1.1. *Drug discovery from plants*

Evolutionary pressure made nature to produce diverse and complex organic molecules, a consequence of the interaction between the organisms and also with their environment for enhancement of organism’s survivability and competitiveness. Living organism is not an isolated system; it takes in chemical components from its environment in the form of organic or inorganic or as photons from the sun to generate order within itself. Likewise, humans utilize natural products containing active ingredients either in crude or purified form for various biological purposes including therapeutics. Since, ancient times humans experiment on plants to identify edible and poisonous ones for food or hunting and execution.

“Eat leeks in March and wild garlic in May, and all the year after the physicians may play.”

Traditional Welsh rhyme

“An apple a day keeps the doctor away.”

Traditional American rhyme

The idea that plants have healing power is dated back to prehistory (Stockwell, 1988; Thomson, 1978). The search for new therapeutic drugs from plants is not new, but is a continuing process. These medicinal properties are generally attributed due to the presence of secondary metabolites. Secondary metabolites are produced by the plants not for growth and development but to interact with the environment. For
instance, paclitaxel, an anticancer agent, present in *Taxus brevifolia* is cytotoxic which prevent the plant from being consumed by herbivores. There are approximately 250000 plant species, and these are the potential reservoir of new drugs, with only about 5-15% has been explored for therapeutic purpose (Jachak and Saklani, 2007; Cragg et al., 1993). According to the World Health Organization, more than 80% of the people living in developing countries depend on plant based medicine for health care (WHO, 2002). The Northeast India represents the transition zone between the Indian, Indo-Malayan and Indo-Chinese biogeographic regions and a meeting place of the Himalayan Mountains and Peninsular India. Because of a wide range of physiographic and ecoclimatic conditions the region is blessed with an immense biodiversity and also represents an important part of the Indo-Myanmar biodiversity hotspot. Further, of the total 15000 plants found in India more than 50% plants are available in NE India (Chakraborty et al., 2012), including a number of medicinal plants. The region is inhabited by numerous tribes and sub-tribes with their own set of customs, rituals and folklore medicinal practices which have little or no influence to the outside world. For these people, medicinal plants play an important position in their healthcare. Due to the lack of hospital or primary health care unit during the time of their illness, people use medicinal plants known to them, or else go to the local medicinal practitioner for treatment. The knowledge of specific plant to be used and the mode of application for a particular disease have been passed from generation to generation through oral route. Therefore, NE India is now a place to explore plants of medicinal importance to be used in health sector.
1.2. Antioxidants

While oxidation is a vital process for living especially to generate energy, it is also a double edged sword. Oxidation also generates damaging molecules known as free radicals which are very reactive and rapidly attacks molecules of the nearby cells which in the long run may results many of the degenerative diseases or pathological processes such as aging, cancer, inflammation, and cardiovascular disease (Dröge, 2002; Markesbery, 1997; Loft and Poulsen, 1996).

Antioxidants are molecules that stabilize or deactivate free radicals before they damage the body cells. Beyond the antioxidant capacity of a biological system, generation of prooxidants generated during metabolism and other activities gives rise to oxidative stress. For maintaining health, a balance needs to be maintained between the prooxidants and antioxidants. Studies have shown that a high intake of antioxidants may decrease the risk of onset of various diseases (Seddon et al., 1994; Chasan-Taber et al., 1999; Engelhart et al., 2002). It has been reported that plants are a good source of antioxidants and a number of phytochemicals having antioxidant property has been identified from it. Therefore, current research on antioxidant search is given to plants.

1.3. Microbial infection

Infection is the invasion and multiplication of microorganisms in body tissues, especially that causing local cellular injury due to competitive metabolism, toxins, intracellular replication, or antigen-antibody response (http://medical-dictionary.thefreedictionary.com/infection).

The harm caused by microbial infection is very high in human. Since the discovery of antibiotics in 1950s man has overcome many of the infectious diseases and
every year two to three antibiotics derived from microorganisms are launched (Clark, 1996). But, the problem regarding the traditional antibiotics is its limited effective life span. Further, the people are aware about the over-prescription and misuse of antibiotics. Because of this, many scientists have shown interest towards plant based anti-infective agents. There are 115 publications in PubMed reporting antimicrobial activities of plants during the period between 1966 and 1994. Interestingly, during a decade between 1995 and 2004 the number was double to 307 indicating the importance of plants as anti-infective agents (Rios and Recio, 2005).

1.4. **Cancer**

Cancer is a complex genetic disease, results from the aberration of a series of molecular events controlling the normal cell division and growth control, mortality, the suicide mechanism in cells; the ability of the cells to migrate; the ability of the cells to attract to them a blood supply (Figure 1.1). Cancer is caused mainly by environmental factors that mutate genes encoding critical cell-regulatory proteins.

