Chapter-1

INTRODUCTION
The regular consumption of fruits and vegetables is important as it helps to reduce the threat of various chronic ailments and even metastasis. These plants develop complex chemical arsenals to survive against the immeasurable number of pathogens such as insects, fungi, viruses and bacteria, thus provide a wide variety of secondary metabolites that can have medicinal value (Newman and Cragg, 2007; Newman and Cragg, 2012). In the recent years, there is great interest to use naturally occurring phytochemicals as chemopreventive agents. On the whole, the natural plant products are preferred world wide as the age old medicinal system like ayurveda has been used for many years to treat various diseases. People can get these botanical supplements very easily from the market on an over the counter basis and feel very contented consuming these products. There are many reports in the literature which show that botanical products strengthen the immune system as well as help in protecting the liver and stomach from the infection caused by bacteria and viruses. These products also act as very strong anti-inflammatory, antioxidant, anti-mutagenic and anti-cancer agents (Miyata, 2007; Espin et al., 2007). The recent findings in the discovery of drugs from plants takes into account many factors that combine all the botanicals, phytochemicals, biological and molecular techniques. In recent years, great attention has been given to plant products in order to prevent metastatic diseases because of their different benefits to health, non toxicity and less other ill effects (Manson et al., 2005).

It has been reported that natural compounds can have modulatory effects in cells via network of a wide range of targets at molecular level which play important role in cell signaling response. The isothiocyanates rich cruciferous vegetables act as anticancer agents by causing the changes in following molecular targets (1) activation of free radical scavenging activity and induction of NF-E2 related factor 2 and arylhydrocarbon receptor (2) reticence of pro-inflammatory reactions via suppression of NF-jB (nuclear factor-jB) and pro-inflammatory enzymes, like cyclooxygenase-2, lipoxygenase and inducible nitric oxide synthase, (3) reticence of cytochrome P450 (CYP) enzyme activity, (4) reticence of histone deacetylase (HDACs), HDACs have many critical roles in controlling the gene expression, cell growth, cell immigration, apoptosis and angiogenesis (5) reticence of phase 1 and induction of detoxify phase II enzyme, mitogen activated protein kinase, c-Jun N-Terminal Kinase 1, protein kinase C (PKC)
Introduction

and (6) promotion of cell cycle arrest and apoptosis (Bax over-expression and Bcl-2 down-regulation, caspases involvement) (Zhang et al., 1992; Dashwood and Ho, 2007). The unbeaten drug to treat cancer should eliminate or cause the death of cancer cells without causing harm to normal cells. The *in vitro* cytotoxicity assay gives us the information that active plant sample causes decrease in the cancer cell number. Many studies in the literature prove that cell death can be triggered via a three well known mechanisms. 1.) Autophagy (programmed cell death II) in which the degradation of organelles occurs while the cell skeleton remains same till the complete death of cell. In this process caspase does not play any role. 2.) Necrosis is the slow sudden death of cell which results into swelling of the cells via abandoned release of cellular contents into the microenvironment. 3.) Apoptosis (programmed cell death I) involves the sequences of changes in the morphology and physiology that causes amplification of free radicals, caspases, cell contraction, chromatin compression and nucleosomal dilapidation. This causes the removal of damaged and detrimental cells which is vital for retaining the tissue homeostasis in higher organisms (Forbes-Hernández et al., 2014).

