely occurring cancer with a total of 10.9 lakh patients in 2008 out of which 9.5 lakh patients died. Prostrate and colon cancers followed the lung cancer in respect of occurrence with 9.03 lakh and 6.63 lakh new cases while liver and
stomach cancers followed lung cancer in cancer mortality in males with 4.78 lakh and 4.64 lakh patients in 2008 respectively (Jemal et al. 2011).

Treatment of cancer differs with the difference in the types of cancer as well as their stage. Different methods like surgery, radiotherapy, chemotherapy, hormone therapy, photo-dynamic therapy, immunotherapy, monoclonal antibodies therapy, gene therapy etc. are used currently, single or in combination of two or more, for the treatment of cancer. The most common mode of treatment for cancer includes radiotherapy, chemotherapy and surgery. In radiation therapy, high energy radiations are used for killing cancer cells by damaging their DNA beyond being able to repair by the cells (Lawrence et al. 2008). When radiation therapy is given with a curative intention, it is given alone or with chemotherapy or surgery or both. In chemotherapy, certain drugs are used for the treatment of cancer some of which damage the mitotic spindle resulting in the disruption of the cell cycle progression of the cancer cells. Vinblastine, vincristine and taxol are included in this category.

Some other drugs like methotrexate, mercaptopurine, and fluorouracil block the synthesis of DNA and RNA. Still other drugs like cisplatin, doxorubicin and detoposide damage the DNA. For the treatment of localized tumors, surgery is used, either alone or in combination with chemotherapy and radiotherapy. Nowadays, an advanced method of surgery called cryosurgery is used in the treatment of localized tumors. This treatment is highly localized, with minimal damage to surrounding tissues, and can be repeated many times. The main disadvantage of this procedure is
that it can be used only on tumors confined to a small area. In addition, cancer cells that have separated from the main tumor mass will be missed.

Among the various methods of therapies used in the treatment of cancer, chemotherapy plays a very important role. However, this therapy has a number of detrimental side effects including fatigue, nausea, vomiting, pain, hair loss, anemia, infection and blood clotting problems. Because of these negative side effects, efforts have focused in identifying new molecular targets that would allow limited cancer treatment side effects (Blagosklonny 2004, Crossman & O’Brien 2004, Ciardiello et al. 2004). The new and recent approach of chemotherapy serves as an attractive alternative to control malignancy (Kapadia et al. 2000). In search for new cancer therapeutics with low toxicity and less side effects, drugs derived from the plants which are used as traditional medicine are promising candidates.

Plants have been used for the treatment of various diseases of human beings and animals since times immemorial. The first written records on the medicinal uses of plants appeared in about 2600 B.C. from the Sumerians and Akkaidians (Samuelsson 1999). 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. The Chinese Materia Medica, which describes more than 600 medicinal
plants, has been well documented with the first record dating from about 1100 B.C. (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 herbal drugs. Dioscorides, the Greek physician (100 A.D.), described in his work “De Materia Medica” more than 600 medicinal plants (Samuelsson 1999) which maintain health and vitality of individuals, and also cure diseases, including cancer without causing toxicity.

More than 50% of all modern drugs in clinical use are of natural products, many of which have the ability to control cancer cells (Rosangkima & Prasad 2004). According to WHO estimates, more than 80% of people in developing countries depend on traditional medicine for their primary health needs. A recent survey shows that more than 60% of cancer patients use vitamins or herbs as therapy (Madhuri & Govind 2008, Sivalokanathan et al. 2005). Over the past decade, herbal medicines have been accepted universally, and they have an impact on both world health and international trade. Hence, medicinal plants continue to play an important role in the healthcare system of a large number of the world’s population (Akerele 1988). Medicinal plants are the most exclusive source of life saving drugs for the majority of the world’s population. Modern pharmacopoeia still contains at least 25% drugs derived from plants and many others, which are synthetic analogues, built on prototype compounds isolated from plants. Interest in medicinal plants as a re-emerging health aid has been fuelled by the rising costs of prescription drugs in the
maintenance of personal health and well-being and the bio-prospecting of new plant-derived drugs. Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of drugs and chemotherapeutics from these plants as well as from traditionally used herbal remedies (Lucy & Edgar 1999).

The use of plant products in the treatment of cancer have been of recent interest (Bauer 2000). Isolation of the vinca alkaloid, vinblastine and vincristine from the Madagascar periwinkle, Catharanthus roseus G. Don. (Apocynaceae) introduced a new era in the use of plant material as anti-cancer agent. These 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 leukemia, lymphoma, advanced testicular cancer, breast and lung cancer and Kaposi’s sarcoma. Discovery of paclitaxel 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 M., Taxus baccata L.) have been used by several Native American Tribes for the treatment of some non-cancerous cases (Cragg & 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,

The basic characteristics of cancers in the human and in other vertebrates are more or less same. They show autonomous growth, disregarding the social order of the multicellular organisms from which they arise. They invade locally, destroying the neighboring cells and tissues. They can spread in the organisms and form metastases.
If left untreated, cancers will almost lead to the death of the individual that they form part of. Humans and animals alike fall victim of this. Therefore, animal experiments remain essential in understanding the fundamental mechanisms underpinning malignancy and to discover improved methods to prevent, diagnose and treat cancer. By using animal model, the interaction between the tumor and host animal as well as the drugs and the host animal can be studied. Among the animals, rodents including mice are widely used in the experiments. There are many advantages in using this animal in the experiments. This small mammal has been in contact with the human beings for a long time (Berry 1970, Moriwaki et al. 1994). They are easy to handle and bread all the year round with a short gestation period. They deliver relatively large progenies and tolerate inbreeding which is very essential for experiments of which genetic homogeneity is required. One advantage of using mice is that its genome has been sequenced completely (Waterston et al. 2002) which will allow its comparison with other mammalian genomes including human beings.