Mortality due to cancer is unacceptably high and is therefore a worldwide concern. Statistics indicate that the total number of cancer deaths in 2007 was 7.6 million, with the deaths higher in developing countries with 62% than developed countries with 38% (American Cancer Society, 2007). By 2050, 27 million new cases and 17.5 million cancer deaths is expected (American Cancer Society, 2007). Therefore, from various angles researchers are working to reduce the threat of cancer. One mode of treatment of cancer is chemotherapy. Analysis of a number of chemotherapeutic drug revealed that 60% of the clinically approved drugs has been derived directly or indirectly from natural resources (Newman et al., 2003). Vincristine, vinblastine,
camptothecin, irinotecan, etoposide, paclitaxel and homoharringtonine are classic examples of plant-derived compounds.

The call for the discovery of more selective and more effective agents to treat cancer has become more urgent. The new generation of anticancer drugs have targets that affect the signals that promote or regulate the cell cycle and apoptosis (Bcl-2 oncoprotein, p53 tumor suppressor gene etc.). Other targets include angiogenesis (growth factors – VEGF, FGF, and PDGF), cell life span (telomerase) and tumor metastasis (Nam and Parang, 2003)

![Diagram showing the fundamental cellular properties altered during oncogenesis](image)

**Figure 1.1. The fundamental cellular properties are altered during oncogenesis.** Six fundamental cellular traits are transformed from normal cells to become cancer (malignant or tumor) cells, as shown here and occurs in a series of steps.

(Adapted from Hanahan and Weinberg, 2000)
1.4.1. The eukaryotic cell cycle

The sequence of events by which a cell duplicates its genome, synthesises the other constituents of the cell and eventually leads to cellular replication and division is termed cell cycle. The eukaryotic cell cycle consists of two basic stages: interphase and mitosis (M). Interphase is the period between mitotic cell divisions; divided into G\textsubscript{1}, S, and G\textsubscript{2} (Figure 1.2.).

G\textsubscript{1} phase lies between the last mitosis and the start of DNA replication when RNAs and proteins are synthesized. S phase is the period where DNA synthesis or replication occurs, leaving the cell with four copies of chromosome. G\textsubscript{2} phase is the period of the cell cycle between S phase and M phase where cytoplasmic materials necessary for mitosis and cytokinesis are assembled. Mitosis (M phase) is the period by which the replicated chromosomes separate, the chromosomes are evenly partitioned to two daughter cells. Under certain conditions such as starvation or when a tissue has reached its final size, cells stop cycling and remain in a waiting state called G\textsubscript{0}. Such post mitotic cells generally exit the cell cycle in G\textsubscript{1}.

Along the stretch of the cell cycle pathway, there are multiple check points that regulate the cell cycle. These checkpoint controls are essential to ensure cell division occurs correctly and arrest the cell cycle upon the occurrence of undesirable events, such as DNA damage, replication stress, and spindle disruption (Qin and Li, 2003). The primary function of the cell cycle check point is to maintain genomic integrity and balance growth and division. The transition between G\textsubscript{1}/S-phase, S/G\textsubscript{2}-phase and G\textsubscript{2}/M phase is tightly controlled by cell cycle checkpoints (Hartwell and Weinert, 1989). One mode of action of the chemotherapeutic drug is to arrest the cell cycle at the cell cycle check points. Chemotherapeutic drugs can be divided into two main classes based on
their action on the cell cycle: cell cycle non-specific and cell cycle specific. Compounds that are cell cycle non-specific can act at several or all cell cycle phases whereas cell cycle specific drugs act only at particular cell cycle phases (Morgan, 2003).

Figure 1.2. The eukaryotic cells continually progress through four stages of the cell cycle in order to duplicate its chromosomes and divide. During G1 and G2 phases, the cell is actively metabolizing but not dividing. In S (synthesis) phase, the chromosomes duplicate leaving four copies of each type of chromosome. During the M (mitosis) phase, the chromosomes are evenly partitioned to two daughter cells and the division of the cytoplasm (cytokinesis) occurs. Cells that are not in the process of dividing enter the G0 phase. There are multiple checkpoints present throughout the cell cycle to ensure cell division occurs correctly and arrest the cell cycle upon the occurrence of undesirable events, such as DNA damage, replication stress, and spindle disruption.
1.4.2. Telomerases

