2. Review of Literature

Cancer is a progressive disease characterized by uncontrolled cell growth, tissue invasion and metastasis. Cancer cell genotype is a manifestation of six essential alterations in normal cell physiology; self sufficiency of growth signals, evasion of apoptosis, limitless replication, angiogenesis, tissue invasion and metastasis (Hanahan and Weinberg, 2002). According to the International Agency for Research on Cancer, in 2012, 8.2 million people died of cancer across the globe. In India alone, 682830 deaths occurred due to cancer with solid tumors of breast, lung and colon accounting for about 26.7% of total cancer deaths (Ferlay et al, 2013).

The development of cancer is a multi-step process. It involves mutation and selection of cells with progressively increasing capacity for proliferation, survival, invasion and metastasis. Tumor initiation occurs due to a genetic alteration leading to abnormal proliferation of a single cell. Tumor progression continues as additional mutations occur, some of which may confer advantages to the cell like rapid growth. Such a descendant may become dominant within the tumor population, a process referred to as clonal selection. Clonal selection continues throughout tumor development as a result of which tumors become malignant and rapidly-growing (Cooper and Hausman, 2007).

2.1 Molecular basis of cancer

2.1.1 Apoptosis

Apoptosis, or programmed cell death, is a mechanism by which cells undergo death to control cell proliferation or in response to DNA damage. The understanding of apoptosis has provided the basis for novel targeted therapies that can induce death in cancer cells or sensitize them to established cytotoxic agents and radiation therapy. These novel agents include those targeting the extrinsic pathway such as tumor necrosis factor-related apoptosis-inducing ligand receptor, and those targeting the intrinsic Bcl-2 family pathway such as antisense bcl-2 oligonucleotides.

Many pathways and proteins control the apoptosis machinery. Examples include p53, the nuclear factor kappa B, the phosphatidylinositol 3 kinase pathway, and the ubiquitin/proteosome pathway. These can be targeted by specific modulators such as bortezomib, and mammalian target of rapamycin inhibitors such as CCI-779 and RAD
Because these pathways may be preferentially altered in tumor cells, there is potential for a selective effect in tumors sparing normal tissue.

2.1.2 Cell cycle

The understanding of the cell cycle is very crucial to the development of cancer. The cell cycle involves a complex series of molecular and biochemical signaling pathways. The cell cycle has four phases.

- The G1 or gap phase, in which the cell grows and prepares to synthesize DNA
- The S or synthesis phase, in which the cell synthesizes DNA
- The G2 or second gap, phase, in which the cell prepares to divide
- The M or mitosis phase, in which cell division occurs

As a cell approaches the end of the G1 phase it is controlled at a vital checkpoint, called G1/S, where the cell determines whether or not to replicate its DNA. At this checkpoint the cell is checked for DNA damage to ensure that it has all the necessary cellular machinery to allow for successful cell division. This involves the interactions of various proteins, a “molecular switch” is toggled on or off. Cells with intact DNA continue to S phase; cells with damaged DNA that cannot be repaired are arrested and “commit suicide” through apoptosis, or programmed cell death. A second such checkpoint occurs at the G2 phase following the synthesis of DNA in S phase but before cell division in M phase.

Cells use a complex set of enzymes called kinases to control various steps in the cell cycle. Cyclin Dependent Kinases, or CDKs, are a specific enzyme family that use signals to switch on cell cycle mechanisms. CDKs themselves are activated by forming complexes with cyclins, another group of regulatory proteins only present for short periods in the cell cycle. When functioning properly, cell cycle regulatory proteins, including CDKs and cyclins, act as the body’s own tumor suppressors by inducing the death of damaged cells. Genetic mutations causing the malfunction or absence of one or more of the regulatory proteins at cell cycle checkpoints can result in the “molecular switch” being turned permanently on, permitting uncontrolled multiplication of the cell, leading to carcinogenesis, or tumor development (Cooper and Hausman, 2007).

