Chapter 1

Introduction

“*For every scientist and medical professional hacking at the roots of cancer, there are tens of thousands hacking at the branches or even studying the leaves of the tree.*” - Henry David Thoreau

1.1 CANCER

Cancer is demarcated as an abnormal growth of cells caused by multiple changes in gene expression leading to a deregulated balance of cell proliferation and cell death. This disease is the contributing factor for major mortality on global level next to cardiovascular diseases.

Cancer is a large group of disease, perhaps even more than hundred. The development of cancer is a sequential multi-step process, that onset from the elementary building block of the organs of body – *the cells*. When the cells continue multiplying irrespective of requirement, they result in a mass/growth of tissue called a tumor. These growths are considered either benign or malignant. Benign growth is termed as non-cancerous and rarely threatens life and do not spread to other parts of the body. Whereas malignant growth is dubbed as cancerous and cells from malignant tumors evolve into a population of cells that can invade normal tissues and affect distant sites by spreading through blood or lymph, causing significant morbidity and mortality. This systemic process of spreading is termed as metastasis. However, the cancer that originates from this process is still referred by the organ of origin. For instance, if cervical cancer spreads to the lungs, it is still called as cervical cancer and not as lung cancer. Different types of cancer arise from variations in the age of onset, rate of growth, state of cellular differentiation, diagnostic detectability, invasiveness, metastatic potential, response to treatment, and prognosis. In many cases causes of cancer are not clearly defined, but both the external (e.g., environmental chemicals and radiation) and internal (e.g., immune system defects, genetic predisposition) factors play a major role. These causal factors may act together or individually to initiate (the initial genetic insult) and promote (stimulation of growth of initiated cells) carcinogenesis.
1.2 PROCESS OF CARCINOGENESIS

Carcinogenesis is a multistep process and characterized by a progression of changes on cellular and genetic level that ultimately reprogram a cell to undergo uncontrolled cell division, in which as many as ten distinct mutations may accumulate before it becomes cancerous.

The cell cycle is regulated by a large number of cellular genes that are expressed or exhibited at different stages of the cycle. These genes code for/or determine growth factors, growth-factor receptors and proteins that control gene functions and cell survival. In all cancers, the process of carcinogenesis starts when the genes involved in cell cycle control and regulation are malfunctioned due to DNA damages caused by exposure to chemicals and radiation. Usually the normal cells with damaged DNA either get repaired or perish. But in the case of cancer, the cell cycle gets distorted due to activation of oncogenes or by the inactivation of tumor suppressor genes. Activation of oncogenes drives abnormal, unregulated cell proliferation and lead to tumor formation, while inactivation of tumor suppressor genes fails to avert the same. Mutations of the tumor suppressor gene such as p53 are found in about 50% of the existing human cancers. Viruses also can induce carcinogenesis by introducing new DNA sequences (oncogenes). Generally, types of carcinogenesis involve chemical oncogenesis, radiation oncogenesis, viral oncogenesis, nutritional oncogenesis, hormonal oncogenesis and genetic oncogenesis, while the latter three are less prevalent than the others.

In a study involving experimental animals, three stages of carcinogenic processes have been identified. They are: (1) initiation, where DNA is irreversibly altered; (2) promotion, which is the multiplication of altered cells; and (3) progression, which involves chromosomal changes, high growth rate, invasiveness and potential to metastasize. There is usually a long time lag between exposure to a carcinogen and the occurrence of cancer. The neoplastic changes start with a reversible increase in cell division caused by an external stimulus, such as hormonal imbalance or chronic irritation that is termed as hyperplasia. The cells then attain a stage, termed as dysplasia, where a series of changes get incorporated slowly to give rise to cancer-like properties to the cells from the properties of normal cells. These
cells are excessively proliferative and are characterized by loss of normal tissue arrangement and cell structure that upon continuous division and accumulation evolve as invasive/metastatic cells. The overview of carcinogenic process is pictorially represented in Fig. 1.1.

