CHAPTER-I
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Introduction

Human life is being targeted by various diseases on this universe. Among them one the deadly disease is cancer, it has become the second largest death rate next to cardiovascular diseases. Cancer is a class of diseases that display uncontrollable growth beyond the normal limits. Normal cells are normally regulated by signals that indicate whether the cell should divide, differentiate into another cell or die. Cells develop into cancerous cell when these signals are lost, resulting in uncontrolled growth, proliferation and invading into surrounding tissue. The cancer may spread to more distant parts of the body through the lymphatic system; around 90 % of cancer is due to tumor spreading i.e metastasis. Tumors can grow and interfere with digestive, nervous, circulatory system and they can release hormones that alter body function (Crosta, 2013).

1.2 Interesting facts about cancer

Cancer starts when a cell begins dividing uncontrollably; eventually developing into a visible mass known as a tumor. The initial mass is known as the primary tumor slowly developed into the secondary tumors form, when cells from the primary tumor break off and become lodged in other parts of the body, eventually forming their own masses. Even when the primary tumor has been removed, there is a chance of developing cancer again, due to cancerous cells, which had already broken away from the primary tumor or metastasized and lodged in distant locations.

Sometimes cancer that occurs when the entire visible tumor has been removed is called recurrent disease. Cancer that returns in the area of the primary tumor is called locally recurrent disease. Cancer that returns in a metastasized form is referred to as distance recurrence. The process of spreading cancer throughout the body is called metastasis.

More dangerous tumors form when two things occur

- A cancerous cell manages to move throughout the body using the blood or lymph system destroying healthy tissue in a process called invasion.
- Cell manages to divide and grow; making new blood vessels to feed itself in a process called angiogenesis.
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Many numbers of things are known to increase the risk of cancer, including tobacco use, certain infections, radiations, lack of physical activity, obesity and environmental pollutants (Anand et al., 2008). All these factors can directly damage genes or combine with existing genetic faults within cells to cause the disease. Approximately five to ten percent of cancerous are hereditary (Kinzler et al., 2002). Certain infectious agents associated with increased cancer risk, including hepatitis B virus (liver), certain subtypes of human papillomavirus (cervix), bacterium Helicobacter pylori (stomach) and human immunodeficiency virus (Alison, 2001).

1.3 History of cancer

Human beings and other animals have had cancer is found in the fossilized bone tumors in human mummies in ancient Egypt and ancient manuscripts will provide the evidence of ancient history of cancer (American Society of Clinical Oncology, 2009). Although the word cancer was not used, the oldest description of the disease is from Egypt and dates back to about 3000 BC.

1.3.1 Origin of cancer word

The origin of the word cancer is credited to the Greek physician Hippocrates (460-370 BC), who is considered as the “Father of Medicine.” Hippocrates used the terms carcinos and carcinoma to describe non-ulcer forming and ulcer-forming tumors. In Greek, these words refer to a crab; most likely applied to the disease because the finger-like spreading projections from a cancer called to mind the shape of a crab. The Roman physician Celsus (28-50 BC), later translated the Greek term into cancer; the Latin word for crab. Galen (130-200 AD), another Roman physician, used the word oncos (Greek for swelling) to describe tumors (American Society of Clinical Oncology, 2009).

1.3.2 Different theories to explain cancer development

From the earliest times, physicians have wondered about the cause of cancer. Several theories were proposed by different scientists to explain the causes of cancers. Some of them are as follows,
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a. **Humoral theory**: Hippocrates believed that the body had four humors (body fluids), blood, phlegm, yellow bile and black bile. The person was healthy when these humors were balanced. They believed that whether there is any changes in these fluids caused disease. An excess of black bile in various body sites was thought to cause cancer (American Cancer Society, 2013).

b. **Lymph theory**: Humoral theory of cancer was replaced by lymph theory, according this form of cancer is by the body fluid lymph. There is continuous and appropriate movement of the fluid parts of the body through the solid parts. Of all the fluids the most important were blood and lymph. Stahl and Hoffman theorized that cancer was composed of fermenting and degenerating lymph, varying in density, acidity and alkalinity. The lymph theory gained rapid support. John Hunter, the Scottish surgeon from the 1700s, agreed that tumors grow from lymph constantly thrown out by the blood (American Cancer Society, 2012).

c. **Blastema theory**: German pathologist Johannes Muller in 1838 demonstrated that cancer is made up of cells and not lymph, he believed that cancer cells did not arise from normal cells; he proposed that cancer cells develop from building elements (blastema) between normal tissue (American Cancer Society, 2013).

d. **Chronic irritation theory**: Virchow proposed chronic irritation as the cause of cancer; but he falsely believed that cancers "spread like a liquid." A German surgeon; Karl Thiersch, showed that cancers metastasize through the spread of malignant cells and not through some unidentified fluid (American Cancer Society, 2013).

e. **The parasite theory**: Another theory in the seventeenth and eighteenth centuries was the "parasite theory." This theory alleged that cancer was an infectious disease that relied upon the transmission of an invisible contagion. The parasite theory persisted off and on until the twentieth century. In 1926 an oncolgical scientist named Johannes Fibiger earned a nobel prize for research erroneously finding that certain worms caused stomach cancer. Later, other scientists disproved this theory.
1.4 Cell biology and mechanism of cancer development

Multicellular organism is made up of a billion of cells; organism can survive only when all its cells function in accordance with the rule that governs cells growth and reproduction. Each cell contains a copy of the genetic plan for our growth and development. This genetic plan comes from the genes located on the chromosomes that we inherit from our parents. The genes contain the information for the body to make all necessary components and chemicals to perform normal functions (National Health and Medical Council, 1999). In each and every cell there are two copies of every gene; one inherited from the mother and one inherited from the father. As we go through our lives, our cells are continually growing and being replaced by new cells. New cells are formed by the existing cells by the process called cell division. Each time as the cell divides it has to make a copy of all of its contents, including the genes, so that new cells have the same set of genes similar to the old cells (Gibbs, 2003). A number of different genes work together to give direction to each cell how to copy its gene properly and how to divide and grow in a controlled and orderly manner.

Cancer results from a series of molecular events that fundamentally alter the normal properties of cells. In cancer cells the normal control systems that prevent cell overgrowth and the invasion of other tissue are disabled. These altered cells divide and grow in the presence of signals that normally inhibit cell growth; they no longer require special signals to induce cell growth and division (Hanahan and Weinberg, 2000). These cells grow; they develop new characteristics; including changes in cell structure, decreased cell adhesion; production of new enzymes. These changes allow the cell and its progeny to divide and grow even in the presence of normal cells that typically inhibit the growth of nearby cells. Such changes allow the cancer cells to spread and invade (Bahls and Fogarty, 2002).

Abnormalities in cancer cells results from mutation in protein encoding genes that regulate cell division. During the time more genes become mutated. Consequently mutations begin to increase in the cell, causing further abnormalities in that cell and daughter cells. Some of these mutated cells die; but other alteration may give the abnormal cell a selective advantage that allows it to multiply much more rapidly than
normal cells. As long as these cells remain in original location they are considered as benign, if they become invasive they are considered as malignant. Cancer cells often metastasis sending cancer cells to distant sites in the body where new tumor may form.

1.4.1 Genetics of cancer

The human genome contains approximately 35,000 genes in each cell, of these only small numbers of genes have been associated with cancer (American cancer society, 2008). Alterations in the same gene often are associated with different forms of cancer. These malfunctioning of genes can be broadly classified into three groups (Hanahan and Weinberg, 2000).

1.4.2 First group called proto-oncogenes

These are a group of genes that cause normal cells to become cancerous when they are mutated (Weinstein and Joe, 2006). Mutations in proto-oncogenes are typically dominant in nature and the mutated version of a proto-oncogene is called an oncogene. Often, proto-oncogenes encode proteins that function to stimulate cell division, inhibit cell differentiation and halt cell death. All of these processes are important for normal human development and for the maintenance of tissues and organs. Oncogenes, however, typically exhibit increased production of these proteins, thus leading to increased cell division; decreased cell differentiation and inhibition of cell death. Thus, oncogenes are currently a major molecular target for anti-cancer drug design (Chial, 2008). More than 40 different human proto-oncogenes are known (Table 5.1). Oncogenes arise as a result of mutations that increase the expression level or activity of a proto-oncogene. Genetic mechanisms associated with oncogene activation include the following:

- Point mutations, deletions or insertions that lead to a hyperactive gene product.
- Point mutations, deletions or insertions in the promoter region of a proto-oncogene that lead to increased transcription.
- Gene amplification events leading to extra chromosomal copies of a proto-oncogene.
• Chromosomal translocation events that relocate a proto-oncogene to a new chromosomal site that leads to higher expression
• Chromosomal translocations that lead to a fusion between a proto-oncogene and a second gene, which produces a fusion protein with oncogenic activity.

1.4.3 Tumor suppressor genes

Tumor suppressor genes are normal genes that slow down cell division; repair DNA mistakes or tell cells when to die (a process known as apoptosis). Proteins made by tumor suppressor genes normally inhibit cell growth prevents tumor formation. Mutations in these genes result in cells that no longer shown normal inhibition of cell growth and division (Yoshida et al., 2000) (Table 5.2). The functions of tumor-suppressor proteins fall into several categories including the following.

• Regulation of genes that is essential for the continuing of the cell cycle. If these genes are not expressed, the cell cycle does not continue; effectively inhibiting cell division.
• Coupling the cell cycle to DNA damage. As long as there is damaged DNA in the cell, it should not divide. If the damage can be repaired; the cell cycle can continue.
• If the damage cannot be repaired, the cell should initiate apoptosis (programmed cell death) to remove the threat it poses for the greater good of the organism.
• Some proteins involved in cell adhesion prevent tumor cells from dispersing, block loss of contact inhibition and inhibit metastasis. These proteins are known as metastasis suppressors (Hirohashi and Kanai, 2003).

1.4.4 DNA repair genes

The third type of genes implicated in cancer is called DNA repair genes. DNA repair gene codes for proteins whose normal function is to correct errors that arises when cells duplicate their DNA prior to cell division. A mutation in DNA repair genes can lead to a failure to repair, which in turn allows subsequent mutations to accumulate. Environmental factors such as ionizing radiations, UV light and chemicals can damage DNA (Table 5.3).
1.5 Cell cycle

Normal cells grow and divide in an orderly fashion. The center of cell proliferation is the cell division cycle, the process by which cell grows, replicates its DNA and then divides to give two daughter cells. The entire process is divided into four sequential phases, of these two phases are most important they are S phase, where DNA replication occurs and phase is mitosis (also known as M phase, when cell undergo division to give two daughter cells (Hartwell and Weinert, 1989). In the concept of cell cycle S phase must follow M phase and that M phase must not start until S phase has been completed. In between S and M phase are two gaps G1 and G2. G1 follows on from mitosis and is a time during the cell cycle when the cell is responsive to both positive and negative growth signals. G2 is the gap after S phase, when cell prepares for entry into mitosis (Fig 5.1).