Wild type p53 acts in response to a wide range of cellular stresses and performs its most important biological functions in response to DNA damage - cell cycle arrest or apoptosis. Following DNA damage, a cell can arrest its progression through the cell
cycle. p53 is involved with cell cycle delays in G1, G2 and mitotic spindle check-point. However, the G1/S arrest is widely observed in accordance with the wild-type p53 status of the cells (Amundson, 1998).

p53 activation can also facilitate apoptosis by the extrinsic death receptor pathway and intrinsic mitochondrial pathway. The p53 protein activates the “death” receptors (belonging to the TNF-R family) and caspase 8 directly - components of extrinsic apoptotic pathway. The intrinsic mitochondrial pathway comprises of the Bcl-2 family of proteins that regulate the mitochondrial membrane permeability and can be either pro-apoptotic or anti-apoptotic. Bax is a pro-apoptotic member of the Bcl-2 family while Bcl-2 is anti-apoptotic; hence a balance between these proteins is important for cell survival (Elmore, 2007). Apoptosis is one of the mainstays for cancer treatment; hence, the development of novel cancer therapeutics is targeted towards these pathways which would be effective in checking malignant cells.

2.2 Targets of cancer therapy

2.2.1 Cell cycle kinases

Cell cycle kinases are proteins that coordinate the complex events that regulate the proper division of cells. When genes mutate in cancer, cell cycle kinases and/or their protein regulators may be deregulated, leading to aberrant cell division and uncontrolled proliferation of cells, both prime hallmarks of human cancer.

While numerous small-molecule inhibitors have been developed to target cell cycle kinases, none has yet been approved for commercial clinical use. The search for synthetic inhibitors of protein kinases as anticancer drugs has been boosted recently by successful approval of a number of molecules that target tyrosine kinases, such as the bcr/abl protein kinases inhibitor imatinib (Gleevec) for the treatment of chronic myelogenous leukemia.

2.2.2 CdK inhibitors

Loss of cell cycle control is a hallmark of cancer, and aberrations in the cyclin-CdK-RB (cyclin-dependent kinase-retinoblastoma protein) pathway are common in breast cancer. Therefore, inhibition of this pathway is an attractive therapeutic strategy (Sutherland et al, 2009).
The cyclin-dependent kinase (Cdk) inhibitor p27 (also known as KIP1) regulates cell proliferation, cell motility and apoptosis. Phosphorylation regulates p27 binding to and inhibition of cyclin-Cdk complexes, its localization and its ubiquitin-mediated proteolysis. In cancers, p27 is inactivated through impaired synthesis, accelerated degradation and by mislocalization. Oncogenic activation of receptor tyrosine kinases (RTK), phosphatidylinositol 3-kinase (PI3K), SRC, or Ras-mitogen activated protein kinase (MAPK) pathways cooperate to inactivate p27 or accelerate its proteolysis in human cancers (Cooper and Hausman, 2007).

2.2.3 Oncogenes

An oncogene is any gene that encodes a protein able to transform cells in culture or to induce cancer in animals. Most of the many known oncogenes, are derived from normal cellular growth-controlling pathways. For example: the ras oncogene is a proto-oncogene that encodes an intracellular signal transduction protein; this oncoprotein provides an excessive or uncontrolled growth promoting-signal.

2.2.4 Tumor suppressor genes

A tumor suppressor gene is a gene that reduces the probability that a cell in a multicellular organism will turn into a tumor cell. Tumor suppressor genes play a critical role in regulating when cells are allowed to divide and increase in number. When DNA damage is detected in a cell, some tumor suppressor genes can stop the cell from multiplying until the damage is repaired. Also, specific tumor suppressor genes can stimulate cells with damaged DNA to commit "cell suicide". When tumor suppressor genes do not function correctly, the cells with DNA damage continue to divide and can accumulate further DNA damage that can eventually lead to the formation of a cancer cell. (Barnes and Camplejohn, 1996)

p53, which has been mentioned earlier, represents the best characterized of tumor suppressors with a clear role in the induction of apoptosis or cellular arrest in response to stresses such as DNA damage. As such, this gene is frequently mutated in cancers, thereby inactivating the protective proapoptotic role of p53 and contributing to the drug-resistant phenotype (Kasibhatla and Tsend, 2003).
2.2.5 Disturbed regulatory mechanisms leading to cancer

Cancer occurs when signal transduction pathways get altered by various factors. These have been studied in detail as they can act as targets of novel cancer drug discovery and development.