![Fig. 1.1: Process of carcinogenesis.](image)

1.3 CLASSIFICATIONS OF CANCER BASED ON TISSUE OF ORIGIN

Cancers are classified by the type of cell that the tumor cells resemble and are consequently presumed to be the origin of the tumor. Based on the tissue of origin, tumors are classified into the following four broad categories.

1.3.1 CARCINOMA

The tumors that principally originate from epithelial cells of ectoderm or endoderm are called carcinoma. The solid tumors in the nerve tissue and the tissues of body surfaces, or their attached glands are examples of carcinomas. These include cervical, breast, skin and brain carcinomas. About 85% of cancers are carcinomas.

1.3.2 SARCOMAS

The term sarcoma is used when the tumor principally consists of connective tissue cells, which are of mesodermal origin. They are solid tumors growing from connective tissue (cartilage, bone and muscle). Although they account for most of the cancers studied in laboratory animals, they constitute only about 2% of human cancers.
1.3.3 LYMPHOMA

The cancers in which there is an excessive production of lymphocytes by the lymph nodes and spleen are termed as lymphoma. Hodgkins’ disease is an example of a lymphoma. Lymphomas constitute about 5% of human cancers.

1.3.4 LEUKEMIA

Leukemia is a type of cancer of the blood or bone marrow characterized by an abnormal increase of immature white blood cells called "blasts". They constitute about 4% of human cancers.

In addition to the types of cancer mentioned above, there might be mixed malignant tumors, e.g. tumors arising from both ectodermal and mesodermal origin.

1.4 TYPES OF CANCER BASED ON ORGAN OF ORIGIN

The type of cancer is usually referred by the organ of origin and not by the organ of occurrence. There are over 100 types of cancer affecting various parts of the body. Each type of cancer is unique with its own causes, symptoms and methods of treatment. A few important types of cancer based on the organ of origin are:

- Blood cancer
- Bone cancer
- Brain cancer
- Breast cancer
- Digestive/gastrointestinal cancers
- Endocrine cancers
- Eye cancer
- Genitourinary cancers
- Gynaecologic cancers
- Head and neck cancer
- Respiratory cancers
- Skin cancer
1.5 CANCER INCIDENCE

Globally, cancer is a major disease with its extensive variations in causative factors, incidence, disease burden, mortality and survival. As a leading cause of death, cancer accounted for 12.7 million new cases and 7.6 million deaths in 2008. Out of which, 70% deaths were in low- and middle-income countries (GLOBOCAN, 2008). According to Parkin et al. (2005), the number of new cancer cases may increase from an estimated 10 million cases in 2000 to 15 million cases in 2020 and could reach 24 million cases by 2050. In United States and other western countries, cancer rates seemed to gradually decrease, but they were found to increase in less developed and developing countries. The cancer type with high frequency of diagnosis is recognized as a most common cancer which might vary considerably across countries (Jemal et al., 2010). Breast cancer in females and lung cancer in males were reported to be the most common cancers with high incidence and mortality in both economically developed and developing countries (Jemal et al., 2011). Overall for all cancer types, the estimated new cases and deaths for 2013 were portrayed in Fig. 1.2.

The incidence rates of cancer in India during 1997 as recorded by National Cancer Registry Programme (NCRP) varied between 52.9 and 81.5 per 100,000 men; and between 56.8 and 95.6 per 100,000 women. About half of the cases among men and one fifth of cases among women were attributable to tobacco use. In India, the most common female cancers are breast, cervical, stomach, lung and colorectal cancer; and the most common cancer among men are lung and bronchus, stomach and hypopharyngeal cancer (NCRP, 1997). The incidence of some sites of cancer like female breast and oesophagus have shown a significant increase over the years, whereas others like cancer of the cervix and oral cavity have recorded a decline (NCRP 2002).
Fig. 1.2: The estimated new cases and deaths for all cancer types for 2013 (American Cancer Society, 2013).