In G1 phase mitogenic stimulation results in activation of cell cycle dependent kinase like cyclin D1/CDK4 and cylin E/CDK2, which activates protein involved in DNA replication and inhibit protein synthesis that retains cells in a non dividing state (Dulic et al., 1994). G1 cells are metabolically active, but not dividing. Cells that are not stimulated to divide in G1 enter G0 state and can remain quiescent for a longer period of time. Activated cells will enter the second phase (S). In the second phase (S) DNA is duplicated and here for the activity of cyclin A/CDK2 is required (Rosenblatt et al., 1992). In G2 phase cells ensure that the DNA is properly replicated and that the conditions are right for the final separation of sister chromatids and cytokinesis in M phase. Several proteins control the timings of the events in the cell cycle, which is tightly regulated to ensure that cells divide only when necessary. The loss of this regulation is the hallmark of cancer. The fundamental task of the cell cycle is to ensure that DNA is faithfully replicated once during S phase and that identical chromosomal copies are distributed equally to two daughter cells during M phase (Heichman and Roberts, 1994).

Oncogenic process exerts their greatest effect by targeting regulators of G1 phase progression (Hunter and Pines 1994). During the G1 phase; cells respond to extracellular signals by either advancing towards another division or withdrawing
from the cycle into a resting state (G0). The transition from G\textsubscript{1} phase to S Phase is often deregulated due to altered gene function.

1.6 Environmental factors in cancer development

Several environmental factors are capable of inducing the majority of human cancer (Tomatis et al., 1990). Environmental factors include only the (natural, man-made) agents encountered by humans in their daily life. These factors are considered as carcinogenic agents; there is a consistent correlation between exposure to an agent and the occurrence of a specific type of cancer. Some of these carcinogenic agents include X-rays, UV light, tobacco products, pollutants and many other chemicals, viruses in the environment and the workplace are some of the potential catalysts of cancer. It is important to remember how these factors increase a person’s risk but do not always "cause" the disease.

1.6.1 Environmental risk factors

**Radiation:** Radiation is the emission (sending out) of energy from any source. High levels of radiation like those from radiation therapies and x-rays. Repeated exposure can damage normal cells and increase the risk of developing leukemia; as well as cancers of the breast, thyroid, lung, stomach and other organs.

**Ultraviolet (UV) radiation:** UV radiations from the sun are directly linked to melanoma and other forms of skin cancer. These harmful rays from the sun cause premature aging and damage the skin. Artificial sources of UV radiation, such as sun lamps and tanning booths, also increase the risk of skin cancer. Around 1.3 million skin cancers diagnosed in the year 2010 could have been prevented by protection from the sun’s rays (International Agency for Research on Cancer, 2010).

**Chemicals:** Long term exposure to chemicals such as pesticides; uranium; nickel; asbestos; radon and benzene can increase the risk of cancer. Such carcinogens may act alone or in combination with another carcinogen, such as cigarette smoke, to increase the risk of cancer and other lung diseases (Table 5.4).
Getting cancer from a chemical depends on the following:

- The kind of chemical you were exposed to;
- How much of the chemical you were in contact with;
- How long the contact lasted;
- How often you were exposed;
- When you were exposed;
- How you were exposed;
- Your general health.

The human body has defenses to guard against all sorts of harmful exposures, including those that may lead to cancer. When something enters into our body, it often goes through a process that allows the body to more easily use or get rid of it. This process is called metabolism. Depending on how a chemical is processed; or metabolized, in the body, three types of carcinogens exist:

a. Chemicals that can cause cancer (direct acting carcinogens).
b. Chemicals that do not cause cancer unless they are changed when they are metabolized (procarcinogens).
c. Chemicals that do not cause cancer by themselves, but can act with another chemical to cause cancer (co-carcinogens). Damage to DNA in cells can lead to cancer. However, cells can often repair DNA damage. If the damage is extreme, the cells may die. Unrepaired DNA damage can lead to mutations; or changes; in the genes and mutations in certain genes can cause cancer.

**Solvents:** Several solvents used in paint thinners, paint and grease removers and in the dry cleaning industry are known or suspected of being cancer-causing agents. These include benzene, carbon tetrachloride; chloroform, dichloromethane (methylene chloride), tetrachloroethylene and trichloroethylene.

**Fibers, fine particles and dust:** Exposures to various fibers; fine particles and dust occur in several industrial settings are associated with increased cancer risks. Exposure can also occur in non industrial settings. Asbestos fibers and all commercial forms of asbestos are human carcinogens. Asbestos exposures account for the largest
percent of occupational cancers, with the greatest risks among workers who smoke. Asbestos fibers are released into the environment from the use and deterioration of more than 5,000 asbestos products, including roofing, thermal and electrical insulation, cement pipe and sheet, flooring, gaskets, plastics, textile and paper products.

**Polycyclic aromatic hydrocarbons (PAHs):** A number of studies have shown the increased incidence of cancer (lung, skin, and urinary cancers) in humans when exposed to the mixtures of Polycyclic Aromatic Hydrocarbons (PAHs). The primary source of PAHs is from burning carbon-containing compounds. PAHs in air are produced by burning wood and fuel for homes. They are also contained in gasoline and diesel exhaust; soot, coke, cigar and cigarette smoke and charcoal-broiled foods. In addition, they are the byproducts of open fires, waste incinerators, coal gasification and coke oven emissions. Foods that contain small amounts of PAHs include smoking, barbecued or charcoal-broiled foods, roasted coffees and sausages.

**Metals in cancer**

**Arsenic:** compounds are associated with many forms of skin, lung, bladder, kidney and liver cancers, particularly when high levels are consumed in drinking water. In addition, occupational exposure to inhaled arsenic, especially in mining and copper smelting, has been consistently associated with an increased risk of lung cancer.

**Beryllium compounds:** are known to cause lung cancer. These compounds are used as metals for aerospace and defense industries, for electrical components; X-ray tubes, nuclear weapons, aircraft brakes, rocket fuel additives, light aircraft construction etc (Table 5.5).

**1.7 Medical drugs in cancer development**

Some drugs used to treat cancer (e.g.; Cyclophosphamide, Chlorambucil, Melphalan) have been shown to increase the occurrence of second cancers; including leukemia. Others that are used as immunosuppressant's, such as Cyclosporin and Azathioprine
for patients having organ transplants also are associated with increased cancer risks, especially lymphoma.

**Estrogens:** used to treat symptoms of menopause and other gynecological conditions have been shown to increase the incidence of endometrial cancer. In addition, some studies have shown an increased risk of breast cancer with estrogen use, but a reduced risk of colon cancer.

**Progesterone:** hormone now used in combination with estrogen for hormone replacement therapy in older women; it helps to protect against the endometrial cancer, breast cancer have recently been shown to be associated with the use of estrogen plus progesterone.

1.8 Viruses and cancer

Many viruses infect humans, but only a few viruses are known to promote human cancer. These include both DNA and RNA viruses (retroviruses). Viruses are believed to be associated with cancer as causative agents, the first human tumor viruses identified was Epstein-Barr Virus (EBV), later several human tumor viruses have been identified, including Kaposi Sarcoma Associated Herpes Virus (KSHV), Human Papilloma Viruses (HPV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Human T-Lymphotropic Virus (HTLV-1) and recently identified Merkel Cell Polyomavirus (MCPyV) (Saha et al., 2009). Tumor viruses are subcategorized as either DNA virus which includes EBV, KSHV, HPV and MCPyV, RNA viruses such as HCV and HLTV-1 (Table 5.6). Tumor virus’s initiates a series of cellular events; which leads to immortalization and proliferation of the infected cells by disrupting the mitotic checkpoints upon infection of the host cell (Fig 5.2).

1.9 Cancer stages

During the diagnosis of cancer, one important aspect is the staging, it is the process used to explain the degree or the spread of the diseases, it is vital for determining the therapy and measuring the prognosis. Location and size of primary tumor determine a stage of cancer and also to determine whether it has spread to other areas of the body.
(Aragon and Zujewski, 2007). Different staging systems are used for all the different cancer types in order to assist in describing the progress of that cancer (Bagchi et al., 2004). The different types, staging used to classify tumors are:

a. TNM: This classification system measures the tumors in three different ways: extent of the primary tumor (T), regional lymph node involvement (N) absence or presence and distant metastases (M) absence or presence (American cancer society, 2006).

b. After the TNM is assigned stages of I (early stages), II, III and IV (more advanced stages) is assigned in addition.

c. Descriptive and statistical analysis of tumor: In situ cancer cells if not invade deeper into the tissue; but are present only in the layer of cells where they developed, invasive if cells have spread to nearby tissue beyond the original layer, local invasive malignant cancer confined entirely to the organ of origin, regional malignant cancer that has extended beyond the limits of organ of origin directly into surrounding organs tissue, distant a malignant cancer that has spread to parts of the body remote from primary tumor either by direct extension of by discontinuous metastasis to distant organs; tissues via the lymphatic system to lymph nodes (Bagchi et al., 2004).

Treatment process is selected for a specific type of cancer can be done only when the stage of cancer is known it is important of decide the right treatment for the specific stage of cancer. After the biopsy, grading is done in the lab where cancer cells are graded according to how much they look like normal cells and also their aggressiveness. Various types of grading system exist and depend on the type of cancer (Table 5.7). The most common type of grading system is Gleason system is the most commonly used grading system which is based on number from 0 to 10, lower the number lower the grade. Grade under 4 mean that the cancer cells looks similar to normal cells and the cancer is less likely to be aggressive, grade 5 to 7 are the intermediates which means that these cancer cells do not look like normal cells and are more likely to be aggressive and more likely to grow faster and grades 8 to 10 means that the cancer is very aggressive in growth (Bagchi et al., 2004).
1.10 Carbohydrates on cancer cell surface

Carbohydrates are bio molecules that have enormous potential for encoding biological information. These combined-molecules (glycoproteins and glycolipids) are responsible for different biological interactions between the cell and the extracellular environment. Regarding the neoplastic cells, the glycosylation of these proteins and lipids is changed, which generates membrane signaling molecules capable of inducing several processes directly related to tumor progression such as cell adhesion, angiogenesis, cellular mitosis and metastasis, in addition, in some cases it may be responsible for inhibition of apoptosis induction triggered by the cells of the immune system (Taniguchi et al., 2012).

Tumor cells display varied patterns of glycosylation in carbohydrates and cell surface proteins. Altered glycosylation are present on cancer cells and some of them are known as progression markers. Each type of cancer displays differential alteration patterns during the different stages of disease. All these changes correlate with the ability of metastatic cancer cells and increase in migration and their ability to evade the immune system.