2.2.6 Retinoblastoma protein

The retinoblastoma protein plays a central role in determining whether a cell will proceed through the G₁ phase of the cell cycle. This control circuit can be disturbed by several alternative genetic and biochemical mechanisms. In case of retinoblastomas, osteosarcomas and small cell lung carcinomas, the pRB protein is absent due to mutations that disable the RB gene (Sellers and Kaelin, 1997). In cervical carcinomas, the pRB protein is sequestered and tagged for degradation by the E7 oncoprotein of type 16 and 18 human pappillomavirus. All such alterations converge on the loss of growth suppression by pRB that exists in a vast majority of tumors (Hahn and Weinberg 2002).

2.2.7 The p53 protein

In normal cells p53 is responsible for temporary arrest of growth cells in response to some damage to the cells till that damage is repaired or by inducing apoptosis which eliminates the damaged cell (Hahn and Weinberg 2002).

The retinoblastoma protein and the p53 tumor suppressor protein have a central role in regulating the cellular response to external signals. Each of the tumor suppressors are regulated by a series of other proteins that together constitute a molecular pathway. Mutations or alterations in expression of any such pathway may lead to cancer. E2F is a family of transcription factors that regulate cell-cycle progression.

The p53 gene has been mutated in more than 50% of the tumors and about 15000 alleles of this gene have been identified (Harris et al, 1996). In some tumors with no sign of p53 mutation, the p53 antagonist is overexpressed, driving the premature degradation of the TP53. It has been seen that the drive to eliminate p53 is evolving and there is pressure on pre-neoplastic cells to eliminate pro-apoptotic mechanisms. Elimination of p53 will eliminate the apoptotic machinery in many types of cancer (Wang et al, 2001).
2.2.8 Telomeres

Telomeres are protective sequences at the end of chromosomes. They are maintained indefinitely by cancer cells for continued cellular proliferation and are very important in the formation of all types of tumors. This phenomenon causes cell immortalization; an intrinsic part of the neoplastic growth (Hahn and Meyerson, 2001).

Telomeres have a dual role in cancer. Telomere attrition limits the replicative life span of a cell; such shortening prevents telomeres from protecting the ends of chromosomes from damage. Widespread death at this point termed “crisis” due to chromosomal instability. Such karyotypic instability not only drives the selection of cells that reactivate telomerase but also promote the acquisition of other mutations that may participate in further oncogenesis.

2.2.9 Mitogenic stimulation

Normal cells are dependent on growth factor availability while cancer cells in contrast have a strongly reduced dependence on external mitogenic stimulation. This is due to the ability of cancer cells to generate constitutive mitogenic signals. For instance, the ras oncogene is found in one quarter of all tumors in humans that encode a mutant protein and a continuous stream of mitogenic signals into the cytoplasm.

Many oncogenes activate mitogenic signaling pathways, such as those controlled by receptor tyrosine kinase and Ras. Mutations or alterations in the expression of each of the members of this pathway are associated with the development of cancer. Normally the binding of a growth factor to a receptor protein kinase recruits and activates the adaptor proteins growth factor receptor – son of sevenless which in turn recruit the small guanosine triphosphate-binding protein Ras. This association activates a cascade of serine-threonine kinases (Raf and mitogen activated and extracellularly activated kinase (MEK) culminating the activation of mitogen-activated protein kinase (MAPK). MAPK moves to the nucleus and modulates the expression of a wide array of genes involved in cell growth and survival. A second pathway activated by growth factors is the phosphatidyl inositol 3-kinase Akt (protein kinase B). Inactivation of the lipid phosphatises and tensin homolog deleted on chromosome 10 (PTEN) also result in activation of this pathway. An inherited loss of PTEN confers susceptibility to many types of cancer.
A similar stream of mitogenic stimulation can also result in alterations in growth-factor receptors on the cell surface. For example, K-ras mutations are highly prevalent in lung, pancreatic and colon cancer (Ellis and Clark, 2000; Bos, 1989), amplification of the receptor HER2/neu is observed in breast cancer and mutations in the BRaf protein, immediately downstream of Ras is seen in many types of melanoma (Davies et al, 2002). A signaling pathway is perturbed in the majority of cancers in humans.