It has been found that most of the drugs from plants exert cytotoxic effects on malignant cells by inducing apoptosis (Elmore, 2007). Stimulation of cell death in metastatic cells is identified as an effective strategy for treatment of cancer. A natural cell death is an important step that plays a vital function in growth and advance of the organism (Sharma et al., 2007). During apoptosis, cell undergoes cascade of changes that finally results in compression of nucleus and disintegration of DNA (Elmore, 2007). Among all, the important identification feature of apoptosis is inversion of phosphatidylserine (PS) from the inner to outer plasma membrane (Mirmikjoo et al., 2009). Apoptotic stimulation has been chosen as a new end point for inventive mechanism based discovery of drug. Simon et al. (2000) reported the involvement of free radicals and mitochondria in stimulation of cell death under physiological and pathological conditions. During initial stages of cell death, the permeability of mitochondrial membranes get enhanced which lead to the discharge of cytochrome C, in addition to the induction of caspases that in turn causes cell death via repression of apoptotic proteins (Hsieh et al., 2013). Mishra et al. (2010) reported the anticancer potential of seed extract of *Ziziphus mauritiana* that induced apoptosis in HL-60 cell line.
and found that it inhibited cell proliferation by inducing apoptosis which was detected with different end points like binding of Annexin V, DNA disintegration and increase in hypo diploid sub G$_0$ DNA content. The cell cycle studies using the flow cytometry after staining the cells with Annexin V/PI showed time related increase in apoptosis, increase ROS that may cause apoptosis via cell death through the liberation of different factors like cyt c, AIF, caspases and post apoptotic necrosis (Qian et al., 2012; Long et al., 2014; Fares et al., 2014 and Kello et al., 2014).

Brassica vegetables have fascinated the scientific world as they are rich in various hydrolytic products of glucosinolates. A large amount of research data gives an ample support to the assumption that daily intake of broccoli and other members of family Brassicaceae is highly useful in decreasing the cancer risk (Block et al., 1992; Lintschinger et al., 1997; Bazzano et al., 2002; Kim, et al., 2004; Barillari et al., 2005; Kim and Milner, 2005; Keum et al., 2005). The beneficial effect of these vegetables is because of the presence of sulphur containing compounds i.e. glucosinolates (Verhoeven et al., 1997). Glucosinolates (GLSs) are a group of thioglycosides, which on hydrolysis give different products having the beneficial effects on human health (Ciska et al., 2000). Enzymatic hydrolysis of these compounds involves the enzyme myrosinase, which occurs in the cells of the plants along with sulphur compounds. In undamaged tissue of the plant, myrosinase is present in compartments separated from its substrate. Glucosinolates get hydrolyzed with the enzyme myrosinase when they come in contact with each other. The products produced as a result of hydrolysis includes an aglucone moiety, glucose and sulphate. Furthermore on rearrangement the unstable aglucone moiety forms the diverse products viz isothiocyanates, thiocyanates, nitriles, oxazolidinethiones and epithionitriles (Bell and Wagstaff, 2014). The structure of side chain and the reaction conditions determine the structure of different hydrolytic products (Fahey et al., 2001). The stability of the major hydrolytic products occur at pH 6 to 7, whereas glucosinolates bearing β-hydroxylated and indole side chains become stable on cyclization. Therefore, the myrosinase is separated from the glucosinolates in plants. This enzyme is present in particular cells called myrosin c and is physically divided from its glucosinolates compounds (Andréasson et al., 2001). Any wear and tear of the cells due to infection caused by microorganisms as well as by chewing, digestion results into
disintegration of cell membrane that allows the reaction of myrosinase with glucosinolates and lead to formation of hydrolytic products. Thus, the modification of glucosinolates myrosinase system also occur due to the damage of enzyme myrosinase while the food preparation (Rungapametsy et al., 2007). Isothiocyanates are known to impose noteworthy defense against cancer triggered by a range of toxicants (Wattenberg, 1978; Morse et al., 1989; Stoner, et al., 1999; Jiao et al., 1997; Hecht 2000; Talalay and Fahey, 2001 and Conaway, et al., 2002). These products play an important role in decreasing the free radical load responsible for causing different chronic ailments by acting as antioxidants (Verkerk et al., 2009). Many workers are of the view that the regular intake of these natural products containing vegetables may help in decreasing the threat of cancer by enhancing the detoxification enzymes as well as via direct reticence of transcriptional factors concerned with cell signaling mechanisms (Hu et al., 2006; Tang et al., 2006; Verkerk et al., 2009). Glucosinolates are ubiquitous throughout 15 families of angiosperms like Brassicaceae, Euphorbiaceae and Tropaeolaceae (Rodman, et al., 1998). The main parts of different members of family Brassicaceae which is used as vegetable are inflorescence, root, leaf etc. It has been reported by many scientists that glucosinolates are present throughout the plant but in varying amounts and types. Although, several reports in the literature show the presence of different kinds of glucosinolates and their hydrolytic products in plants but only 3–4 of these predominate (Holst and Williamson, 2004). A large number of glucosinolates exist due to the variations in the structure of side chain. Presently, above one hundred and twenty hydrolytic products of glucosinolates have been profiled, all which share a general structure having sulphur attached to side chain and found in the seeds, roots, stems and leaves of plants (Chen and Andreasson, 2001).