In experimental cancer chemo-preventive studies, attempts are made to identify agents which can prevent the initiation of tumors, delay or arrest the development of tumors, extend cancer latency periods, reduce cancer metastasis and mortality and prevent recurrence of secondary tumors. The major focus of research in chemotherapy for cancer in recent times includes identification, characterization and development of new and safe chemo-preventive agents (Kellof 2000).
The emphasis on screening new agents derived from pure natural products and their extracts, for antitumor activity, necessitates in vitro evaluation in cell culture and then in vivo in appropriate tumor bearing animal models. Evaluation of the cytotoxic potentials of the plants can be done in vitro and cells cultured are used for such type of experiments. In vitro testing of the cytotoxic activities is comparatively fast and large number of samples can be tested in a very short period. By using in vitro models to address questions of chemo-sensitivity, one limits the study to cell intrinsic properties found in cultured cells, which simplifies the system and focuses the initial investigation on tumor cell responsiveness. Testing of chemo-sensitivity using reduction of tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide or MTT) is a sensitive, accurate and efficient in the in vitro evaluation of anti-cancer agents (Mosmann 1983). The MTT is a pale-yellow substrate that produces a dark blue formazan product when incubated with live cells. The amount of formazan produced is directly proportional with the number of live cells, and dead cell including immediately lysed ones does not have any role in the formazan production. This formazan can be solubilised and measured its optical density. For any given tumor cell line the optical density of the solubilised formazan product obtained after incubating tumor cell lines with MTT is directly proportional, over a large range, to the number of cells (Carmichael et al. 1987). The MTT assay for chemo-sensitivity may, therefore, be directly compare with assays counting the total number of cells in the culture before and after a treatment.
Another method which is commonly used nowadays for anti cancer drug screening is the colorimetric cytotoxicity assay also known as SRB assay. First described by Skehan et al. (1990), the assay used sulforhodamin B (SRB) which is a bright-pink aminoxanthene dye with two sulfonic groups that bind to basic amino acid residues under mild acidic conditions and dissociate under basic condition (Lillie 1977). The binding of the SRB is stoichiometric; the amount of dye extracted from the stained cells and its optical density is directly proportional to number of cells and its cellular protein content. The end point of the SRB assay is colorimetric, non-destructive and indefinitely stable and is very rapid (Griffon et al. 1995, Perez et al. 1993). The test is very sensitive that it can detect densities as low as 1000-2000 cells per well in a microtitre plate which is comparable to fluorescent dye staining methods and is superior to those of other protein staining methods using conventional visible dyes (McCaffrey et al. 1988, Skehan et al. 1990). With its high level of sensitivity, adaptability to the 96 well formats and endpoint stability, SRB assay is practical and has been widely used for drug toxicity testing against different types of cancerous and non cancerous cell lines (Monks et al. 1991).

Cell cycle transition is an ordered, tightly-regulated process that involves multiple checkpoints that assess extracellular growth signals, cell size and DNA integrity. Cancer cells differ from normal cells in many important characteristics. These include the loss of differentiation, self-sufficiency in growth signals, limitless replicative potential, increased invasiveness, and decreased drug sensitivity.
(Hanahan & Weinberg 2000). The hallmark of cancer is deranged growth control (Pardee et al. 1978). Checkpoints are defective in cancer cells (Hartwell & Kastan 1994) and control mechanisms are usually lost by mutation. Molecular analysis of human tumors has shown that cell cycle regulators are frequently mutated, which underscores how important the maintenance of cell cycle commitment is in the prevention of human cancer. Defective cell cycle regulation and checkpoint mechanisms have implications for cancer detection and treatment. The frequent loss of cell cycle regulation in human cancer has revealed targets for possible therapeutic intervention. Indeed, restoring proper restriction point control to cancer cells might allow them to return to a quiescent state. Alternatively, it could take advantage of their uncontrolled proliferation to facilitate apoptotic death or to specifically exposure cancer cells to cytotoxic treatments (Chen et al. 1999).

Manipur which is located between 23°47' - 25°41' NL and 92°58' - 94°47' EL is blessed with a wide range of physiographic and eco-climatic conditions and the geographical ‘gateway’ for much of India’s endemic flora. This region represents an important part of the Indo-Burmese mega-biodiversity ‘hotspot’, one of the 32 global biodiversity ‘hotspots’ recognized currently. The region also falls in the biogeographic tri-junction of the Indian, the Himalayan and the oriental landmasses. Manipur is very rich in indigenous conventional medicinal and non-medicinal food plants which contain aromatic and bioactive compounds. These plants have been used in the treatment of various ailments including cancer but they have not been
studied systematically. Attempts have been made to evaluate the anti-cancer property of four plants which have been used for the treatment of cancer in traditional medicine but not investigated systematically.