Certain changes in glycans occur frequently in neoplastic cells and may be considered "tumor-specific", establishing a correlation between the stage of disease progression and prognosis. Thus, due to the intrinsic role of carbohydrates in the tumorigenesis, the glycosylation process as well as the identification of glycosylated antigens has been intensively focused. Changes in glycosylation involve not only interaction with endogenous but also with exogenous lectins, that alter the response of cancer cells. The knowledge of interaction with cancer cells and how it affects the biology of tumor, will explain the role of carbohydrates in the acquiring malignant status and its inhibition. From the past few years lectins have become a well established for understanding the varied aspects of cancer and metastasis. Lectins are the dynamic contributors to tumors cell recognition (surface markers), cell adhesion and localization, signal transduction, cytotoxicity and apoptosis.
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Several studies have focused on the lectin ability to shown preferential agglutination of cancer cells; where lectin can be used in the detection of malignant changes in transformed cells due to the changes on cancer cells surface. It has been observed that expression of glycoconjugates is often altered in tumor cells. Abundant N-acetylglucosamine (α1; 3), N-acetylglucosamine/glucose and galactose (β1; 4), N-acetylglucosamine (α; 2), mannose (α1; 6) residues were observed in epithelium of tumor cells (Chan et al., 2001).

1.11 Cancer globally

It has been estimated that 12.7 million cancer cases around the world in 2008, of these 6.6 million cases were in men and 6.0 million in women. This number is expected to increase to 21 million by 2030 (World Cancer Research Fund International, 2012). Research suggests that one-third of cancer deaths can be avoided through prevention. Although proven ways to prevent cancer exist, these services and technologies are not widely available in low and middle-income countries (Center for Disease Control and Prevention, 2013).

Among many cancer types breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death among females; accounting for 23 % of the total cancer cases and 14 % of the cancer deaths. Lung cancer is the leading cancer site in males, comprising 17 % of the total new cancer cases and 23 % of the total cancer deaths. Cancer survival tends to be poorer in developing countries, most likely because of late stage of diagnosis and limited access to timely and standard treatment (Cancer Global Statistics, 2013).

1.12 Cancer situation in India

Among many diseases, cancer has become a big threat to human beings globally, in spite of good technological innovations in diagnosis and treatment cancer it is still a big threat to our society (Kotnis et al., 2005). Cancer is the second most diseases for maximum deaths in world after cardiovascular disorders (Jemal et al., 2007). A census of cancer patients was compiled from 2004 to 2010 in India, the increasing trends of cancer patients during the last few decades, the number of cancer patients
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have been predicted to double by the end of 2015 and 2020 in India (Fig 5.3). The entire summary of these data shows that number of male and female cancer patients increased continuously up to 2009, thus it is clear that number of cancer cases has increased gradually with time (Indian council of medical Research, 2009). In India, around 5, 55, 000 people died of cancer in 2010, an estimated 71 % of all cancer deaths in India occur in people 30 to 69 years of age (Dikshit et al., 2012). Among men 30 to 69 years of age, about half of all of cancer deaths were from oral and pharyngeal (23 %), stomach (13 %) and lung (11 %) cancers. Among women in the same age group, about half of all cancer deaths were from the cervix (17 %), stomach (14 %), breast (10 %), oral and pharyngeal (10 %) cancers.

As per ICMR (Indian Council for Medical Research, 2009) incidence of breast cancer has nearly doubled in the last 24 years. One in every 22 women is likely to develop breast cancer and it is fast becoming the number one cancer among urban women. As per the International Agency for Research on Cancer (IARC), India could see around 250,000 new cases of breast cancer by 2015. Cancer prevalence in India is estimated to be around 2.5 million, with over 8, 00,000 new cases and 5, 50,000 deaths occurring each year due to this disease (Nandakumar, 2001). Indians are at high risk of acquiring cancers due to high rates of smoking, tobacco use, occupational risks and unhygienic residential living conditions. Cancer has a huge impact on the Indian economy; estimation of expenditure of cancer patients includes both medical and non medical cost (Popkin et al., 2001).

1.12.1 Indian states and cancers

The state wise distribution of different cancer patients in India is shown in (Fig 5.4). From the figure, it clearly shows that lung cancer is the most common cancer in various states like Jammu and Kashmir, Himachal Pradesh, Delhi, Uttarakhand, Rajasthan, Maharashtra, Jharkhand, West Bengal, Andhra Pradesh, Kerala, Tripura and Manipur. It is also clear that cervical cancer is the second most common form of malignancy in the female population of Himachal Pradesh, Haryana, Rajasthan, Goa, Tamil Nadu, West Bengal while it stands at third position in females of Punjab, Andhra Pradesh and Uttar Pradesh. Breast cancer is the most common form of cancer
in the women of Himachal Pradesh, Delhi, Rajasthan, Nagaland and Goa and the second most common form of malignancy in females of Punjab, Maharashtra and Gujarat. In Tripura, breast cancer represents the third most common form of cancer in women folks. The Figure (Fig 5.4) also dictates that stomach cancer is the third most commonly reported cancer in Sikkim, Arunachal Pradesh, Tamil Nadu, Mizoram and Goa sates. It is the second most common cancer in Andhra Pradesh and Nagaland and the third most common type of malignancy in Jammu and Kashmir. Oral cancer stands at second and third positions in Goa and Assam states, respectively. Head and neck cancer patients have been observed in Tripura. Oesophageal cancer is a common type of malignancy after lung cancer in Jammu and Kashmir; Assam and Karnataka. Of course; Gall bladder cancer is not frequent in India, but it has been diagnosed in certain parts of Punjab, Uttar Pradesh and Bihar. Tongue cancer is the most common type of cancer in Madhya Pradesh; especially in Bhopal while it stands at second position in Goa. Oropharyngeal cancer is prevalent in Haryana and Meghalaya. This Figure also shows that some other types of cancers viz. skin, laryngeal and non-Hodgkin’s lymphoma are rare in India.

Cancers of ovary, prostate and brain have been reported only in some places in Rajasthan. The prostate and brain cancers were found in males of Rajasthan. This clearly indicates that an increased number of cancer patients every year due to various factors, these factors need to be controlled for their eradication. India is a growing country need attention on this, diet and lifestyle are the important factors to control the spreading of cancers hence Indians should be careful about these facts; cancer is disturbing the growing economy of the country which can be saved by proper handling of these diseases (Imranali et al., 2011).

1.13 Cancer prevention

Cancer prevention is action taken to lower the chance of getting cancer. Cancer is not a single disease, but a group of related diseases. Many things in our genes; our lifestyle and the environment around us may increase or decrease our risk of getting cancer. The WHO world cancer report (AACR, 2013) provides clear evidence that healthy lifestyles, public health action, government and health practitioners, personal
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care prevent as many as one third cancer worlds wide. Reduction of tobacco and alcohol use is the single greatest avoidable risk factor for cancer mortality worldwide, causing an estimated 22% of cancer deaths per year. Tobacco smoking causes many types of cancer; like the lung; esophagus, larynx (voice box), mouth, throat, kidney, bladder, pancreas, stomach and cervix (WHO, 2006). Alcohol use is a risk factor for many cancer types including cancer of the oral cavity, risk of cancer increases with the amount of alcohol consumed. Dietary modification is another important approach to cancer control. There is a link between overweight and obesity to many types of cancer, such as oesophagus, colorectum, breast, endometrium and kidney. Regular physical activity and the maintenance of a healthy body weight along with a healthy diet will considerably reduce cancer risk (American Cancer Society-Cancer Facts and Figures, 2006).

Infectious agents are responsible for almost 22% of cancer deaths in the developing world and 6% in industrialized countries. Viral hepatitis B and C cause cancer of the liver, human papilloma virus infection causes cervical cancer; preventive measures include vaccination and prevention of infection and infestation (WHO, 2006). More than 40 agents, mixtures and exposure circumstances in the working environment are carcinogenic to humans and are classified as occupational carcinogens (Siemiatycki et al., 2004).

1.13.1 Cancer treatment

With the advancement of research and technology in the area of cancer many advanced treatments are available to get rid of the cancer, possible in the earlier diagnosis. A few methods that are employed in the cancer treatment are discussed below.

a. Surgery: Surgery is the oldest form of cancer treatment. About 60% of cancer patients will undergo surgery, either by itself or in combination with other therapies. It provides the best chance to stop many types of cancer and it plays a part in diagnosing, staging and supporting cancer treatment. There are several types of cancer surgery:
1. **Curative surgery**: simply involves removal of a cancerous tumor. It works best on localized cancers that haven't yet spread to other parts of the body, and is often followed by radiation therapy or chemotherapy to make sure all cancerous cells have been removed.

2. **Preventive surgery**: is used to keep cancer from occurring. Many colon cancers can be prevented by removing precancerous polyps before they become malignant. A woman at very high risk for breast cancer may decide to have her breasts removed rather than worry about getting breast cancer later in life (www.mdanderson.org).

3. **Reconstructive surgery**: returns the body to normal or near-normal appearance or function following cancer treatment. The most common restorative surgery is breast reconstruction after a mastectomy. Facial reconstruction and testicular implants are also examples of reconstructive surgery (www.mdanderson.org).

b. **Radiation therapy**: a wide range of malignancies is treated with radiation and it has become a standard treatment. Radiation therapy uses high-energy radiation (x-rays, gamma rays and charged particles) to shrink tumors and kill cancer cells (Lawrence and Rosenberg, 2011). Radiation therapy kills cancer cells by damaging their DNA or creates charged particles within the cells that in turn damage the DNA, cancer cells whose DNA is damaged beyond repair stop dividing or die. When the damaged cells die, they are broken down and eliminated by the body's natural processes.

c. **Chemotherapy**: Cancer treatment through chemotherapy was introduced more than 50 years ago into the clinic (Johnstone and Ruefli, 2002). A chemotherapeutic agent is a natural or synthetic compound that can suppress; inhibit the development and progression of cancer. Secondary metabolite from natural products such as plant and microbes contribute towards chemotherapy and play a major role in cancer treatment. Phytochemicals present in the plants may function as chemopreventive or chemotherapeutic agents (Bagchi et al., 2004). Chemotherapy drugs can be divided into several groups based on factors such as how they work; their chemical structure and their relationship to another drug.
Because some drugs act in more than one way, they may belong to more than one group (Table 5.8). One of the properties of the anticancer drug is that, it cannot differentiate well between cancer and normal cells and attack both normal cells and cancer cells that actively growing, but normal cells have better repairment system than cancer cells during the chemotherapy treatment. Chemotherapy is usually given in cycles and for every chemotherapy cancer treatment received, it will be followed by a rest period after that. It is important to make sure normal cells of the body can recover from any damage caused by the anticancer drugs before the next treatment.

**d. Hormonal therapy:** Hormonal therapy is one of the major modalities of medical treatment for cancer, others being cytotoxic chemotherapy and targeted therapy (Biotherapeutics). It involves the manipulation of the endocrine system through the exogenous administration of specific hormones; particularly steroid hormones, or drugs which inhibit the production or activity of such hormones (hormone antagonists). Because steroid hormones are powerful drivers of gene expression in certain cancer cells; changing the levels or activity of certain hormones can cause certain cancers to cease growing; or even undergo cell death. Hormonal therapy is used for several types of cancers derived from hormonally responsive tissues, including the breast, prostate, endometrium and adrenal cortex.