1.6 BREAST CANCER

Breast cancer is the most common cancer in women and it makes up one tenth of all new cancer diagnoses made worldwide (Ferlay et al., 2004). There was a transferal in trend from the previous decade during which the most common cause of cancer death was cervical cancer. Breast cancer accounted for 23% (1.38 million) of all new cancer cases and 14% (458,400) of all cancer deaths in 2008. Each year in the United States, more than 226,870 women and about 2,190 men are diagnosed with breast cancer (NCI, 2012). According to American Cancer Society (2013), 232,340 new cases of invasive breast cancer are expected among women and 2,240 new cases are expected in men during 2013. Breast cancer incidence rates were also increased by 140% in Miyagi (Japan) from 1973-1977 to 1998-2002, 40% in Chennai (India) from 1983-1987 to 1998-2002 and 4.5% per year in Kampala (Uganda) from 1991-2006. The overall incidence of breast cancer among different countries has been graphically presented in Fig. 1.3. As a result of early detection and improvement the death rates of breast cancer were found to decrease in North America and several European countries. In contrast, many African and Asian countries including Uganda, South Korea and
India, have observed an increase in mortality due to changes in reproductive patterns, physical inactivity and obesity. About half of the breast cancer cases and 60% of the deaths were estimated to occur in economically developing countries (Jemal et al., 2010; 2011). The worldwide scenario of breast cancer is depicted in table 1.1.

Breast cancer in India appears to be a different disease from that in the West, with early onset, short disease free interval and high mortality (ICMR, 2006). Presently, 75,000 new cases occur in Indian women every year and 1 in 58 women suffer from breast cancer in urban India. Overall incidence of breast cancer in India is depicted in Fig. 1.4.

**Table 1.1:** Incidences and deaths of Breast cancer disease worldwide
(Ferlay et al., 2004).

<table>
<thead>
<tr>
<th>Country</th>
<th>Incidence</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td>18.7</td>
<td>5.5</td>
</tr>
<tr>
<td>Zimbabwe</td>
<td>19</td>
<td>14.1</td>
</tr>
<tr>
<td>India</td>
<td>19.1</td>
<td>10.4</td>
</tr>
<tr>
<td>Japan</td>
<td>32.7</td>
<td>8.3</td>
</tr>
<tr>
<td>Brazil</td>
<td>46</td>
<td>14.1</td>
</tr>
<tr>
<td>Singapore</td>
<td>48.7</td>
<td>15.8</td>
</tr>
<tr>
<td>Italy</td>
<td>74.4</td>
<td>18.9</td>
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<tr>
<td>Switzerland</td>
<td>81.7</td>
<td>19.8</td>
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<tr>
<td>Australia</td>
<td>83.2</td>
<td>18.4</td>
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<tr>
<td>Canada</td>
<td>84.3</td>
<td>21.1</td>
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<tr>
<td>Netherlands</td>
<td>86.7</td>
<td>27.5</td>
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<tr>
<td>UK</td>
<td>87.2</td>
<td>24.3</td>
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<tr>
<td>Sweden</td>
<td>87.8</td>
<td>17.3</td>
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<tr>
<td>Denmark</td>
<td>88.7</td>
<td>27.8</td>
</tr>
<tr>
<td>France</td>
<td>91.9</td>
<td>21.5</td>
</tr>
<tr>
<td>United States</td>
<td>101.1</td>
<td>19</td>
</tr>
</tbody>
</table>
Fig. 1.3: Trends in female breast cancer incidence rates, 1973-2006 (Jemal et al., 2010).
Fig. 1.4: Chart showing incidence of breast cancer in India (NCRP, 2006).

1.6.1 ANATOMY OF THE BREAST

The breast of an adult woman is a tear-shaped, milk producing gland. Each breast is a hemispheric projection attached to the front of the chest wall on either side of the sternum by the pectoral muscles and dense connective tissues. These strands of connective tissue called as the suspensory ligaments of the breast (Cooper’s ligaments) run between the skin and deep fascia to support the breast. Each breast has a pigmented projection called the nipple surrounded by a circular pigmented area named the areola. The nipple has a series of closely spaced openings of ducts called lactiferous ducts for the secretion of milk.