Eukaryotic chromosome terminates with a special sealing structure known as telomeres that confers protection of chromosome shortening and ensures cell survival. Telomerase, a reverse transcriptase synthesise and maintain the telomeres. In normal somatic cells, which show little or no telomerase activity in synthesizing new telomeres at the ends of replicating chromosomes, the telomeric DNA progressively shortens with each cell division. Extensive shortening of telomeres is detected as a kind of DNA damage; as a result p53 is activated, leading to p53-triggered apoptosis. Most tumor cells, despite their rapid proliferation rate, stabilises by expressing telomerase (Hahn et al., 1999; Hahn et al., 2001). Many researchers believe that telomerase expression is essential for a tumor cell to become immortal, and specific inhibitors of telomerase have been suggested as cancer therapeutic agents. Telomere length in humans is primarily controlled by three major components, hTERT, TEP-1, and hTR. The hTERT promoter site (-181 bp) has two c-Myc binding regions and c-Myc has been shown to regulate telomerase activity by regulating hTERT expression (Hao et al., 2008). In the field of new anticancer drug discovery, one of the most exciting strategies is to design new drugs that target telomerase or telomeres (Hahn et al., 1999; Takakura et al., 1998).

1.4.3. Apoptosis

Apoptosis - the evolutionary conserved process of programmed cell death - is a set of ordered events that enables the selective removal of cells from tissue and is essential for homeostasis and proper function of multicellular organisms (Los et al., 2003). It is activated only by appropriate stimuli. Inhibition of apoptosis can lead to cancer and induction of apoptosis is one way of cancer therapy. Failure to activate
apoptosis represents one of the major obstacles to the successful treatment of cancer with drugs (Cummings et al., 2004). Therefore, targeting components of apoptotic machinery is an essential means for the development of therapeutic strategy.

Two distinct pathways of apoptosis have been identified (Figure 1.3) as the extrinsic or death receptor-mediated pathway requires caspase-8 (Zapata et al., 2001) and intrinsic or mitochondria-initiated apoptosis occurs through caspase-9 (Hockenbery et al., 1990).

Extrinsic or death receptor-mediated pathway

Triggering of a death receptor by an extracellular death signal leads to the activation of the adaptor protein FADD and caspase-8, an initiator caspase that in turn triggers the caspase cascade and execute cell death. In some cells, caspase 8 interacts with the intrinsic apoptotic pathway by cleaving Bid (a proapoptotic member of the Bcl-2 family), leading to the subsequent release of cytochrome-c (Ghobrial et al., 2005).

Intrinsic or mitochondria-initiated apoptosis

The mitochondrion is a crucial control point in the induction of apoptosis. The second pathway is the intrinsic or mitochondrial pathway that when stimulated leads to the release of cytochrome-c from the mitochondria. The release of cytochrome c is preceded by changes in the permeability of the mitochondrial membrane and Bcl-2 family members play a very important role in mitochondrial membrane permeability and cytochrome C release upon apoptotic stimulation. The Bcl-2 family includes proapoptotic members (e.g.Bax) and antiapoptotic members (e.g. Bcl-2) (Reed, 1990). Antiapoptotic Bcl-2 members suppress apoptosis by blocking the release of
cytochrome-c, whereas proapoptotic members induce apoptosis. Thus, the fate of a given cell—survival or death are dependent on the ratio of Bax to Bcl2.

The cytochrome C once released to the cytosol triggers Apaf-1 and binds to procaspase 9 to form apoptosome during which caspase -9 is activated. Active caspase-9 then activates caspase-3, which subsequently activates the rest of the caspase cascade, eventually resulting in apoptosis.

The extrinsic and intrinsic apoptotic pathways are regulated by proteins such as the p53.

1.4.4. The tumor suppressor p53

p53 (protein 53), a nuclear phosphoprotein is a tumour suppressor encoded by the TP53 gene (Matlashewski et al., 1984; Isobe et al., 1986; Kern et al., 1991). It acts as a transcription factor regulating downstream genes important in cell cycle arrest, DNA repair, and apoptosis. The p53 protein is an essential checkpoint that arrests cells with damaged DNA in G₁ or G₂, allowing time for DNA repair before the cell tries to enter the S or M phase. But if the cell is committed to division or the damage cannot be repaired, then p53 triggers a program cell death through the extrinsic and intrinsic apoptotic pathways (Ghobrial et al., 2005). These outcomes have been implicated in an individual's ability to suppress tumors and to respond to many types of cancer therapy. Further, p53-dependent apoptosis promotes deleterious side effects of chemotherapy (Brady and Attardi, 2010).
Figure 1.3. Apoptosis can occur through extrinsic and (or) intrinsic pathway. The extrinsic or death receptor pathway is triggered through the pro-apoptotic death receptor on the cell surface. The intrinsic or mitochondrial pathway is initiated within the cell which when stimulated leads to the release of cytochrome-c from the mitochondria and activation of the death signal. Both pathways converge to a final common pathway involving the activation of caspases that cleave regulatory and structural molecules leading to death of the cell (Adapted from Ghobrial et al., 2005).
1.4.5. Tumor metastasis