**1.14 Natural compounds a potential source of anticancer agents**

Cancer is a group of disease characterized by uncontrolled growth and spread of abnormal cells. Cancer chemoprevention is an important strategy to reduce the cancer burden. The use of natural products and nutraceuticals in modern medicine for the prevention or treatment of cancer is an important aspect. Nature has been an always an attractive source of new therapeutic candidate; compounds have a tremendous chemical diversity found in thousands of species of plants, animals, marine organism and microorganism as potential anticancer agents (Newman and Lansky, 2007). Around 75-80% of the pharmaceutical active compounds are derived from the natural source. A large number of such natural compounds have been identified as having some potential cancer chemo preventive value, inhibiting mutagenesis, inducing apoptosis, which are critical characteristics of chemoprevention (Table 5.9).
a. **Plant based anticancer agents**

The plant has a long history of use in the treatment of cancer. Anticancer agents from the plant source started in the early 1950s with the discovery and development of Vinca alkaloids, Vinblastine and Vincristine. This led to the discovery of many novel chemotherapeutic agents showing wide ranges of cytotoxic activities (Cassady and Douros, 1980).

b. **Microorganism as the source of anticancer agents**

Antitumor antibiotics are also the important anticancer agents isolated from various microorganisms (bacteria, fungi) and already used in the treatment. Anthracycline, bleomycin, actinomycin are clinically useful agents, most of these agents exhibit antitumor activity mainly by inhibiting topoisomerase II (Binaschi et al., 2000). Anticancer agents from the microorganism may be an alternative source of anticancer agents.

c. **Marine as a source of anticancer agents**

Marine organisms are the richest source of natural products; an advance in the deep-sea collection and aquaculture technology gives a significant number of compounds having potential anticancer agents (Schwartmann et al., 2000). Diverse class of bioactive compound includes terpenes, steroids, peptides, alkaloids have been reported. Nature still today is a rich hub of active principles agent’s for cancer cells. Natural compound exerts a specific action on pathways involved in the disease development. By serious research, new bioactive compounds can be screened by various sources; it may be a new class of compound potentially having pharmaceutical significance.
1.15 Endophytes

Every process in the environment is carried out by the endless capacity of microorganisms that transforms the world around them, the microorganism is ubiquitous that are distributed everywhere. People began to investigate seriously on microbes as a source for bioactive natural products only after the Pasteur’s discovery of fermentation caused by living cells, which led the foundation for research in the microbial community. Gradual progress in the microbial science led to the development of the antibiotic era by Fleming via the discovery of penicillin from the *Penicillium notatum* (Strobel and Daisy, 2003). Furthermore the discovery of microbes for the applications that provide a broad spectrum of utility in medicine (e.g. anticancer and immunosuppressant functions), agriculture and industry because in the development of novel technology for screening process in medicine and agriculture (Lanen and Shen, 2006).

From last few years’ research has been made to isolate a special class of microorganisms called endophytes, which has been shown to have the potential for accumulation of various bioactive metabolites that can be used directly or indirectly as therapeutic agents for numerous diseases (Strobel et al., 2003). This is the beneficial substitutable approach for efficiently producing scare and valuable bioactive compounds (Gunatilaka, 2006).

1.15.1 What are endophytic microorganisms?

Endophytic microorganisms are the microbes that colonize; living; the internal tissue of plants without causing any immediate; overt negative effects (Bacon and White, 2000). Another classical definition is the microorganisms that colonize plant tissues without producing any apparent symptoms (Hirsch and Braun, 1992). Plants may serve as a reservoir of large numbers of these microorganisms. Endophytes are microorganisms (mostly fungi and bacteria) that inhabit plant hosts for all or part of their life cycle. They colonize the internal plant tissues beneath the epidermal cell layers; intracellular space of stems; petioles; roots and leaves of plants (Strobel and Long, 1998) without causing any apparent harm or symptomatic infection in their
host. Approximately, there are nearly up to 300,000 plant species on earth, each individual plant is the host to one or more endophytes, and many of them may colonize in certain hosts. It has been estimated that there may be as many as one million different endophytic fungal taxa, thus endophytes may be hyperdiverse (Strobel and Daisy, 2003).

Endophytic microorganisms fall into several identifiable classes often in relation to their plant organ source; with the major group as fallows (Stone et al., 2000): a) Endophytic calvicipitaceae, b) Fungal endophytes of dicots, c) Endophytic ascomycota, d) Other systemic fungal endophytes, e) Fungal endophytes of lichens, f) Endophytic fungi of bryophytes and ferns, g) Endophytic fungi of tree bark, h) Endophytes of root, i) Fungal endophytes of galls and cysts, k) Prokaryotic endophytes of plants (including endophytic bacteria and actinomycetes). Other microbial forms such as *Mycoplasma*, *Rickettsia* and *Archaebacteria*, most certainly exist in plants as endophytes but no evidence for them has been presented so far. It could be noted that fungi are most frequently encountered endophytes (Staniek et al., 2008). Endophytes have been shown to be a potential source of bioactive and structurally diverse natural products and secondary metabolites.

1.15.2 Discovery of endophytes

The existence of the endophytes has been known for over one hundred years. The evidence of plant-associated microorganisms found in the fossilized tissues of stems and leaves revealed that the plant-microbe associations might have evolved from the time that higher plants first appeared on the earth (Strobel et al., 2003). The symbiotic association of plants and microbes most probably belong to an earlier time to the emergence of vascular plants (Rodriguez et al., 2009).

In certain environments; some microbes appear to penetrate plant tissue; actively invading cell wall by hydrolyzing cell wall components using hydrolytic enzymes such as, cellulose, pectinase and/or through wound. Some bacterial endophytes are believed to be originated from the rhizospheric microflora which penetrate through root hairs and colonize root tissue (Misko and Germida, 2002). During long-term co-
evolution of endophytes with their host plants, the endophytes seem to have developed the adaptation towards the microenvironment inside the plant tissue, which may involve cross talk between plant host and the endophyte or exchange of genes between host and residing microbe (Stierle and Strobel, 1993; Germaine et al., 2004).

1.15.3 Diversity and distribution of endophytes

Endophytes are ubiquitously present in plants growing in tropical, temperate and boreal forests with the hosts ranging from herbaceous plants to woody trees in various habitats including extreme arctic alpine and xeric environments to mesic temperate and tropical forests (Shamoun and Sieber, 2000). There are approximately 300,000 different plant species existing on earth and considering that each individual plant is a host to one or more endophytes, one can estimate existence of at least one million endophytes. Nearly all vascular plant species examined to date were found to harbor endophytic bacteria and fungi (Arnold, 2007). The endophytes are presumably ubiquitous in the plant kingdom; the diversity of which may depend on the host species, geographical location and surrounding environment. Various groups of microbial endophytes viz. fungi, bacteria and actinomycetes have been studied.

1.15.4 Host endophytes interaction

There is a complex relationship between endophytes and their host plants. Host-endophyte interactions can range from mutualism through commensalism to parasitism; as the interactions are often depending on the genetic dispositions of the two partners, their developmental stage and nutritional status; but also on environmental factors (Schulz and Boyle, 2005).

Commensalism provides benefit to the endophytes by enabling an undisturbed existence and nutrient supply without affecting the host. The mutual relationship benefits the endophytes through provision supply of energy, nutrients, shelter as well as protection from environmental stress. On the other hand fungal endophytes indirectly benefit plant growth by producing special substances mainly secondary metabolites and enzymes, which are responsible for the adaptation of plants to abiotic stresses such as light, drought and biotic stresses, such as herbivore, insect and

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nematode attack or invading pathogens. Under certain conditions, endophytes may become parasitic and become pathogens causing symptomatic infection. Endophytes of certain plant could be a pathogen of other plants, depending on the balance between pathogenicity and endophytism of the microorganism in the different hosts (Saikkonen et al., 2004). The asymptomatic colonization of endophytes is a balanced antagonistic interaction between host plant and endophytes and as long as endophytic virulence and plant defense are balanced, the interaction remains asymptomatic. Once the host-endophyte interaction becomes imbalanced either disease results in the host plant or the plant defense machinery kills the pathogenic endophytic fungus (Schulz and Boyle, 2005).

Interaction is balanced or imbalanced depends on the general status of the partners, the virulence of the fungus and the defenses of the host and both virulence and defense being variable and influenced by environmental factors, nutritional status and developmental stages of the partners. Hence, commensalism and mutualism require a sophisticated balance between the defense responses of the plant and the nutrient demand of the endophyte (Kogel et al., 2006).

1.16 Biological role of endophytes

Endophytes play vital roles in various aspects of life varying from its effects on host plants to its effects to environmental and human life. Endophytes are capable of synthesizing bioactive agents that can be used by plants for defense against pathogens and/or stimulating plant growth and other agents have been proven useful for novel drug discovery process.

a. Roles of endophytes on host plants

Plants are benefited by endophytes in various ways; the potential functions of endophytes have not been clearly defined, but in the most cases, the presences of endophytic microorganisms in the host plants are beneficial to their host plants. Endophytes can actively or passively promote the plant growth through a variety of mechanisms, as endophytic metabolites provide a variety of fitness to host plants
enhanced by increasing plant resistance to biotic and abiotic stresses, as well as enhance plant growth (Barraquio et al., 1997).

**b. Role of endophytes in host growth and nutrient uptake**

One of the potential functions of endophytes is especially root mycorrhizal fungi are the facilitation of plant nutrient uptake, which in contrast leads to growth stimulation. Improved nutrition and growth may have positive indirect effects on the other well-known functions, such as greater stress tolerance or pathogen resistance in plants (Kageyama et al., 2008).

**c. Role of endophytes in production of phyto-hormones**

Endophytes enhance the growth of host plant by producing phytohormones without any changes in the host nutrient uptake; it enhances the biomass of host by producing the growth hormones production. The use of culture extracts of endophytic fungi enhance the plant growth this indicates that soluble agents in culture may stimulate host this shows that endophytic fungi produce phytohormones *in vitro* as well as *in vivo* (Selim et al., 2012).

**d. Role of endophytes in hosts tolerance to stress**

Endophyte residing in the host plant helps to tolerate and withstand environmental stress such as drought, salts and high temperatures. Endophytic microorganisms actively response to various biotic and abiotic stress factors that which hampers the overall agricultural scenario (Berg et al., 2005).

**e. Stimulation of plant secondary metabolites**

Secondary metabolites are the group of compounds that does not play important role in the basic life function of the plant life, but play a role in the adaptation of plants to their environment. Endophyte plant association could be subjected to stimulate the production of secondary metabolites by the host plant. Observation indicates that endophytic infections alters pattern of gene expression in the host plant. It has been studied that endophytic fungi *Fusarium* sp of *Euphorbia pekinensis* could promote the
growth and increased its terpenoids content (Yong et al., 2009). A similar situation was observed in suspension cultures of *Taxus cuspidate*, to which the addition of fungal endophyte culture supernatants led to the yield of paclitaxel; which was 1.8-fold of the yield from control (Li et al., 2009). It seems that co-culturing with endophytic elicitor is an alternative way to enhance plant secondary metabolites.

**f. Endophytes in defense mechanism**

Endophytes play a major role in the defense mechanism of plant against various plant pathogens, some endophytic species may induce plant defense mechanism to attack pathogen and it has been reported that antibiotic substance was produced by endophytic fungi, which inhibit the growth of plant pathogen (Strobel et al., 2002; Wang et al., 2007).