Within each breast is a mammary gland that consists of 15 to 20 lobes or compartments, separated by a variable amount of adipose tissue. In each lobe there are several smaller compartments called lobules, composed of grapelike clusters of milk-secreting glands termed as alveoli are embedded in the connective tissue. After production of milk, it passes from the alveoli into a series of secondary tubules and then into the lactiferous duct.
The synthesis, secretion and ejection of milk are collectively called as lactation, which is associated with pregnancy and childbirth. Milk production is stimulated largely by the hormone prolactin from the anterior pituitary, with contributions from progesterone and estrogens, and the ejection of milk is stimulated by oxytocin that is released from the posterior pituitary. Each breast also consists of many blood vessels and lymph vessels that are connected to the lymph nodes in surrounding parts of the body. The pictographic anatomy of the breast was given in Fig. 1.5.

1.6.2 TYPES OF BREAST CANCER

The types of breast cancer broadly fall into five categories depending upon its origin.
1.6.2.1 DUCTAL CARCINOMA

Ductal carcinoma originates in the cells which line the breast's ducts beneath the nipple and areola (Fig. 1.6). Between 85% and 90% of all breast cancers are ductal. If the cancer is ductal carcinoma in situ (DCIS), it is well contained, not invasive, and can be very successfully treated.

![Diagram of ductal carcinoma stages](Source: bccancer.bc.ca)

**Fig. 1.6:** Figure portraying stages of ductal carcinoma (Source: bccancer.bc.ca).

1.6.2.2 LOBULAR CARCINOMA

Lobular carcinoma begins in the lobes or mammary glands. The lobes are located deeper inside the breast under the ducts. About 8% of breast cancers are lobular. Lobular carcinoma in situ (LCIS) is limited within the lobe and does not spread (Fig. 1.7).
Fig. 1.7: A depiction of lobular carcinoma *in situ* (Source: cancer.gov).

Fig. 1.8: Types of invasive carcinoma of the breast (Source: breastcancercare.org.uk).
1.6.2.3 INVASIVE (INFILTRATING) BREAST CANCER:

Invasive or infiltrating breast cancer is the second most common group of breast cancers that invade nearby tissue. It has the potential to spread out of the original tumor site and invade other parts of the breast and body. There are several types and subtypes of invasive breast cancer (Fig. 1.8).

1.6.2.4 INFLAMMATORY BREAST CANCER:

Inflammatory breast cancer is the less common, but most aggressive of breast cancers, taking the form of sheets or nests, instead of lumps. It starts in the soft tissues of the breast, just under the skin or it appears on the skin (Fig. 1.9).

![Fig. 1.9: A representation of inflammatory breast cancer (Source: virtualmedicalcentre.com).](image)

1.6.2.5 PAGET'S DISEASE OF THE NIPPLE/AREOLA:

Paget's disease is the least common type of breast cancer named after Sir James Paget, who first noticed the relationship between changes in the nipple and the underlying breast cancer. Cancer of the nipple often looks like a skin rash or rough skin. It resembles eczema and can be itchy. The itching and scabs (if scratched) are signs that cancer may be under the surface of the skin and is breaking through. Paget's is usually treated with a mastectomy, because the cancer has by then invaded the nipple, areola and the milk ducts.
1.7 CHEMOPREVENTIVES

In spite of advances in clinical treatment of cancer malignancies, the reduction in rate of such cancer mortality is not satisfactory as per National Cancer Institute (Sporn and Suh, 2000). Also, to develop therapies suitable for the full gamut of cancers is depicted as Herculean and almost impossible (Evan and Vousden, 2001). Such obscurity in developing cancer therapies is due to its molecular complexity. Recent progress in tumor targeting technology has augmented the likelihood that cancer prevention relies increasingly on interventions collectively termed ‘chemoprevention’ (Tamimi et al., 2002). Cancer chemoprevention is a pharmacological approach to intervene the use of agents to inhibit, reverse or retard carcinogenesis (Surh, 2003) and such agents are termed as chemopreventive agents (Tamimi et al., 2002). Extensive research in the last few years has revealed that regular consumption of certain fruits and cruciferous vegetables can reduce the risk of acquiring specific cancers (Kelloff et al. 1996a). Systematic, molecular target based evaluation of such dietary agents is one important strategy for discovering better chemopreventive agents (Kelloff et al. 1996b).