### 2.2.10 Angiogenesis

This is yet another very important factor in tumorigenesis. Incipient tumors cannot grow in size till they gain access to the vascular system. Cancer cells have an ability to release signals to attract and stimulate endothelial cells. As a result, capillaries form direct networks with the existing vasculature providing nutrients and oxygen to the host and removing the metabolic wastes. Cancer cells secrete proangiogenic factors; vascular endothelial growth factor, basic fibroblast growth factor and downregulate the expression of anti-angiogenic factors such as thrombospondin-1 (Hanahan and Folkman, 1996). An array of signaling pathways lead to the formation of new blood vessels providing nutrition to the cancerous cells.

### 2.2.11 DNA damage

DNA damage can be induced by exogenous physical agents, endogenous genotoxic agents, ROS and radiations. The consequences of DNA damage are two-fold. After misrepair or replication of the damaged template, surviving cells can undergo permanent change in their genetic constitution in the form of mutations or chromosomal aberrations which may lead to cancer (Bartek et al, 2007). Alternatively, it can lead to cell death or senescence that may lead to aging. Therefore, DNA damage and genome maintenance are very important aspects of cancer. Most mutations and large genomic alterations that are relevant to cancer originate from DNA damage.

### 2.3 Oxidative stress in cancer

Increased generation of reactive oxygen species (ROS) has been observed in cancer. ROS can stimulate cell proliferation, promote genetic instability, and induce adaptive responses that enable cancer cells to maintain their malignant nature. The oncogenic signals such as Ras and Bcr-Abl promote ROS generation, contributing to oxidative stress in cancer cells.
The redox reactions, with simple transfer of electrons affect almost all complex biological processes, and have profound effects on cell proliferation, cell fate, and various pathological processes. In recent years, ROS stress in cancer cells and its potential therapeutic implications have emerged as a promising area of research (Waris and Ahsan, 2006).

Mutations caused by oxidative DNA damage include a range of specifically oxidized purines and pyrimidines, alkali labile sites, single strand breaks and instability formed directly or by repair processes (Wang et al., 1998). Therefore, if not repaired they can lead to carcinogenesis. It has been understood that all the four bases are modified by ROS, mutations are usually related to modification of GC base pairs, while that of AT base pair rarely leads to mutations. In human tumors, G to T transversions are the most frequent mutations in the p53 suppressor gene (Harris et al., 1993). Initiation of cancer in humans by ROS is further supported by the presence of oxidative DNA modifications in cancer tissue (Takeuchi and Morimoto, 1993).

Reactive oxygen species (ROS) might also function as a double-edged sword. A moderate increase of ROS may promote cell proliferation and survival. However, when the increase of ROS reaches a toxic threshold, it may overwhelm the antioxidant capacity of the cell and trigger cell death. Under physiological conditions, normal cells maintain redox homeostasis by controlling the balance between ROS generation (pro-oxidants) and elimination (antioxidant capacity). Normal cells can tolerate a certain level of exogenous ROS stress owing to their ‘reserve’ antioxidant capacity. In cancer cells, the increase in ROS generation from metabolic abnormalities and oncogenic signaling may trigger a redox adaptation response, leading to an upregulation of antioxidant capacity. Therefore, cancer cells would be more dependent on the antioxidant system and more vulnerable to further oxidative stress induced by exogenous ROS-generating agents or compounds that inhibit the antioxidant system. This might constitute a biochemical basis to design therapeutic strategies to selectively kill cancer cells using ROS-mediated mechanisms (Trachootham et al., 2009).