Brassica oleracea L. var. italica Plenck (Broccoli) belongs to family Brassaceace is the rich source of natural products and is used as functional food. Dr. Stephen de Felice gave the term “Functional foods” (nutraceuticals) as distinct products which are used to improve the health by adding new ingredients or more of existing ingredients (Moreno, et al., 2006; Sharma, et al., 2014). Broccoli is very popular due to the presence of high amount of sulphur compounds like dithiolthiones and isothiocyanates, which are reported to enhance the activity of detoxifying enzymes which eliminate the toxic compounds
Broccoli might have originated from wild or cultivar forms in Roman times from the eastern Mediterranean region. The white-headed cauliflower and broccoli have come from Italy and northern Europe, during the last four centuries that became secondary centers of diversity. Broccoli that can withstand hot, humid and tropical climate has originated in India during the last two centuries. It has spread throughout the world from United States in the last fifty years. Broccoli is grown on a small scale in tropical Africa with highland areas in many countries and occasionally in West Africa in lowland areas during winter season (Buck, 1956). Though, this plant has been documented in ancient text and used in traditional system of medicine yet, it has not been explored for its bioactivities. Based upon the background of diversified therapeutic values, of plants and their uses in cancer treatment in traditional system of medicine coupled with the fact that anticancer potential of *Brassica oleracea* L. var. *italica* Plenck has not yet been explored, the present study was planned. It is pertinent to mention that plant extracts and the constituents present in them exert the anticancer activity by scavenging the free radicals and reducing the inflammation, therefore, the extracts were also explored for their antioxidant as well as anti-inflammatory activities.

The Main objectives framed in the present study were:

- Preparation of different extracts from seeds, sprouts, leaves and florets of different varieties i.e. Palam Samridhi, Palam Vichitra and Punjab 1 of *Brassica oleracea* L. var. *italica* Plenck by method of Liang *et al.*, (2006).

- Fractionation, isolation and characterization of extracts using column chromatography and spectroscopic techniques such as FTIR (Fourier Transformation Infrared Spectroscopy), NMR (¹H & ¹³C) and mass to elucidate the chemical structure of isolated compounds

- Evaluation of antioxidative properties of different extracts using:
  - Electron and hydrogen donating potential in molybdate ion reduction assay (Prieto *et al.*, 1999) and DPPH free radical scavenging assay (Blois, 1958) respectively.
Introduction

- Antioxidant activity potential in superoxide anion radical scavenging assay (Nishikimi et al., 1972) and hydroxyl radical scavenging in DNA nicking assay (Lee et al., 2002).
- Mechanistic studies of extracts/isolated compounds using different methods.
  - Morphological analysis of cancer cells for apoptotic features with DAPI staining of cells for nuclear morphology using confocal fluorescent microscopy (Bhushan et al., 2007) and scanning electron microscopy (SEM) (Rao et al., 2009; Sharma et al., 2008).
  - Cell cycle analysis by flow cytometry (Singh et al., 2007; Hu et al., 2010).
  - Reactive oxygen species (ROS) generation by flow cytometry and spectrofluorometry (Bhushan et al., 2007; Deng et al., 2013).
  - Alteration of mitochondrial membrane potential (MMP) by spectrofluorometry (Deng et al., 2013).
- Evaluation of anti-inflammatory properties was done using:
  - Cyclooxygenase (COX) (ovine) inhibition with kit of Cayman Chemicals Company, USA (Kaur et al., 2010).