**1.17 Bioactive compounds from the endophytes**

Plants has been recognized as ‘bio-factories’ of potentially valuable therapeutic compounds. Many complementary and alternative medicines have enjoyed increased popularity in recent years (Joseph and Priya, 2011). Drugs obtained from the natural source play a significant role in the prevention and treatment of human diseases. From the past few years there has been an increasing interest on the investigation of novel bioactive compounds from plants. But slow growing rate and harvesting of rare endangered species pose a risk and imbalance in the biodiversity of plants. Recent and scenario shows that discovery rate in active chemicals compounds are declining (Lam, 2007).

Due the long period of co-evolutional, friendly relationship was gradually observed between each endophytic microorganism and its host plant. Some endophytes have developed the ability to produce same or similar bioactive substance as those originated from the host plant. This could be beneficial to understand the relations between the endophytes and their host plant and to develop an approach for producing scarce and valuable bioactive compounds (Gunatilaka, 2006).
Introduction

Endophytes are chemical synthesizer inside the plants (Owen and Hundley, 2004). Bioactive compounds produced by endophytes have been promising potential usefulness in safety and human health concern (Strobel and Daisy, 2004). Endophytes provides a broad variety of bioactive secondary metabolites with unique structure, including alkaloids, benzopyranones, chinones, flavanoids, phenolics acids, quinines, steroids, terpenoids, xanthones and others. Such bioactive metabolites find wide-ranging application as antibiotics, immunosuppressant, antiparasitics, antioxidant and anticancer agents (Gunatilaka, 2006). These endophytes can mimic the chemistry of their respective host plant and make almost similar bioactive natural products or derivatives (Owen and Hundley, 2004). In the growth of human population as well as in technological advances; new diseases are immerging and old diseases started gaining active, to fight against these requires the discovery and development of new drugs to combat them. We need useful compounds to provide assistance and relief in all aspects of the human condition. Therefore, alternative sources are outmost essential since organic synthesis are not yet economically feasible and high cost makes it unavailable to people in the under developed countries of the world. Endophytes are the microorganism that includes bacteria and fungi living within the plant tissue these could be the alternative source.

a. Antimicrobial compounds from endophytes

The haunt of new novel antimicrobial compounds from endophytes is an important alternative to fight against drug resistance human pathogens. Antimicrobial metabolites are low molecular weight organic natural substances made by microorganism that are active at low concentration against other microorganism. The antimicrobial compounds can be used not only as drugs by humankind but also as food preservative in control of food spoilage and food borne disease (Liu et al., 2004). To till date studies have been reported a large number of antimicrobial compounds like alkaloids, peptides, steroids, terpenoids, phenols, quinines and flavanoids. Many bioactive compounds including antifungal, antibacterial and antiviral compounds have been isolated from endophytes (Pongcharoen et al., 2008) (Table 5.10).
b. Antioxidant compounds

Natural antioxidants are commonly present in medicinal plants, vegetables and fruits. Polysaccharides from plants and microorganisms have extensively studies as potent antioxidants. Many antioxidants compound posse’s anti-inflammatory, anti-atherosclerotic, antitumor, antibacterial activities in high or lower level (Owen and Hundley, 2004; Cozma, 2004). It has been reported that metabolites from endophytes can be a potential source of novel natural antioxidants (Liu et al., 2007). It has been reported that the phenolic content in the endophytic extract contribute to the antioxidant activity (Huang et al., 2007) (Table 5.11).

c. Anti cancer agents from endophytes

The discovery of the paclitaxel (taxol) producing endophytic fungus Taxomyces andreanae from Taxus brevifolia (Stierle and Strobel, 1993) evoked the interest in endophytes as potential new sources for therapeutic agents. This early work set the stage for a more comprehensive examination of the ability of other Taxus species and other plants to yield endophytes producing taxol. On the other hand, endophytic fungi were found to produce interesting bioactive metabolites not related to the natural products produced by their host plants. For example; chaetomelic acids A and B, isolated from the culture of an endophytic Chaetomella acutisea, were found to be specific inhibitors of farnesyl-protein transferase (Ishii et al., 2000). Extracts of Alternaria alternata an endophytic fungus isolated from Coffea arabica displayed moderate cytotoxic activity towards HeLa cells in vitro (Fernandes et al., 2000). According to the WHO, 80 % of the world’s population of developing countries depends on plant-derived medicines for health care. More than 50 % of the all drugs in clinical use are derived from the natural source. Whereas slow growth rate and extinction of endangered species make pose a risk hence microbial route of bioactive compounds especially against cancer has upsurge in recent years with the invention of taxol from endophytic fungi provided a new source of anticancer agents from the endophytes of medicinal important plants (Table 5.12).
d. **Other bioactive compounds from endophytes**

Endophytic fungi are also known as producers of many other metabolites of biological interest, such as anti-inflammatory, anti-diabetic, anti-malarial and immunosuppressant agents, as well as insecticidal and anti-nematodes agents (Table 5.13). Immunosuppressive drugs are commonly used to prevent the rejection during transplantation process and to treat autoimmune disorders such as rheumatoid arthritis. The endophytic fungus *Fusarium subglutinans*, isolated from *T. wilfordii*, produces the immunosuppressive but noncytotoxic diterpene pyrones subglutinol A and B (Lee *et al.*, 1995). Many reports reported that endophytic fungi are capable of producing anti-inflammatory compounds such as phomol and mevinin acid, isolated from the endophytic fungus *Phomopsis* (Weber *et al.*, 2005). Highly desirable search for sustainable and economically feasible new source bioactive compounds has tempted various researchers, which indeed have drawn attention proclaiming endophytes creating a huge biodiversity as independent bio-factories.
1.18 *Viscum album* L. (European Mistletoes)

**General introduction**

Parasitic interactions between organisms play a fundamental role in ecosystems. Parasites are organisms that obtain sustenance from, another organism and complete at least part of their life cycle on their host (Hawksworth and Wiens, 1996). The interacting partners often belong to different families or even kingdoms. Based on the degree of chlorophyll content and nutrient uptake, two major types of plant parasites can be distinguished. Hemiparasite with green leaves contain chlorophyll and perform photosynthesis, but is dependent upon host plants for water and nutrient uptake. Holoparasites have low chlorophyll content or lack chlorophyll altogether and are therefore often conspicuously pale green to brown. These plants take all nutrients from their host plants.

One well-known group of parasitic angiosperms is *V. album* (European mistletoe). Mistletoes are a group of obligate hemi-parasitic plants that belong to the families Viscaceae and Loranthaceae within the order Santalales (Barlow, 1983). In the past, Viscaceae have often been placed inside Loranthaceae, because of differences in floral structure, embryology and chromosomal traits, the distinction of the two families is mostly accepted (Calder, 1983).

The term mistletoe was first applied to the European mistletoe, *V. album*. In the seventeenth and eighteenth centuries when new species were discovered all over the world, all plants that showed similarities in morphology and life-history of *V. album*, where it is classified as mistletoes (Calder, 1983; Barlow, 1983). Nowadays, mistletoes are flowering plants that show some degree of parasitism. Mistletoe types include root-parasitic, terrestrial shrubs (e.g. *Nuytsia floribunda*), common epiphytic stem parasites (e.g. *V. album*) and even parasitic species that produce only flowers and fruits on the surface of the host (e.g. *Viscum minimum*). All mistletoes are shrubs and develop a haustorium to contact the host xylem for water and nutrient uptake hence they are classified as hemi parasites.
1.26 History of mistletoe

Theophrastus (371–287 BC) described mistletoe in his botanical treatise Historia Plantarum as an evergreen plant growing on pine and fir trees and used for feeding animals during harsh winters. He acknowledged that mistletoe does not grow on the earth and its seeds are spread through bird excreta that feed on mistletoe berries. Celtic Druids considered mistletoe sacred primarily because of the ability of this plant to remain flourishing green even in dead winter without having roots in the earth. They considered this plant a symbol of ever-lasting life.

Pliny the Elder (23–79 AC) the legendary Roman naturalist and author of Historica Naturalis reported that the Druids followed ceremonious removal of mistletoe growing on oak trees using a golden sickle on the sixth day after new moon. They considered the plant to augment fertility and an antidote for poisons. They believed it to possess miraculous properties to cure every illness due to its’ all healing abilities.

1.27 V. album

V. album is a species of mistletoe in the family Santalaceae; commonly known as Mistletoe; European Mistletoe or common Mistletoe. It is native to Europe and western and southern Asia. V. album is an evergreen plant that grows hemi-parasitically on the stems of its host by deriving water and minerals from it, this mistletoe never touches the earth and blooms during winter (Zuber and Widmer, 2000).

Area of distribution

The area of distribution of V. album (European mistletoe) is Central Europe (from North Africa to Southern England and Southern Scandinavia); Southwest-and East Asia to Japan.

Host specificity

V. album is able to infect a large number of host plants. A compilation of the host range (Barney et al., 1998) has shown that 384 taxa of shrubs and trees may become
infected under natural conditions. Three widely distributed subspecies of *V. album* that differ in host specificity have been recognized (Ball, 1993).

The European population is divided into three sub-species that have different hosts.
2. *V. album* L. sp. *abieteris* Beck; growing on *Abies* sp.

**Scientific classification**

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<tr>
<td>Species:</td>
<td><em>V. album</em></td>
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**Binomial name**

*V. album* L.

**Classification**

The mistletoe was one of the many species originally described by Linnaeus. Its species name is the Latin adjective albus "white". It and the other members of the genus *Viscum* were originally classified in the mistletoe family Viscaceae; but this family has since been sunk into the larger family Santalaceae.

**Subspecies**

Several subspecies are commonly accepted. They differ in fruit colour, leaf shape, size and most obviously of the host trees utilized, taxonomy and morphology (Blamey and Wilson, 1989).
Geographical distribution

Mistletoes have been known for a very long time. The occurrence of *V. album* in southern Europe and was first reported by Theophrast (287-371 BC) and was mentioned by others such as Aristoteles and Plinius (23-79AD). *V. album* is native to most parts of Europe. It also occurs in the south of Great Britain; Asia.

Habitat

The habitat is topographically and ecologically defined by the host tree. Host size and canopy characteristics determine where mistletoes can grow (Dawson *et al*., 1990). *V. album* takes water and dissolved inorganic compounds directly from the xylem of its host. *V. album* can grow in a temperature-sphere with an average temperature above 15 °C in the warmest month and an average above 8 °C in the coldest month. Flowers tolerate frost down to 8 °C. *V. album* is a light-demanding species, especially for germination.