2.4 Plants as anticancer agents

Plant derived compounds are of great significance to cancer therapy. The first cures in human cancer (Hodgkin’s Lymphoma) were achieved only after the addition of vinca alkaloids vincristine (Catharanthus roseus), Apocynaceae (Johnson et al., 1963) to prednisone, procarbazine and mechlorethamine (MOPP regimen) (DeVita et al., 1970).
The combination of the epipodophyllotoxin etoposide (derived from mandrake root *Podophyllum peltatum* and the wild chervil *P. emodi*, Berberidaceae, bleomycin and cisplatin is highly effective regimen against testicular cancer (Williams et al., 1987). Etoposide is also one of the most active agents against small cell lung carcinoma.

The more recent developments of the structurally and mechanistically novel taxanes (extracted from the barks of *Taxus baccata, T. brevifolia*, Taxaceae) and camptothecins isolated from the barks and wood of *Camptotheca acuminata*, Nyssacea in the 1990s represented a landmark in the history of cancer research due to their highly-potent efficacy against solid tumor. Paclitaxel is used for the treatment of ovarian, breast and non-small cell lung carcinoma (McGuire et al., 1996). Irinotecan and topotecan are semi-synthetic in nature as they have been synthesized from the lead molecule; camptothecin. These are being used for colorectal cancer and a second-line therapy in ovarian carcinoma respectively (Creemers et al., 1996)

### 2.5 Selection of plants for anticancer activity

#### 2.5.1 *Memecylon umbellatum*

- *Memecylon umbellatum* Burm. (Synonym: *Memecylon edule*) belongs to the family melastomataceae. It’s a large ornamental shrub or small tree found in the coastal regions of Deccan peninsula, eastern parts of India and the Andaman Islands. The leaves are known to possess cooling and astringent properties and are useful in leucorrhoea, gonorrhoea, eye troubles and bruises (The Wealth of India, 1962). The plant is also used in herpes, snake-bite (Rajakumar and Shivanna, 2009), colic, skin diseases bronchitis (Majumdar et al., 2009), dysentery and jaundice (Song and Kim, 2011).

- Preliminary phytochemical analysis of the plant revealed the presence of steroids, tannins, terpenoids, flavonoids, gums, resins, oils, phenols and saponins (Murugesan et al., 2011).

- A novel butenolide, umbelactone (4-hydroxymethyl-3-methyl-but-2-ene-4,1-olide) has been isolated from the chloroform extract of the plant. Other constituents which have been isolated are β-amyrin, sitosterol, ursolic acid, sitosterol-β-D-glucoside (Agarwal, 1978), malic acid, tartaric acid and oleanolic acid (Murugesan et al., 2011).

- In a comprehensive screening of medicinal plants for a wide range of pharmacological activities by the Central Drug Research Institute, Lucknow, it
was reported that *M. umbellatum* showed anti-cancer potential against Lewis lung carcinoma in mice (Dhar et al., 1968) and cytotoxicity in brine shrimp lethality assay (Rumzhum et al., 2012). The plant also showed anti-amphetamine, anti-viral and spasmolytic activity (Dhar et al., 1968).

- The leaves of *M. umbellatum* showed anti-diabetic, analgesic, anti-microbial, and wound healing activity in animal models (Amalraj et al., 1998; Himashu et al., 2010; Satya et al., 2003; Puratchikody et al., 2007).

### 2.5.2 *Nardostachys jatamansi*

*Nardostachys jatamansi* (Synonym: *Nardostachys grandiflora*) is an erect, perennial herb found in Alpine Himalayas. It is commonly known as Indian Spikenard or Jatamansi. It is used for treatment of various types of cancer (Hartwell, 1982; Saetung et al., 2005), cough, asthma, bronchitis, edema, piles, arthritis, gout, fractures, skin diseases, palpitations of heart, insomnia and epilepsy (Khare, 2007).