Pre-clinical and clinical studies in chemoprevention field yielded many valuable data in preventing the onset of disease and suppressing the progress of their growth, making chemoprevention a challenging and a very rational strategy in future researches (Meiyanto et al., 2012).

More than 600 potential chemopreventives have been identified and about 40 promising chemopreventive agents and agent combinations, inclusive of abundant diet-derived agents, have been clinically evaluated for major cancer targets such as breast, prostate, lung and colon (Tanaka, 1994; Kelloff et al., 2000). Such chemopreventive phytochemicals include genistein, resveratrol, diallyl sulfide, S-allyl cysteine, allicin, lycopene, capsaicin, curcumin, 6-gingerol, ellagic acid, ursolic acid, silymarin, anethol, catechins and eugenol which might possess several untapped therapeutic values. These agents have been seen to suppress cancer cell proliferation, induce apoptosis, suppress the expression of anti-apoptotic proteins, inhibit growth factor signalling pathways, NF-κB, AP-1 and JAK-STAT activation pathways,
angiogenesis and cyclooxygenase-2. These chemopreventive agents also have been very recently found to reverse chemoresistance and radioresistance in patients undergoing cancer treatment. Thus, these chemopreventive agents have potential to be used as adjuncts to current cancer therapies (Dorai and Aggarwal, 2004). The various molecular targets that aimed at cancer treatments are presented in the Fig. 1.10.

![Molecular targets](image)

**Fig. 1.10:** Molecular targets of chemopreventive agents in cancer (Dorai and Aggarwal, 2004).

Chemopreventive agents can be used not only to prevent cancer, but also to treat cancer. Because of their pharmacological safety, most chemopreventive agents can be used in combination with chemotherapeutic agents to enhance the effect at lower doses and thus minimize chemotherapy-induced toxicity. Tumor cells use multiple cell survival pathways to prevail, and thus agents that can suppress multiple pathways have great potential for the treatment of cancer. Selective estrogen receptor modulators (SERM) are trending as a key factor for breast cancer chemoprevention, as estrogen exposure levels play a vital role in breast cancer pathogenesis. Tamoxifen, an antiestrogen, is well known for its effect in reducing breast cancer risk in women (Fisher et al. 1998). Natural products like, Soy isoflavones are also gaining importance as SERMs (Kelloff et al., 2000).
Chemoprevention by the aid of synthetic agents results in side effects in the tenure of treatment (Wattanapitayakul et al., 2005). One such example is doxorubicin, a chemotherapeutic agent commonly used in treatment of breast cancer. It has exhibited low efficacy thus leading to its resistance and toxicity on normal tissues (Fimognari et al., 2006). Therefore, the focus of researchers inclines on the natural metabolites from plant sources in the hunt for a new potent chemopreventives.

1.8 MEDICINAL PLANTS

The use of plants as medicines is probably as old as Human kind itself. The first written record to the use of plants for its medicinal properties dates back to 2600 BC from the Sumerians and Akkaidians (Samuelsson, 1999), followed by the Egyptian ebers papyrus of 1500 BC for its mention of plants in treatment of cancer. The Chinese Materia Medica of 1100 BC has documented the record of more than 600 medicinal plants (Cragg et al., 1997). Susruta and Charaka had discussed the usage of plants as medicine in their Ayurvedic system of medicine in 1000 BC (Kappor, 1990). The Greeks have also substantially contributed to the rational development of the herbal drugs in 100 AD (Samuelsson, 1999).

In recent years, plants are considered as the best sources for drug components, since it exhibits lesser side effects and believed to offer protection from carcinogenic exposure. (Krishnaiah et al., 2007; Rates, 2001). There is considerable interest in screening the plant extracts in modern drug discovery programs, since structurally novel chemotypes with potent and selective biological activity may be obtained (Kinghorn, 2000). In USA, under the NCI (National Cancer Institute) program, over 35,000 plants were screened for anti-cancer activity between 1960 and 1986. More than 1,50,000 plant species have been studied so far and many of them were reported to contain therapeutic constituents (Ishii et al., 1984; Hoyos et al., 1992).

