INTRODUCTION

Cancer is a disease characterized by loss of cellular growth control (Kamb, 1995). It is a hyperproliferative disorder that involves transformation, dysregulation of apoptosis, proliferation, invasion, angiogenesis and metastasis (Aggarwal et al., 2006). Usually the timing of cell division is under strict control, involving a network of signals to ensure the integrity of organs and tissues. Mutations in one or more nodes in this network can trigger cancer. Charaka and Sushruta samhitas, two well-known Ayurvedic classics, describe cancer as inflammatory or non-inflammatory swelling and mention them as either Granthi (minor neoplasm) or Arbuda (major neoplasm) (Balachandran and Govindrajan, 2005).

Within the last 50 years, major advances have been made in our understanding of the basic biology of cancer (Aggarwal et al., 2006). Cell is the basic unit of life. ‘Cell cycle’ or ‘cell division cycle’ denotes the orderly sequence of events by which a cell duplicates its genetic material (the chromosomes) and divides into two identical daughter cells (Malumbres and Barbacid, 2001). Two classes of genes: proto-oncogene and tumor suppressor genes, work together to regulate cell division in normal cells. All cancers involve the malfunction of genes that control cell growth, division and death. However, most of the genetic abnormalities that affect cancer risk are not hereditary, but instead result from damage to genes (mutations) that occur throughout one’s lifetime. The majority of human cancers result from exposure to environmental carcinogens; these include both natural and manmade chemicals, radiation, and viruses. It is also clear that apart from exposure to carcinogens other factors such as the genetic predisposition have been documented. Carcinogens in the diet that trigger the initial stage include moulds and aflatoxins (for example, in peanuts and maize), nitrosamines (in smoked meats and other cured products), rancid fats and cooking oils, alcohol, additives and preservatives. A combination of foods may have a cumulative effect and when incorrect diet is added to a polluted environment, smoking, UV radiation, free radicals, lack of exercise and stress, the stage is set for DNA damage and cancer progression. Aberrant activation of the cell cycle can be achieved by induction of positive regulators (often encoded by proto-oncogenes) or through inactivation of negative regulators (often encoded by tumour-suppressor genes). Induction of positive regulators is caused by overexpression or mutations leading to permanent protein activity. Inactivation of repressors is caused by deletion, mutation or promoter hypermethylation. All mechanisms can be found in human cancer (Tessema et al., 2004).
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Cancers are classified by the type of cell that resembles the tumor and therefore, the tissue presumed to be the origin of the tumor. The following general categories are usually accepted:

**Carcinoma**: Malignant tumors derived from epithelial cells. This group represents the most common cancers, including the common forms of breast, prostate, lung and colon cancer.

**Leukemia and Lymphoma**: Malignant tumors derived from blood and bone marrow cells. Other lymphatic tissues include the spleen, the tonsils and the thymus gland.

**Sarcoma**: Malignant tumors derived from connective tissue or mesenchymal cells.

**Mesothelioma**: Tumors derived from the mesothelial cells lining the peritoneum and the pleura: such as nerves, muscles, cartilage, joints, bone or blood vessels.

**Glioma**: Tumors derived from glia, the most common type of brain cell.

**Germinoma**: Tumors derived from germ cells, normally found in the testicle and ovary.

**Choriocarcinoma**: Malignant tumors derived from the placenta.

One in eight deaths worldwide are due to cancer, it is the second and third leading cause of death in economically developed and developing countries, respectively (Pereira, 2009). Cancer affects 1 in 3 of us in our lifetime. It also affects people at all ages with the risk for most types increasing with age. Over 70% of cancers happen to people who are over the age of 60 (Kamal et al., 2010). Minna (2008) reported that lung cancer, the most common cause of cancer-related death in men and women, is responsible for 1.3 million deaths worldwide annually. In 2010, according to National Institutes of Health, about 569,490 Americans were expected to die of cancer, more than 1,500 people a day (Minna, 2008; Mariotto et al., 2006). Garcia et al. (2007) reported that the burden of cancer is increasing in developing countries as childhood mortality and deaths from infectious diseases decline and more people live to older ages. The corresponding estimates for total cancer deaths in 2007 are 7.6 million (about 20,000 cancer deaths a day), 2.9 million in economically developed countries and 4.7 million in economically developing countries. By 2050, the global burden is expected to grow to 27 million new cancer cases and 17.5 million cancer deaths simply due to the growth and aging of the population (Jemal et al., 2007).