- The rhizomes are rich in sesquiterpenoids – jatamanshic acid, jatamansone, patchouli alcohol, nor-seychelanone, seychellen, α and β patchoulene, valeranone, valeranal, nardol, calarenol, nardostachone, n-hexacosanyl arachidate and isovalerate, n-hexacosanol, calaren, n-hexacosane, β-sitosterol, terpenic coumarins – oroselol, jatamansin and sclindin. An alkaloid actinidin was also reported (Khare, 2007; Medicinal plants of India, 1987)

- It is one of the constituents of Thai traditional formula composed of 12 medicinal plants for treating cancer and showed cytotoxicity against CORL-23 (large cell lung carcinoma) cells and PC-3 (prostate cancer) cells and is non-toxic to normal human fibroblast cells (Saetung et al., 2005).

- Roots and rhizomes of *N. jatamansi* showed anti-convulsant, anti-Parkinson’s, hepato-protective and anti-oxidant activity in animal models (Vidya et al., 2005; Ahmad et al., 2006; Ali et al., 2000; Sharma and Singh, 2012).

Cancer arises due to extensive genetic instability accumulating in tumor genomes over years making it difficult to treat leading to high morbidity and mortality. An improved understanding of the signal transduction pathways and their roles in cancer suggest that simultaneous inhibition of multiple targets may optimize the therapeutic benefit.

Natural products have provided us numerous lead compounds against cancer and there is a growing trend for the development of natural product-like libraries which are
low-cost, safe, have the ability to target multiple pathways and selective to cancer cells with minimal toxicity to normal cells. The Indian sub-continent has great botanical diversity and widespread use of traditional systems of medicine, however, a small number of plants have been evaluated scientifically for their potential clinical benefit.

Based on extensive literature survey, we selected *Memecylon umbellatum* and *Nardostachys jatamansi* as candidate plants for evaluation of their anticancer potential. The plants have been used traditionally for inflammation and cancer, however; scientific evidence supporting the claims is very limited.

The leaves of *Memecylon umbellatum* have been used traditionally to treat various infections and inflammatory conditions. The plant grows widely in the Western Ghats and is found to be rich in steroids, tannins, terpenoids, flavonoids, gums, resins, oils, phenols and saponins. Of these phytoconstituents, terpenoids, flavonoids, and phenols are known to possess anticancer activity. Phenolic compounds have been intensely studied for their antitumor, pro-apoptotic and anti-angiogenic effects (Carocho and Ferreira, 2013, Chahar et al, 2011, Wahle et al, 2010, Huang et al, 2012). The leaves were used traditionally for infections and inflammatory conditions. The common underlying mechanisms of inflammation and cancer were also taken into consideration while selection of plants. A novel butenolide, umbelactone (4-hydroxymethyl-3-methylbut-2-ene-4,1-olide), β-amyrin, sitosterol, ursolic acid and sitosterol-β-D-glucoside have been isolated from the plant. Hence, the plant is a rich source of terpenoids and terpenoids have known anticancer activity. Recent efforts into the research and development of anti-cancer drugs derived from natural products have led to the identification of different terpenoids that inhibit cancer cell proliferation and metastasis via various mechanisms. (Huang et al, 2012).

Additionally, in a comprehensive screening of plants for pharmacological activities by the CDRI, Lucknow in 1968, *M. umbellatum* extracts showed anti-cancer activity against Lewis lung carcinoma (LLC) in mice and cytotoxicity in brine shrimp lethality assay (Dhar et al, 1968). In the LLC model in mice, the air-dried leaves of *Memecylon umbellatum* were powdered mechanically and extracted by three cold percolations with 50% ethanol. The combined extracts were then concentrated under reduced pressure, and dried in vacuum desiccators. The MTD was established at 2000 mg/kg in albino mice (i.p.). However, further details on the anticancer activity are not described in the publication by Dhar et al (1968) since the objective of the study was
comprehensive screening of 237 plant species for various biological activities and establish a library of plants with therapeutic activities and could be explored further based on the screening results. The extract was evaluated in the LLC model in mice which is a well-established reproducible syngeneic model for lung cancer. LLC is a cell line established from the lung of a C57BL/6 mouse bearing a tumor resulting from the implantation of primary LLC. As per available literature, Kellar et al (2015), the cell line is highly tumorigenic and is primarily used to model metastasis as well as evaluate the efficacy of chemotherapeutic agents in vivo. The advantage of the LLC model is that implanted cells are immunologically compatible with the murine system, unlike the widely used xenograft models in which human cells are implanted into mouse tissue. As a result, LLC models can be created on an immunocompetent murine background and true immune and toxicity responses can be evaluated with respect to targeted therapies and tumor growth. No further anti-cancer studies have been carried out for this plant.