Drugs have been customarily isolated as single plant extracts/fractions, or have been mixtures of fractions/extracts from different plants. These drugs were used subsequent to their evaluation of safety and efficacy in model systems and humans (Dahiru et al., 2006). Numerous plant products in the form of
decoction, tincture, tablets and capsules have been clinically used for the treatment of different kinds of cancer (Ram and kumari, 2001). This has been scientifically defined as “the use of non-cytotoxic nutrients or pharmacological agents to enhance physiological mechanisms that protect the organism against mutant clones of malignant cells” (Morse and Stoner, 1993; Kinghorn, 2000).

Plant anticancer agents were initially categorized as four structural classes, the *Catharanthus* (Vinca) alkaloids (vinblastine, vincristine, vinorelbine), the epipodophyllotoxins (etoposide, etoposide phosphate, teniposide), the taxanes (paclitaxel and docetaxel), and the camptothecin derivatives (irinotecan and topotecan) (Cragg, et al., 1997). Subsequently, several other flavonoids, terpenoids, alkaloids and phenylpropanoids were isolated from plant sources which were known to possess anticancer properties (Kintzios, 2006; Park et al., 2008). To prove the same, most of the plant-derived compounds were known to have undergone a number of preclinical and clinical trials (Shu, 1998).

Among many recent advances in cancer chemotherapy, plant sources have played an important role in contributing to the arsenal of approximately 60 cancer chemotherapeutic drugs on the market (Kinghorn, 2000). Cytotoxic phytochemicals such as vinca alkaloids or paclitaxel (Taxol) were often used in oncology as highly potent drugs and/or serve as model for synthetic compounds (Thatte et al., 2000; Pandi et al., 2011). There have been a plethora of reports in the scientific literature documenting the chemopreventive potential of phytochemicals such as Lupulone, Hesperidin and blueberry in various cancer cell lines (Park et al., 2008; Lamy et al., 2009; Adams et al., 2010). Also, tests in experimental systems (*in vitro* and *in vivo*) have demonstrated that most of the phytochemicals act by interfering with several cell signalling pathways and lead to cell cycle arrest, apoptosis and/or differentiation induction in cancer cells (Chathoth et al., 2008).
1.9 MEDICINAL PLANTS SELECTED FOR THE STUDY

Medicinal plants selected for the present study were reportedly used as traditional medicine by diverse tribal and ethnic groups. The collected specimens were confirmed at Arya Vaidya Pharmacy (Coimbatore) Ltd., Kanjikode, Palakkad, India and at the University of Calcutta, Kolkata, West Bengal, India.

A brief report of the medicinal plants selected for the study is as follows:

1.9.1 *Rheum emodi* Wall. ex Meissn.

![image](A) plant at natural habitat, B) dried rhizome.

**Fig. 1.11.** *Rheum emodi* Wall. ex Meissn, A) plant at natural habitat, B) dried rhizome.

*Rheum* species, commonly known as Rhubarbs, has been cultivated for over 5000 years for its medicinal properties and has its origin in the mountains of the North-western provinces of China and Tibet. It was first mentioned in the Chinese herbal Pen-King, wherein it has been listed as a purgative and stomachic (Foust, 1992). This genus includes a large number of perennial stout herbs. In India, it is distributed in the temperate and subtropical regions of Himalaya from Kashmir to Sikkim, at a height of 2800 and 3800 m above mean sea level (Nautiyal et al., 2003). The botanical name of Himalayan rhubarb is *Rheum emodi* Wall. ex Meissn. (kingdom: Plantae; division: Magnoliophyta; class: Magnoliopsida; order: Caryophyllales; family: Polygonaceae; genus: *Rheum* L.) (Sharma, 2009). The rhizomes of *R. emodi* have great significance for its traditional use in Ayurvedic, Unani and folk systems of medicine (Singh et al., 2010). It has also been used as an ingredient in many polyherbal formulations used for the treatment of various diseases,
in particular the regulation of blood fat, hepatitis and cancer, and is officially listed in the Indian Pharmacopoeia (Singh et al., 2005).