Morphology

*V. album* is a semi-parasitic woody perennial commonly found on the oaks and deciduous trees. The plant is small, dioecious and shrubby with evergreen leathery entire leaves, foliage leaves are opposite, rarely 3 (4-5) whorled, sessile, oviate-oblong, obtuse, leathery and yellowish-green. In general leaf length ranges from (1.3-2) and (8 -10.7) cm, with a minimum width of 0.3 cm and a maximum of 4.3 cm. The foliage leaf internodes are 1 - 9 cm long. The length of leaves and internodes increases during the first five years after germination and decreases slowly thereafter. Shape and size of leaves may vary considerably, not only within an individual, but also between different individuals of the same host tree or of different host trees (Langbehn and Weber, 1995). Leaves have a faint but characteristic odor and a bitter taste. The inflorescence is a dichasium subtended by a pair of usually fused bracts (bracteal cup). The flowers are inconspicuous, sessile, small and yellowish-green. *V. album* is dioecious. Staminate and pistillate flowers are usually 3 (-5) in triads with one terminal and two lateral flowers. Mistletoe is propagated by birds that eat the berries and then excrete seeds; or smear them on branches by wiping the sticky pulp
off their beaks. Under appropriate conditions; seed germinate and roots penetrate the branch of the host tree (Fig 5.5).

**Nutrition of *V. album***

*V. album* is hemiparasitic; i.e. it depends for water and mineral nutrition on its respective host, but is able to produce carbohydrates by photosynthesis. It contains all pigments, chlorophyll A and B as well as carotenoids that are necessary for photosynthesis (Luther and Becker, 1986). As there is no phloem connection between mistletoe and host, organic substances from the host are only transported via the xylem. This transport includes amino acids, cyclohexols and thiols (Richter and Popp, 1992). Mistletoe has a much higher transpiration rate than their respective hosts. Mistletoe transpiration is higher on hosts with low nitrogen content than on hosts with higher nitrogen content in their xylem sap. All mistletoes including *V. album* usually have a higher mineral content than the respective host and especially the infected host branch.

**1.19 Bioactive chemical compounds from *V. album***

Mistletoe has been used in folk medicine from past ancient times. Today it is applied as a remedy for a broad spectrum of different diseases, such as epilepsy, diabetes, hypertension, cancer and arthritis. Modern day research indicates that a bioactive component in the mistletoe plays a valuable role treating above mentioned diseases. The main ingredients of the *V. album* extract are lectins, steroids, triterpenes, sesquiterpenes, lactones, flavanoids, alkaloids, organic acids, amino acids and peptides.

**a. Mistletoe lectins**

Lectins are the glycoproteins; these are of complex structure made up of both proteins and carbohydrates. They capable of binding to the surface of different cells and induces biochemical changes in them. Lectins will recognize specific carbohydrates residue and bind to them on the cell surface; nuclear structure and to components of ECM (Extra Cellular Matrix). European mistletoe lectins (ML) were (ML-I, ML-II,
ML-III), all these lectins were type II ribosome binding protein; lectin of V. album was D-galactose, N-acetyl-galactosamine. Lectin isolated shows the increase metabolic activity of lymphocytes and it was toxic to the variety of cancer lines.

b. Flavonoids

Flavonoids are most commonly known for their antioxidant activity. They are the “nature’s biological response modifiers”; due the strong ability to modify the body's reaction to allergens; viruses and carcinogens. Flavonoids like Flavoyandrinin-A, Flavoyandrinin-B have been reported (Ohta and Yagashita, 1997) also Quercetin and their derivatives have been reported (Lorch, 1993) (Table 5.14).

c. Phenylpropanoids

It is commonly known as cinnamic acids are related to flavanoids, the best known Phenylpropanoids are Caffeic acid, Ferullic acid and Sinapic acid have been isolated from V. album (Pfuller, 2000) (Table 5.15). Cinnamic acid is used in flavors, synthetic indigo and certain pharmaceuticals, though its primary use is in the production of the methyl, ethyl and benzyl esters for the perfume industry.

d. Triterpenes

It constitutes a large and varied class of hydrocarbons, which are widely distributed. There is a rich content of triterpene such as β-amyrin, β-amyrin- acetate, betanulic acid (Pfuller, 2000) (Table 5.16). Triterpenoids and their derivatives act at various stages of tumor development, inhibit initiation and promotion of carcinogenesis, induce apoptosis, suppress tumor angiogenesis, invasion and metastasis through regulation of various transcription and growth factors as well as intracellular signaling mechanisms (Liby et al., 2007).

e. Alkaloids

It is of naturally occurring chemical compounds containing basic nitrogen atoms, they have important pharmacological effects. But alkaloids like substance in V. album. Such as phenylethylamine, choline and acetylcholine have been reported (Khwaja
et al., 1986). Alkaloids from Korean mistletoe inhibited the growth of cultured leukemia cells and increased the life span of leukemic mice. Because of the extreme liability of these alkaloids, their structures have not yet been defined. It has been proposed that the alkaloids are glycoconjugates with proteins and lectins from *V. album* (Pfuller, 2000).

f. Cyclopeptides

Cyclic compounds may be produced by peptide chains are called cyclopeptides. In the cyclic peptides, the two ends are linked together producing a circular topology. This property gives the cyclopeptides many advantages over linear peptides. When the two ends are joined together, it makes it almost impossible for digestive enzymes to attack the peptide. Viscumamide is the only cyclopeptide discovered in the Loranthaceae family (Okumura and Sakurai, 1972).

1.20 Biological activity of *V. album* and their pharmaceutical impact

In recent years bioactive compounds derived from the natural resource, mainly from plants and endophytes have been used intensively to prevent a wide variety of diseases. Where these natural compounds have some advantages over synthetic ones, being obtained easily, economically and have negligible side effects. European mistletoe has been used for decades as an alternative treatment and adjuvant cancer therapy, particularly in Germany, Austria and Switzerland. A number of biological activities such as anticancer, antimycobacterial, apoptosis, including antiviral and immunomodulatory activities have been reported.

a. Anticancer and immunomodulation

The main ingredients of *the V. album extract* are its three ribosome inactivating proteins or lectins ML-1, ML-2 and ML-3. These are glycoprotein binding with D-galactose and N-acetyl-galactosamine. They cause cells to agglutinate and inhibit protein synthesis at the ribosomal level. Lectins are structurally similar to two highly biologically active toxic proteins, ricin and abrin. Among these, ML-1 is most studied because of its antitumor and immunomodulating activities. ML-1 stimulates the Th-1
response, though cytokine-dependent cytotoxicity is provoked, which plays an important role in the natural defense mechanism against tumor cells. A Th-2 response provokes the production of IL-4, IL-5, IL-10, thus B cells increase their production of IgG2 and IgG4. It has been reported that VAA-1 was capable of inducing cytotoxicity and induced apoptosis through caspase-3 activation, induces the nuclear fragmentation on against human A549 lung cancer cells (Siegle et al., 2001) (Table 5.17).

b. Hypotensive effect

The hypotensive effect is documented to active components of *V. album*, the exact nature of the hypotensive effect of mistletoe is unclear, it has been reported that activity is mainly due to an inhibitory action of excitability of the vasomotor center in the medulla oblongata. Crude extract of *V. album* leaves has significantly reduced the blood pressure but had no effect on the heart rate (Ofem et al., 2007).

c. Cardiovascular effect

Use of *V. album* extract shows the effects on cardiovascular diseases, Viscotoxins have been shown to induce reflects bradycardia and posses negative inotropic effect on cardiac muscle, as well as vasoconstriction. Phoratoxin shows the action of skeletal muscle fibersn. Phenylpropanoids also plays a role in mistletoes cardiovascular effects through a postulated inhibition of cyclic monophosphate phosphodiesterase (Deliorman et al., 2000).

d. Mistletoe in diabetes

Diabetes mellitus was characterized by the lack of the pancreatic hormone insulin, the *V. album* extract has been documented for treating diabetes. Tea prepared from the leaves of the plant extract showed 1 to 2 fold stimulation of insulin secretion from the pancreatic B- cells, providing the evidence that insulin releasing natural compound in the *V. album* that may contribute to anti-diabetic property of the plant (Gray and Flatt, 1999).
1.21 Lectins

Lectins or glycoproteins are a class of multivalent carbohydrate binding proteins of non immune origin which recognize diverse sugar structure with a high degree of specificity in non catalytic manner. The term lectin was coined by William Boyd in 1954. Lectins are multivalent in nature and can bind to the carbohydrate moieties on the surface of erythrocytes and agglutinate the erythrocytes without altering the properties of carbohydrates (Lam and Ng, 2011). Lectins are distributed extensively in nature. Hundreds of lectin has been isolated from many sources like plants, viruses, bacteria, vertebrates, invertebrates. They have been implicated in cellular signaling, malignancy, host pathogen interactions, scavenging of glycoprotein from the circulatory system, cell-cell interactions in the immune system, differentiation and immune responses and protein targeting to cellular compartments (Sharon, 2008).

1.21.1 Groups of lectin

Lectins were classified into three groups based on their overall structure and properties (Peumans and Van Damme, 1995)

a. Merolectins: Proteins that consist of exclusively of a single carbohydrate binding domain. These proteins are incapable of precipitating glycoconjugates or agglutinating cells because of monovalent nature. Monomeric mannose binding proteins from orchid and class I chitinases that possess a chitin binding domain and a catalytic domain; all these come under this category (Collinge et al., 1993).

b. Hololectins: These are exclusively made of carbohydrates- binding domain. Since hololectins have multiple binding sites they are fully capable of agglutinating cells and precipitating glycoconjugates. Most plant lectins belong to this subgroup (Rudiger, 1998).

c. Chimerolectins: These are fusion proteins composed of one or more carbohydrate binding domain and unrelated domain with a well-defined biological activity. The number of binding sites, chimerolectins behaves as merolectins or hololectins. For example, the type 2 Ribosome inactivating proteins (RIPs) ricin and abrin consist of a
toxic A chain (which has the N glycosidase activity characteristic of all RIP's) and carbohydrate binding B chain with two carbohydrate binding sites to agglutinate cells. Based on the number of sugar-binding sites chimerolectins behave as either merolectins or homolectins. Based on their molecular structure lectins are further classified into three classes: simple lectins, mosaic lectins and macro molecular lectin (Sharon and Lis, 2004).

**d. Simple lectins:** These have the molecular weight below 40 kDa with a small number of subunits, which may contain an additional domain binding site. All known plant lectins are galactose- specific lectins come under this class.

**Multidomain lectin:** These types of lectins consisting of several kinds of domains, but among this domain only one of which may possess a carbohydrate binding site. These lectins have a wide range of molecular weight. Many of these lectins are monovalent.

**Macromolecular assemblies:** These types of lectins are commonly observed in the form of fimbriae or pilli in bacteria. Fimbrial filament is made up of polymers of the major subunit (Gaastra and Svennerholm, 1996). Usually a one subunit possesses a carbohydrate combining site and is responsible for the binding activity and sugar specificity of the fimbriae.