In addition, a pilot study was carried out to determine the anticancer activity of methanol extract of leaves of *Memecylon umbellatum* (MUM) in colon cancer cells (HCT-116). The extract was also tested for the selectivity towards cancer cells as compared to African green monkey normal kidney cells (Vero). The cell growth inhibition was tested by sulforhodamine assay in both cancer and normal cells at various concentrations. The extract exhibited dose-dependent cytotoxicity in both cells with varying indices of selectivity. MUM was found to exhibit cytotoxicity in HCT-116 cells with an IC\(_{50}\) of 276.63 ± 11.75 µg/mL. The cytotoxic selectivity was also found to be ~2-fold towards cancer cells as compared to normal cells. MUM also demonstrated apoptosis, the preferred mode of cell death for anticancer agents in HCT-116 by acridine orange/ethidium bromide (AO/EB) staining. Taken together, the results suggested that *Memecylon umbellatum* can act as a lead for the development of potential anticancer agents with selectivity towards cancer cells (Chaudhary et al, 2017).

*Nardostachys jatamansi* is a well-known Ayurvedic drug for its CNS and cardiotonic effects which is also used traditionally for treatment of a range of cancer types as reported by Hartwell. It is also used in traditional Thai medicine for the treatment of cancer. The rhizomes are rich in sesquiterpenoids like jatamansone, valeranone, seychellen, nardol, calarenol, nardostachone and alkaloids. Sesquiterpenes are a promising class of natural compounds in cancer drug discovery since they are selective towards cancer cells with 3 compounds; artemisinin, thapsegargin and
parthenolide currently being evaluated in clinical trials (Ghantous, 2006). In recent reports, the plant was also found to possess cytotoxicity against lung, prostate (Saetung et al., 2005) and neuroblastoma cancer cells (Pandita et al., 2012). However, further systematic studies are requisite to explore the detailed anti-cancer activity and establish its phytochemical and mechanistic basis.

Antioxidants can be promising anticancer agents; and they can be useful in quenching free radicals. Dietary antioxidants like vitamin C, vitamin A can selectively act as anticancer drugs. They protect against oncogenic transformation by radiation and free radical-producing anticancer drugs and also reduce the painful side-effects of chemotherapy (Borek, 1983).
2.6 Samples of plant parts used in the study

1. *Nardostachys jatamansi*

![Rhizomes of *Nardostachys jatamansi*](image1)

2. *Memecylon umbellatum*

![Leaves of *Memecylon umbellatum*](image2)
2.7 References


Ellis CA, Clark G. The importance of being K-Ras. Cell Signal, 2000; 12, 425-34.


Harris CC. p53 tumor suppressor gene: from the basic research laboratory to the clinic--an abridged historical perspective. Carcinogenesis, 1996; 17: 1187-98.

Hartwell JL. Plants used against cancer. Quaterman, Lawrence, MA, 1982; 654-656.


Medicinal plants of India. Vol.2. ICMR publications, New Delhi, 1987; 312-323.


Takeuchi T, Morimoto K. Increased formation of 8-hydroxydeoxyguanosine, an oxidative DNA damage, in lymphoblasts from Fanconi’s anemia patients due to possible catalase deficiency. Carcinogenesis, 1993; 14: 1115-20.