Metastasis, the ability of tumor cells to spread to other locations in the body, where a new colony is established. Metastases are the major causes of cancer induce death because eradication of metastatic disease is difficult to treat. It consists of a series of sequential steps which includes invasion of extracellular matrix (ECM), detachment, intravasation, transport in the circulation, extravasation, adhesion to endothelial cells in a new tissue and proliferation (Isaiah J. Fidler, 1990). Controlling metastasis is one of the major challenges in cancer chemotherapy.

1.5. Bioactivity guided isolation

In the field of natural product drug discovery research, searching for the lead compounds from natural products is like burning the hay to find the needle. Bioactivity guided isolation is a typical protocol to locate and isolate the exact bioactive agent from natural origin. It is a step-by-step separation process starting from the crude to lead molecule based on biological activity. Generally, the crude fractions are first tested for biological activity; fractions which show activity are further purified and tested until the key component is identified.

The genus *Cephalotaxus* has received a great level of scientific interest as it contains anticancer potential ingredients (Takano et al., 1996, Takano et al., 1997; Kuo at al., 2000; Morita et al., 2002). Homoharringtonine, an alkaloid isolated from *Cephalotaxus harringtonia* was recently approved by USFDA for the treatment of adult patient with chronic myeloid leukemia (http://www.fda.gov/NewsEvents/)
Newsroom/Press Announcements/ucm325895.htm). In view of the importance of the Cephalotaxus genus, we searched for unexplored species within this genus to check for anticancer potential components. Cephalotaxus griffithii Hook. f., a gymnosperm in the family Cephalotaxaceae, is another important species commonly known as Griffith's plum yew (Figure 1.4). It is a shrub or small tree and found up to an altitude of 2000 m and is distributed in North East India, western Sichuan province in China, and Myanmar (Shanker, 2008). C. griffithii mostly remained unexplored due to remoteness of location and limited accessibility of the habitat of this species. So far, only two studies from C. griffithii have been attempted. Kamil et al. (1982) isolated and characterized six flavonoids, Phutdhawong et al. (2002) carried out chemical analysis of volatile oil from needles of C. griffithii.
1.7. *Oroxylum indicum* (L.) Benth. ex Kurz

*Oroxylum indicum* (L.) Benth. ex Kurz (Bignoniaceae), commonly known as Midnight horror, is a deciduous tree well known among ethnic communities of South Asia including India for its medicinal property (Figure 1.5). The tree was distributed throughout the greater part of India but now the existence of *Oroxylum indicum* in natural population is highly threatened and has been categorized as vulnerable (Gokhale and Bansal 2006). A good stand of this plant is found to occur in the mountains of

![Image of Oroxylum indicum](image-url)
North East India. Different ethnic communities of the region use this plant for the treatment of various ailments and as food supplement. For example, Mao (2002) reported that the bark is taken for curing gastric ulcer and a paste made of the bark powder is used for treating mouth cancer, scabies and other diseases. The paste of the bark powder has also been found to be effective when applied to wounds to kill maggots, and decoction of fresh bark fed for de-worming of animals. Scientific research has progressively accumulated knowledge on biological potential of extracts of *Oroxylum indicum* stem bark (OIB) as antioxidant (Mishra et al., 2010), antimicrobial (Islam et al., 2010; Das and Choudhury 2010), hepatoprotective (Bichitra et al., 2011) and in exhibiting cytotoxic activity against B-16 (murine melanoma), HCT-8 (human colon carcinoma), CEM and HL-60 (leukemia) tumor cell lines (Costa-Lotufo et al., 2005) and HeLa cells (Siriwatanametanon et al., 2010). However, detailed reports on the systematic study of OIB to investigate the bioactive principle with reference to a specific biological activity are not available.
1.8. Research aim

The overall aim of this research is to understand the multiple pharmacological potential of *Cephalotaxus griffithii* and *Oroxylum indicum* and to elucidate the bioactive principle with respect to anticancer activity.

1.9. Research objectives

1. Extraction and testing of extracts for biological activity.
2. Fractionation of the most potential bioactive extract (cytotoxic extract).
3. Screening of different fractions / compounds for their cytotoxic activity and mechanism(s) study of cell death.
4. Chemical characterization of the most potential fractions/ compounds.