**1.22 Historical prospective**

It is generally believed that the earliest description of lectin was by Stillmark in 1888 an agent that can agglutinate animal erythrocytes from the extract of castor bean (*Ricinus communis*) this hemagglutinin was toxic and was named as ricin. Boyd and Shapleigh in 1949 coined the term lectin (Latin; ‘legere’; to select or choose) based on their observations that some plant seed extracts could distinguish between human blood groups. Hellin (1891) discovered abrin, the toxic protein from jequirity beans. During the 1890's, Ehrlich worked with ricin and abrin, and discovered immunospecificity and reversibility of the antigen-antibody reaction as some of the fundamental principles of immunology. One of the first lectins to be crystallized was Concanavalin A from *Jackbean* by Sumner. Sugar specificity and inhibition of
Introduction

hemagglutination of the lectin was reported by Watkins and Morgan in 1952 (Table 5.18). Lectins are widespread in distribution and have been isolated from microorganisms, plants and animal. Over the years, numerous lectins have been isolated from plants as well as from microorganism and animals (Sharon and Lis, 2004).

The structures of lectins have been deduced. Although most of the well characterized lectins are from plants, fungal lectins are receiving increased attention due to their importance from basic as well as applied aspects. This compilation gives a comprehensive account of fungal lectins with respect to their occurrence, purification, physicochemical properties, biological roles and applications. The lectin induced agglutination of cells has originally served as the most common assay to detect and quantify lectin activity in a variety of organisms (Goldhar, 1994). Even the lectins were isolated first from plants, the presence of these lectin outside plants has been demonstrated. They are distributed in all types of organisms. A brief account of lectin occurring in different organism is mentioned below.

a. Viral lectins: Viruses’ example Influenza viruses interact with their target cells by means of lectin carbohydrate interaction. Interaction of influenza virus with erythrocytes and other cells by recognizing V-acetyl neuraminin acid present on the cell surface this interaction is necessary for infection. This would be critical for mediating cellular attachment. The detailed knowledge of interaction provides the basis for designing the antiviral drugs that would block the viral attachment to the cells (Sharon and Lis, 1989).

b. Protozan lectins: For the infection to develop involves attachment of the pathogen to a host cell receptor most the protozoa that infects humans and animals have lectins on their surface. Well studies pathogenic amoeba Entamoeba histolytica, Hartmannella vermiformis. A lectin sugar interaction plays a role in adherence. Two lectins isolated and characterized from Entamoeba histolytica, one is specific for β-1, 4 linked oligomers of GlcNAc (Kobiler and Mirelman, 1981) and the other for Gal and GalNAc (Petri et al., 1989).
c. **Slime molds lectins**: Lectin from slime molds induces mold to aggregate through cell-cell recognition; this is a key event in the differentiation of organism from their single-cell; vegetative form to an aggregated form this process involves the lectin in cell-cell recognition. Galactose specific lectin, Discodin I has been produced by *Dictyostelium discoideum* during its developmental stages. The lectin is present on the surface of aggregating cells (Sharon and Lis, 1989).

d. **Fungal lectins**: Fungal lectins have become a rich source for new lectins about 82% arising from mushrooms, 15% from micro fungi and 3% from yeast (Singh *et al.*, 2010). They serve as storage proteins, growth and morphogenesis, host recognition, adhesion, defense and in the mating process. Fungal lectin are reported to participate in the formation of primordial, creation of mycelium structures to facilitate, penetration of parasitic fungi into the host organism as well as mycorrhization (Guillot and Konska, 1997).

e. **Bacterial lectins**: Most of the bacterial strain produces surface lectins commonly in the form of fimbriae. Best characterized lectin is mannose specific fimbrial lectins of *Escherichia coli*. Bacterial surface lectins plays an important role in the infection by adhering to epithelial cells of the host, *E. coli* to the urothelial surface via type-1 fimbriae-uroplakin interactions may play a role in their colonization (Sharon, 1987).

f. **Animal lectins**: Animals are capable of producing varieties of lectins both intracellular and extracellular. Animal lectins discovered were found to be naturally multivalent; either because of their defined multisubunit structure or by virtue of having multiple carbohydrate-binding sites within a single polypeptide. Animal lectins are classified into different groups having different carbohydrate specificity (Table 5.19).

g. **Plant lectins**: Plant lectins are of heterogenous group of proteins due to their difference in their molecular structure, biochemical properties and carbohydrate-binding specificity (Van Damme *et al.*, 1998). Plant lectins occur in all parts of plant seeds, vegetative tissue, such as leaves, bark, stem, rhizomes, bulbs and tubers (Peumans and Van Damme, 1995). Amount of lectins may vary seeds contains about
50% of total seed proteins, vegetative tissue contains 20% of total vegetative proteins.

Plant lectins can be classified based on different characteristics. According to one scheme, they are classified based on their carbohydrate-binding specificity; into several specificity groups. Using this criterion, plant lectins have been distinguished as mannose, mannose/glucose, mannose/maltose, Gal/GalNAc, GlcNAc/(GlcNAc)$_n$, fucose and sialic acid binding lectins (Van Damme et al., 1998). Another classification of plant lectins is based on evolutionary and structurally related proteins. Seven lectin families are classified namely (Table 5.20).

1. **Legume lectin**: Legume lectins are the best known lectin family (Sharon and Lis, 1990) and best characterized family of plant lectins. Concanavalin -A (ConA) was the first plant lectin to be purified and crystallized and the first lectin whose primary and three dimensional structures were resolved. Legume lectins are dimeric or tetrameric proteins, each subunit (25-30 kDa) consisting of one carbohydrate binding site. A wide range of carbohydrate- binding specificities were observed in legume lectins.

2. **Monocot mannose binding lectins**: Monoct mannose binding proteins are the relatively new groups of lectins. These lectins are specificity towards mannose. All monocot mannose binding proteins are built up of 1, 2, 3 or 4 subunits of 12 kDa exhibit specificity towards mannose. So far, these lectins have been found in six different monocot families, namely; Alliaceae, Amaryllidaceae, Araceae, Bromeliaceae, Liliaceae and Orchidaceae (Van Damme et al., 1998). They are reported to occur in various vegetative tissues such as leaves, flowers, ovaries, bulbs, tubers, rhizomes, roots. Other than mannose specific lectin complex sugar specificity has also been reported to occur in some monocots, N-acetyl-D-lactoseamine specific lectin from *Arisaema flavum* (Singh and Kamboj, 2004), *Arundo donax* (Kaur et al., 2005) etc. Monocot mannose binding proteins receive a lot of attention because of their potent antiviral (Balzarini et al., 2006) and anti-insect properties (Gatehouse et al., 1995).

3. **Chitin-binding proteins**: Chitin binding proteins is largest lectin family and containing hevein domain(s). The term hevein, a small 43 amino acid residue protein
found in the latex of the rubber tree (*Hevea brasiliensis*) (Lee et al., 1991). Chitin-binding lectins are ubiquitously found in plants. Their occurrence has been reported in several plant families; namely Gramineae, Solanaceae, Phytolaccaceae, Urticaceae, Papavaroacaeae and Viscaceae (Peumans et al., 1995).

4. **Type 2 ribosome- inactivating proteins (RIP):** Type 2 ribosome inactivating proteins are chimerolectins composed of the polynucleotide adenosine glycosidase domain (A-chain) tandemly linked to a galactose specific domain (B-chain). The chain A and B are linked by disulfide bonds. Type 2 RIPs occur in several families; namely Euphorbiaceae, Fabaceae, Viscaceae, Passifloraceae, Ranunculaceae, Lauraceae, Sambucaceae, Cucurbitaceae and Iridaceae. All of these types 2 RIP share a high sequence similarity both in the A and the B chain, and have a similar overall folding and three dimensional structures. In spite of their similarities type 2 RIP strongly differ from each other with respect to their catalytic activity, substrate specificity and cytotoxic properties. Most of type 2 RIP preferentially binds either Gal or GalNAc. Some type 2 RIPs also show antiviral activity *in vitro* against plant viruses, hence might be involved in the plant’s defense against these viruses (Barbieri et al., 1993).

5. **Jacalin related lectins:** Jacalin is one of the two lectins which occur in mature seeds of *Artocarpus integrifolia* (Jackfruit). Based on their specificities, the lectins in this family are divided into two subgroups. The galactose-specific subgroup comprises Jacalin and a few other Moraceae lectins, which exhibit specificity towards galactose and are built up of subunits consisting of a short β chain of about 20 residues and a long α chain of 133 residues. Jacalin-related lectins are reported to occur in plants from several taxonomically unrelated families; namely Moraceae (jacalin), *Artocarpus hirsuta* lectin (artocarpin), Convolvulaceae (calsepa, conarva), Asteraceae (heltuba), Gramineae (barley and wheat lectins) and Musaceae (banana lectin) (Peumans and Van Damme, 1998). Jacalin was the first lectin in this family to be isolated, sequenced, crystallized and three-dimensional structure determined in complex with methyl-α-D-galactose (Sankaranarayanan et al., 1996).
6. Cucubitaceae phloem lectin: Cucubitaceae phloem lectins are dimeric proteins composed of two identical subunits of about 24 kDa. This lectin binds oligomers of GlcNAc (Wang et al., 1996). These lectins consist of unglycosylated subunits of about 25 kDa and are dimeric in nature. The three-dimensional structure of none of these lectins has been reported so far. Most probably, the Cucubitaceae phloem lectins are involved in the plant defense mechanism.

1.23 Carbohydrate specificity

Lectin differs from other plant proteins by their capacity to bind simple or complex carbohydrates. Based on the amino acid sequences of available lectins, it is deduced that the carbohydrate binding property of most lectins resides in a polypeptide sequence which is termed as carbohydrate-recognition domain. Several comments have been made with respect to the carbohydrate binding specificity of lectins. Lectins display a broad range of specificity; lectins have high affinity for oligosaccharides than for simple sugars and structurally diverse lectins may recognize the same sugars.

Lectins can be subdivided into carbohydrate specificity groups according to their preferential binding to simple sugars (Table 5.21). Based on their specificity lectins are subdivided in to mannose/glucose, Gal/GalNAc, GlcNAc/(GlcNAc)n, fucose and sialic acid binding lectins were recognized, further a novel specificity group monocot mannose binding lectins, mannose/maltose binding lectins (Peumans and Van Damme, 1995) were identified.

The force involved in carbohydrate-lectin interactions are a subject of considerable interest, based on the polyhydroxylic and hydrophilic nature of sugars, polar interactions such as hydrogen bonds and dipole interactions play a dominant role in carbohydrate-protein interactions, experimental evidence support for this view from the X-ray crystallographic studies on Concanavalin A-methyl α-mannopyranoside complex. The binding with simple or complex carbohydrate conjugates is reversible and non covalent. The specificity of lectin towards carbohydrates can be defined on the basis of Hapten inhibition test, in which various sugars or saccharides are tested.
for their capacity to inhibit the property of hemagglutination of erythrocytes. The binding property of many lectins can be affected by more than one carbohydrate moiety, this is because each lectin molecule posses two or more carbohydrate-binding sites that are essential for their ability to agglutinate cells or to react with complex carbohydrates. Hapten inhibition test is commonly used for identification of lectin specificity in present days.