India is in an epidemiological transition phase and cancer is now one of the leading causes of morbidity and mortality. It is estimated that there are 2 million cancer patients in India with 0.7 million new cases each year. More than 35% of cancer cases in men are
related to the oral cavity, larynx and pharynx (all tobacco related). About 40% and 30% of cancer cases in women are cervical and breast cancer, respectively, in India (Seth et al., 2005).

Cancer treatment involves medical procedures to destroy, modify, control, or remove primary, regional, or metastatic cancer tissue. The most important therapies employed for the treatment of cancer are surgery, radiation therapy and chemotherapy. Surgery is the earliest, most widely used therapy for the excision of a tumor. It is most effective if the cancer is localized. Radiation therapy is dosing of radiation to kill cancer cells and stop them from spreading. Radiation kills or slows not only the growth of cancer cells, it can also affect nearby healthy cells. It can be used to cure and to stop or slow down the growth of cancer. Sometimes both the surgery and radiation therapy are used. Radiation shrinks the size of the cancer before surgery, or it may be used after surgery to kill cancer cells that remain in the body. Chemotherapy involves the systemic administration of anticancer drugs that travel throughout the body via circulatory system. It is the use of drugs to destroy cancer cells. In essence, it wipes out all the cancerous cell colonies including metastasized cancer cells. Chemotherapy has played a major role in cancer treatment for half a century. Years of testing and research have proved it to be an effective cancer treatment.

Important chemotherapeutic agents are alkylating agents, antimetabolite and natural products (Chabner and Roberts, 2005; Takimoto and Calvo, 2008). In the continuing search for agents that may treat or ameliorate the affliction of cancer, natural products have provided an endless supply of active compounds that are increasingly being exploited. Indeed, natural products have been the mainstay of cancer chemotherapy over the last three decades and plant extracts have been used to treat cancers for even longer (Deorukhkar et al., 2007). The chemotherapy in oncology is focused on application of cytostatic and/or cytotoxic drugs with potential to kill malignant cells. Such drugs may be obtained using chemical synthesis or isolated from plants or different fungi. Such cytotoxic and cytostatic drugs are known to frequently induce DNA damage and/or block cellular division. Martinkova et al. (2009) reported that currently approved anti-cancer drugs are sorted into several groups according to known mechanisms inducing DNA damage and cellular death, which include: (i) alkylating cytostatic agents (melphalan, chlorambucil, cyclophosphamide and ifosfamide, derivatives of nitrosourea – carmustine
and lomustine, busulfan, dacarbazine, temozolomide and procarbazine); (ii) anti-
metabolites (methotrexate, 5-fluorouracil and its derivative capecitabine, 
cytosinarabinoside, gemcitabine, mercaptopurine, fludarabine, cladribine, hydroxyurea); 
(iii) anti-cancer antibiotics (doxorubicine, idarubicine, epirubicine, mitoxantron, 
bleomycine and mitomycin C); (iv) plant alkaloids (vinca alkaloids – vincristine, 
vinblastine and vinorelbine, podophyllotoxin alkaloids – etoposide and teniposide, 
camptothecin analogs – topotecan and irinotecan, taxanes – paclitaxel and docetaxel); and 
(v) other drugs (platinum compounds – cisplatin, carboplatin, oxaliplatin; L-asparaginase; 
amascrin and others).

The overwhelming contribution of natural products to the expansion of the 
chemotherapeutic arsenal is evidenced by the fact that 50% of all the anticancer drugs 
approved worldwide between 1940 and 2006 were either natural products or natural 
product derived (Newmann and Cragg, 2007). Many of these novel anticancer agents 
have a history of use in traditional medicine, notably in Traditional Chinese Medicine. The 
most prominent among them are: (i) camptothecin, isolated from the Chinese ‘happy tree’ 
*Camptotheca acuminata* (Sriram *et al.*, 2005); (ii) podophyllotoxin from *Podophyllum 
peltatum*, originally used by Native Americans but also found in large amounts in 
*Podophyllum emodi var. Chinense* (KeeChang, 1999) and (iii) paclitaxel initially obtained 
from *Taxus brevifolia* and present in high quantities in *Taxus chinensis* (Zhang *et al.*, 
2000).