There are also scientific reports subsequent to the traditional use of *R. emodi*, where crude extracts of *R. emodi* were reported to possess hepatoprotective activity in CCl4-treated male rats, and in CCl4-treated cultured primary rat hepatocytes (Ibrahim et al., 2008). *R. emodi* was also reported to have an immuno-enhancing effect through the release of various cytokines (Kounsar and Afzal, 2010), thus playing a role in chemoprevention. Phytoconstituents from diverse *Rheum* species have been reported for its various biological properties (Matsuda et al., 2001). From *R. emodi*, the polar extracts have been previously reported for their promising antioxidant, cytotoxic and apoptotic properties (Babu et al., 2003; Rajkumar et al., 2010a; 2011a). Also, petroleum ether and chloroform extracts of *R. emodi* rhizomes have been reported to possess a moderate degree of antibacterial and antifungal activities with its reported compounds revandchinone-1, revandchinone-2, revandchinone-3, revandchinone-4 and b-asarone. Some of the well known compounds such as chrysophanol, physcion and emodin were also found to be present in *R. emodi* (Babu et al., 2003).

1.9.2 *Oroxylum indicum* (L.) Vent.

Fig. 1.12: *Oroxylum indicum* (L.) Vent., A) tree at natural habitat, B) dried stem bark.
Oroxylum indicum (L.) Vent. (also known as Shivnak, Sonapatha, Shyonaka or Midnight horror) is a deciduous tree belonging to Bignoniaceae family characterized with few branches. The bark decoction of O. indicum was previously reported for its use in treating cancer but lacks specific chemopreventive records (Mao, 2002). The plant was also reported to be used in the treatment of other ailments like gastric ulcer, scabies and tonsil pain. O. indicum bark extracts were furthermore reported to possess antiproliferative/cytotoxic, and apoptotic properties (Lambertini et al., 2004; Kumar et al., 2010; Rajkumar et al., 2011b). In addition, the bark extracts were found to possess anti-inflammatory activity (Laupattarakasem et al., 2003) and anti-microbial activity (Uddin et al., 2003). Previous phytochemical studies of O. indicum led to the identification of ellagic acid (Maitreyi et al., 2008), 5,7-Dihydroxy flavone (chrysin) (Babu et al., 2006), 5-hydroxy-8-methoxy-7-0-β-Dglucopyranuronosyl flavone (Nair and Joshi, 1979), Stigmast-5-en-3-ol (Rasadah et al., 1998), 5,6,7-trihydroxy flavone (baicalein) (Chen et al., 2003; Chen et al., 2005), 4',5- Dihydroxy-7-methoxy isoflavone (pratensol) (Polya, 2003), 3-(4- hydroxy phenyl)-2-propenoic acid and 3, 4', 5, 7-tetrahydroxy-flavonol (Islam et al., 2010).

1.10 OBJECTIVES OF THE PRESENT STUDY

Traditional medication has been harnessing an intervention of plants for medicinal use since decades. Most of the medicinal plants mentioned in the ancient literature lack scientific evidence. There are reports for the use of plant sources in traditional medicine for its cancer curative properties. Even if the general properties of such plants have been studied; specific investigations to include its targeted chemotherapeutic properties are lacking.

Hence, the present study focuses on isolating and identifying a potent plant compound with target specific chemotherapeutic properties against breast cancer by testing the following hypothesis:

‘Compound(s) isolated from selected medicinal plants might possess the ability to activate Caspase-3 in the human breast cancer cells, thus leading the cells towards apoptotic cell-annihilation.’
The objectives of the study include:

- Assessment of cytotoxicity of the crude extracts obtained from selected medicinal plants.

- Analysis of the induction of apoptosis by caspase-3 activation by the plant extracts.

- Isolation of compounds from the most efficient extract that activates caspase-3 by TLC and Column chromatography.

- Determination of bioactivity (cytotoxicity, apoptosis inductivity and caspase-3 activation potential) of the isolated compounds.

- Characterization of the most efficient compound by FTIR, NMR, Mass spectroscopic analyses.