1.24 Biological applications of lectin

Lectins are the proteins widely distributed in nature; they are acting as mediators of a wide range of biological events that involve the crucial step of protein-carbohydrate recognition, such as cell communication, host defense, antimicrobial activity, immunomodulatory, antitumor activities etc. (Sharon, 2007).

a. Antimicrobial lectins: Many human pathogens utilized cell surface glycans as receptors to initiate the adhesion and infection (Zem et al., 2006). For example, *E.coli* binds to host through mannoses, *Neisseria gonorrhoea* specifically binds through N-acetyl-galactosamine, *Psuedomonas aeruginosa* specifically binds through fucose. Host pathogen interactions are multivalent and therefore the binding events are of high affinity and suited for host invasion (Nimrichter et al., 2004). The attachment of bacteria is mediated by glucan binding lectin (GBL), with regard to bacterial surface lectins that play an important role in the initial step of adherence to the host tissues. Lectins from plants and other source will display antimicrobial effect by interfering in this process showing anti adherence agents (Islam et al., 2009).

Antibacterial activity on Gram-positive and Gram-negative bacteria occurs through the interaction of lectin with components of the bacterial cell wall including teicoic and teichuronic acids, peptidoglycans and lipopolysaccharides. The study revealed that the isolectin I from *Lathyrus ochrus* seeds bind to muramic acid and muramyl dipeptide through hydrogen bonds between ring hydroxyl oxygen atoms of sugar and carbohydrate binding site of lectin and hydrophobic interactions with the side chains of residues Tyr100 and Trp128 of isolectin (Bourne et al., 1994). Lectins from *Datura stramonium, Robina pseudoacacia* agglutinated *Streptococcal* group C bacterial cells
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(Kellens et al., 1994) preventing them from attaching to human cell surfaces (Table 5.22)

Similarly, inhibition of fungi growth can occur through lectin binding to hyphas resulting in poor absorption of nutrients as well as by interference on spore germination process. The polysaccharide chitin is a constituent of fungi cell wall and chitin-binding lectins showed antifungal activity; impairment of synthesis and/or deposition of chitin in cell wall may be the reasons of antifungal action (Selitrennikoff, 2001). Probably the carbohydrate-binding property of lectin is involved in the antifungal mechanisms and lectins of different specificities can promote distinct effects.

b. The antiviral activity of lectins: Most the enveloped viruses and HIV virus belongs to retrovirus category are typically covered by virally encoded glycoprotein’s that contribute to half of their molecular weight. Most of the lectins antiviral activity is due to the ability to bind mannose containing oligosaccharide present on the surface of the viral envelope. The agents that binds specifically and stringy interact with glycans disturbs the interaction between viral envelope and cells of the host; this prevents the further co-receptor interaction. Most the antiviral therapeutics inhibit the viral life cycle. Lectin prevents the penetration of viruses to the host cells. It has been reported that the antiviral potential of monocot mannose binding lectin from Amaryllidaceae (Balzarini, 2006) has displayed antiviral activities, similar type of results reported in lectin extracts of C.latifolium (Zvetkova et al., 2001). It may be noted that potent antiviral agents asscytovirn and cyanovirn (Barrientos et al., 2003) are also mannose binding proteins other than plant lectins.

c. Lectin immunomodulatroy activities: Immune system is a complex system within the biological system that protects the body against diseases. The immune system is complex of cells connected in a network manner, in order to function properly an immune system must detect a variety of agents from viruses to cancer cells, and distinguish them from the organism’s own healthy tissue. The immune system has two phases of activity innate and adaptive immunity. In innate immunity there will the
activity of cells and cytokines in a nonspecific way. The adaptive immune response is composed of another set of cells that acts in a specific way, such cells are responsible for the production of antibodies. Thus, the use of molecules capable of inducing cell recruitment as well as cytokine production and lymphocyte proliferation are of special scientific interest. Korean mistletoe (*V. album* L. var. *coloratum*) is traditionally used as a sedative, analgesic, anti-spasmylytic, cardiotoxic and anticancer agent, in Korea. An important lectin has been isolated from this plant and its immunomodulatory activity was analyzed (Lee and Sugden, 2008). A recent study demonstrated the immunomodulatory activity of ConBr, a lectin isolated from *Canavalia brasiliensis* seeds. The assays showed that ConBr was able to induce *in vitro* proliferation of splenocytes with minimal damage to the cellular structure. Furthermore, ConBr increased in the production of cytokines such as IL-2, IL-6 and IFN-γ production and decreased IL-10. These findings indicate the potential immunomodulatory effect of this lectin in conjunction with the intrinsic role of carbohydrates in intercellular communication related to the inflammatory process.

d. Antitumor effect of lectins: Cancer is a deadly disease that displays uncontrolable growth beyond the normal limits. Treating cancer from past many years new approaches are employed in the treatment. It is important in cancer therapy that the treatment targets only affected cell, leaving normal cells undisturbed, which is difficult especially in chemotherapy. Over the past few years lectins have been found to have anticancer properties (Fig 5.6). Several reports have been reported about the use of lectin to inhibit tumor growth, by inducing cytotoxicity, apoptosis and down regulation of telomerase activity and inhibition of angiogenesis. A number of plant lectins have been in preclinical and clinical trials as potential drugs for treatment of cancer (Ernst et al., 2003). Till now plant lectins are divided into 12 different families, according to their carbohydrate binding specificities. Among the various lectin families ricin- B family, legume lectin domain and GNA family have been widely reported to have antitumor properties (Liu et al., 2007) (Table 5.23).
1.25 Molecular mechanisms of lectin induced anticancer properties

Rcin is the toxin lectin and one of the most potent toxin known, belongs to type II ribosome inactivating proteins. Other plant lectins relatively closer in structure and function to ricin are *Abras pectorius* seeds, *Adenia digitata* roots, *V. album* leaves and *Adenia volkensi* roots (Barbieri et al., 1982).

Rcin is composed of two distinct N-glycosylated polypeptides joined by disulphide bonds. One polypeptide chain is (A chain) and the other is a B - chain. B-chain polypeptide of ricin is responsible for the attachment on the cell surface by interaction with galactosyl residues of membrane glycoproteins, surface bound ricin enters the cell by classical receptor mediated endocytosis. The B - chain is responsible for attachment and penetration. A-chain polypeptide inside the cell inactivates the 60s ribosome subunits. Ribosome exposed to A-chain cannot bind to EF-2 and stops protein synthesis. It has been reported that mammalian ribosomes are particularly sensitive to ricin than a plant ribosome (Olsnes and Phil, 1982).

It has been reported that ricin-B family induces apoptotic cell death through up regulating caspase-8 and down regulating caspase 3/7 in L5540 Hodgkins lymphoma cells (Polito et al., 2009). Similarly, lectin (ricin) cytotoxic mechanism involving caspase activation, apoptosis, chromatin condensation, nuclear fragmentation, DNA release and externalization of membrane phosphatidylinerine in human monoblastic leukemia U937 cells (Kim et al., 1993). Abrin also known lectin of type II ribosome in activating protein, also found to induce apoptosis by stimulating caspase-3 expression mediated apoptosis, results also report that abrin mediated necrosis can involve lysosomal membrane permeabilization and the release of cathepsins from lysosomes. It has been suggested that abrin-mediated death pathway appears to depend on which two events occurs first, lysosomal membrane permeabilization or loss of MMP that may decide cancer death by apoptosis or necrosis (Bora et al., 2010).

Mistletoe lectins (MLs), well studied type II ribosomal inactivating proteins (RIPs II), which has received much attentions for their antiproliferative and apoptosis inducing
mechanism on cancer cells. They are heterodimeric glycoprotein that consists of A-
chain and B-chain. B-chain plays an important role in determining selective
cytotoxicity by interacting with certain sugars –chains or sugar receptors on the cell
surface; A-chain inhibits protein synthesis by disrupting 28s ribosome. Recently it has
been reported that Mistletoe lectins (MLs I, MLs II) possess anti-proliferative activities
against various cancer cell lines (Human acute lymphoblastic leukemia cells, human
hepatocarcinoma cell, human A459 lung cancer cells (Ahmad and Hoessli, 2008).
Besides apoptosis inducing activities of (MLs I, MLs II) had also found to posses
antiproliferative and apoptosis inducing activities by activating MAPK signaling and
altering cellular signaling pathway that may modulate the apoptotic response.

Expression of glycoconjugates is often altered in tumor cells. Abundant N-
acetylglucosamine (α1, 3) N-acetylglucosamine/ galactose and galactose (β1, 4) N-
acetylglucosamine (α, 2) mannose (α1, 6) residues were observed in dysplastic
epithelium tumor cells as evidenced by labeling by the N-acetylglactosamine-
specific and complex type oligosaccharide-specific lectins. The binding of these
lectins to androgen-independent rat prostatic carcinoma was revealed, indicating that
these sugar residues are common in some dysplastic and neoplastic prostatic cells
(Chan et al., 2001). The above mentioned discoveries of the lectins suggest that they
posses various biological activities and that closely related to their corresponding
molecular structures.

1.26 Need of research

Endophytes are the unexplored producers of metabolites. These are the
microorganisms that reside the plant. An endophytic fungus is one among the large
untapped resource, which may have economically important applications in medicine
and biological control agents. The plant selected for research is medicinaly important
plant and has wide variety of medical application in the treatment of cardiovascular
diseases, anticancer, arthritis, immunomodulatory activities, etc. Plant V. album
known to produce bioactive compounds like glycoproteins (lectins), alkaloids,
flavanoids, terpenoids etc. Among these bioactive compounds, plant V. album is
known for the presence of toxic protein, glycoprotein (lectin), commonly referred as
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*V. album* agglutinin (VAA). There three different types of lectin from *V. album* has been reported, namely (VAA-1, VAA-II, VAA-III), among these lectin the potential important is VAA-1, it belongs to ribosomal in activating protein. The VAA-I has been reported to have immunomodulatory activity, cytotoxic and apoptosis activity inducing DNA fragmentation and it is used as adjuvant in the treatment of cancer. Recently recombinant DNA technology has employed in the production of mistletoe lectin (VAA-I) in *E. coli*.

Presence of various bioactive compounds in *V. album* has made us to select the plant *V. album*. The present investigation was carried out to isolate and identifies endophytic fungi from *V. album*, isolate bioactive compounds from endophytic fungi and analyze its anticancer activities, mechanism of action on human cancer cell lines. An anticancer compound obtained from endophytes may be the new alternative source of anticancerous compounds.
OBJECTIVES
The research aims

The core aim and focus of this research was to isolate and identify endophytic fungi from the medicinal plant (*V. album*), to isolate, characterize the anticancer agent from endophytic fungi and to evaluate its anticancer potential, mechanism of action on the cancer cell. The technologies used include affinity chromatography, haemagglutination assay, SDS-PAGE, PAS staining, antioxidant, anti-inflammatory assay, antibacterial and MIC assay, cytotoxicity assay, mechanism of action on cancer cell lines and MALDI-TOF-MS.

Objectives

- A collection of *V. album* plant growing on different host, Karnataka, India.
- To isolate and characterize the endophytes from *V. album*.
- To isolate the anticancer agents from endophytes.
- To screen the compound against cancer cell lines to know their efficacy and mechanism of action.
- To identify the compound analyze its physical and structural parameters.