During past decade, the evidence is gradually being shown that many cancer 
chemotherapeutic agents induce a cell death process known as programmed cell death, or 
apoptosis. Recently interest has been focused on the manipulation of apoptotic processes 
in the treatment and prevention of cancer (Yi *et al.*, 2003). Thus, inducing apoptosis is an 
efficient method of treating cancers (Hu and Kavanagh, 2003). Apoptosis, or programmed 
cell death, is an essential event that plays an important role in organism development and 
homeostasis (Liu *et al.*, 2006). During apoptosis, cell experiences a cascade of events that 
ultimately result in nucleus condensation and DNA fragmentation (Nagata *et al.*, 2003). 
Apoptotic cells can be recognised by characteristic morphological patterns e.g., cell 
shrinkage, condensation of cytoplasm, nuclear fragmentation, formation of apoptotic 
odies and loss of cell surface structures, etc. (Christop, 2003). Certain products from 
plants are known to induce apoptosis in neoplastic cells but not in normal cells. It has
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It has become increasingly evident that apoptosis is an important mode of action for many antitumor agents, including ionizing radiation, alkylating agents such as cisplatin and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), topoisomerase inhibitor etoposide, cytokine tumour necrosis factor (TNF), taxol and N-substituted benzamides such as metoclopramide and 3-chloroprocainamide. Apoptotic induction has been a new target for innovative mechanism based drug discovery. It is thus considered important to screen apoptotic inducers from plants, either in the form of crude extracts or as components isolated from them. Understanding the modes of action of these compounds should provide useful information for their possible application in cancer prevention and perhaps also in cancer therapy (Taraphdar et al., 2001). Based upon the background of diversified therapeutic values of plants and uses in cancer disease in folklore/traditional Indian system of medicine and available scientific literature the aim of the present study was to evaluate the following plants and to identify active fraction/isolates for anticancer activity.

Ocimum viride (Common names: Mosquito plant, Fever plant), a native of Western Africa, and Ocimum carnosum (Common name: Alfavaca), an exotic South American species belonging to family Lamiaceae, have been introduced at Indian Institute of Integrative Medicine (formerly Regional Research Laboratory), Jammu, India (Sobti et al., 1981; Khosla et al., 2000). The whole plants and their essential oils have many applications in traditional medicine, especially in Africa and India. Preparations from the whole plants and leaves of O. viride are used as anticonvulsant, antidiarrhoeal, treatment of cold, fever, chest pains and treatment of catarrh and bronchitis (Gill, 1992). Isu (2006) have demonstrated that hot aqueous extract of O. viride possesses anti-bacterial properties. Sobti et al. (1977) reported that O. viride is a good source of thymol. Furthermore, in another study Khosla and Bhasin (2000) reported that O. carnosum is a rich source of elimicin and methyl eugenol. Elimicin is of immense pharmaceutical value and forms the base material for the formulation of antibacterial drug (Khosla et al., 2000). In the present study, the essential oils of aerial parts of O. viride and O. carnosum in full mature influorescence stage have been analysed.

Vallaris solanacea (Common name: Ramsar), is a climbing shrub belongs to family Apocynaceae. The latex of V. solanacea is mildly irritant, applied to wounds and sores (Kirtikar and Basu, 2000; Adhikari et al., 2010). Vagdevi et al. (2011) reported that crude extract of V. solanacea possess considerable antimicrobial activity. Plant is also
used to cure toothache and as dentifrice (Sharma, 2009). Seeds of *V. solanacea* are very rich in cardiac glycosides (Kaufmann *et al.*, 1965). Furthermore, Vohra *et al.* (1966) identified one of the glycosides of *V. solanacea* as o-acetyl-solanoside. In the present study, isolates from aerial parts of *V. solanacea* have been analyzed for their anticancer potential.

Briefly, the objectives of the present study are as follows:

- Extraction followed by fractionation of active agents from aerial parts of *Vallaris solanacea*.
  - Preparation of Extracts (95% alcoholic, 50% aqueous-alcoholic and aqueous).
  - Fractionation of extracts with solvents based on polarity (Hexane, Chloroform, n-Butanol and Water).
- Mechanistic studies of promising isolates using different techniques:
  - Microscopic studies
    - Light microscopy
    - Fluorescence microscopy
    - Scanning electron microscopy
  - Flowcytometric studies
    - Cell cycle analysis
    - Annexin V/PI lebelling
    - Change in mitochondrial membrane potential ($\Delta\psi_{mt}$)
  - DNA fragmentation assay
  - Fluorimetric assay
    - Reactive Oxygen Species (ROS) detection
  - Colorimetric assays
    - BrdU incorporation
    - Changes in cytochrome c and Bcl-2 levels
    - Nitric oxide (NO) production
    - Estimation of Caspase activity