Cancer is the second leading cause of death all over the world. Consumption of vegetables and fruits may reduce the risk of development of cancer. Epidemiological studies have demonstrated that consumption of green leafy vegetables may delay the onset of various cancers such as lung, breast, colorectal cancer etc. Crucifers are widely consumed in many parts of the world. There have been no reported concerns with respect to their tolerance and safety in humans (Shapiro et al., 2006) [84].

Crucifers are important sources of glucosinolates (GLs) whose de-generated products like isothiocyanates were attributed to chemopreventive activity. Cruciferous vegetables in particular have attracted a great deal of attention since they are rich in aromatic and aliphatic Isothiocyanates. Glucosinolates are anionic, hydrophilic plant secondary metabolites and play an important role in the prevention of cancer and other chronic and degenerative diseases (Fahey et al., 2002; Halkier, 1999) [85, 86].

Brassica oleraceae belongs to the Cruciferae family is inexpensive and is easy to grow, harvest and store [43]. Brassica oleraceae colour can be used as a natural food colour. Brassica oleraceae is a rich source of anthocyanins, mainly acylated anthocyanins such as cyanidin 3, 5-diglucoside, cyanidin 3-sophoroside-5-glucoside and cyanidin 3-sophoroside-glucoside acylated with sinapic acid (Wu et al., 2006) [87]. In general, acylated anthocyanins contain two sugar molecules (glucose and sophorose) and several aromatic acids (Tanchev 1969; Dyrby et al., 2001; McDoughall et al., 2007) [40, 88, 89]. The Brassica oleraceae anthocyanins transition from purple-red to pink-red to blue-green between pH levels of 3 and 6 respectively (Hagiwara et al., 2001) [90].
2.1 Isolation of bioactive fractions from *Brassica oleraceae*

*Brassica oleraceae* (*Brassica oleraceae var rubra*) contains similar amounts of glucoraphanin and glucobrassicin but in addition, appreciable amounts of glucoiberin, progoitrin, sinigrin, gluconapin and glucoerucin, while neo-glucobrassicin occurred at trace levels (Kim and Milner 2005) [91]. Glucosinolates present in *Brassica oleraceae* were reported to be Glucoraphanin, Glucobrassicin, Sinigrin and Progoitrin etc.

2.1.1 Glucosinolates

Glucosinolates and their breakdown products are of particular interest in food research because of the alleged anticarcinogenic properties. The hydrolysis products include Isothiocyanates (ITC), nitriles, indoles and oxazolidinethiones from which ITCs and Indoles reported to have anticancer properties (Steven et al., 2004) [92]. ITCs are the putative bioactive constituents of cruciferous vegetables.

Agudo et al. (2008) [93] stated that the consumption of cruciferous vegetables its active constituent glucosinolates is relatively low within Europe, which in turn is lower than in North America and several Asian populations. A considerable number of epidemiological studies revealed an inverse relationship between consumption of Brassica vegetables (broccoli, *Brassica oleraceae*, brussels sprout, kale, cauliflower, cabbage) and risk of cancer in various human organs. Kim et al. (2004) [94] stated that the levels of leaf glucosinolates are regulated during plant development. The level of glucosinolates increases in response to mechanical damage or insect feeding.

Zhou et al. (2005) [95] used a three step process to purify main glucosinolate from *Brassica oleraceae*. The steps involved were extraction with methanol, separation and purification by chromatographic column on alumina support, followed by a reversed-phase separation by octadecyl (C18) silica.

Barillari et al. (2005) [96] measured the two important glucosinolate viz., glucoraphasatin (GRH) and glucoraphanin (GRE) from *Raphanus sativus* sprouts and seeds
during different stages of development. They found that in comparison to the seeds, the GRE content in sprouts decreased with development whereas GRH content increased steadily up to a 25-fold increases.


Aires et al. (2009) [99] demonstrated the potential of these glucosinolates and their respective enzymatic hydrolysis products on bacteria isolated from the human intestinal tract. Various collaborative research states that Indole compounds will be produced as a consequence of breakdown of glucosinolates by enzyme myrosinase which is present in intact tissue. Many researchers support that the chemopreventive effect of Brassica vegetables and their constituents in various animal and clinical experiments (Murillo and Mehta, 2001) [100].

Xavier et al. (2008) [101] studied the optimization of column process and optimization conditions for extraction of anthocyanins from Brassica oleracea. A well-known analytical method used for glucosinolates is desulfonation of glucosinolates with sulfatase, followed by analysis using a reverse phase HPLC gradient system (Bjerg and Sorensen, 1987; Bjorkqvist 1988) [102,103]. In the procedure of glucosinolates extraction myrosinase should be inactivated to prevent their loss. From this perspective, mixed alcohol and water or boiling water at almost its boiling point (around 60–100°C) was selected as the glucosinolate extraction solution (Ciska and Kozlowska, 2001) [104].

For the extraction of glucosinolates from radish root, samples should be freeze-dried or cut into small cubes, with subsequent extraction using hot methanol (70–80% in water, at temperatures higher than 70°C) or using boiling water to prevent the hydrolysis reaction of glucosinolates by internal myrosinase (Visentin et al., 1992) [105]. A simple extraction procedure for separation and purification of glucosinolates from crude plant homogenates by high speed counter current chromatography was reported (Fahey J W et al., 2003) [106].
Minchinton et al. (1982) [107] reported a method for separation of desulphoglucosinolates by RP-HPLC. Kaushik and Agnihotri (1999) [108] developed a procedure for extraction of intact glucosinolates by HPLC.

Kiddle et al. (2001) [109] reported that glucosinolates was poorly hydrolyzed by myrosinase in the presence of denaturants such as methanol. Hence 80 % methanol is chosen as the best solvent for its hydrolysis with intermittent shaking of the mixture during hydrolysis.

2.1.2 Myrosinase

The enzyme myrosinase (thioglucoside glycohydrolase EC 3:2:3:1) catalyses the hydrolysis of glucosinolates. Myrosinase is found in plant cells in a separate compartment from glucosinolates. When the plant cells are damaged, e.g. by cutting or chewing, the myrosinase comes in contact with the glucosinolates and hydrolysis occurs. All kinds of processing lead to a certain degree of glucosinolate hydrolysis by myrosinase or other chemical reactions. Myrosinase cleaves the glucosinolates forming glucose, hydrogen sulphate and either a thiocyanate, nitrile, or an isothiocyanate (ITC) depending on the starting glucosinolate, reaction pH and availability of ions (Kliebstein et al., 2005) [110]. For determination of total glucosinolate content, colorimetric measurement of glucose released by myrosinase hydrolysis seems most appropriate.

2.1.3 Isothiocyanates

Glucosinolate hydrolysis products make a significant contribution to the typical flavour of Brassica vegetables. Sulforaphane (SFN) is an aliphatic ITC that has shown activity as a antioxidant agent and has aroused interest as a possible cancer-preventive agent. The effects of processing on glucosinolate levels in vegetables have been reviewed by De Vos and Blijleven (1988) [111]. Bertelli et al. (1998) [112] reported the solid phase extraction and quantification of sulforaphane in Broccoli by RP HPLC. Liang et al. (2006) [113] reported a simple easy convenient method of separation of sulforaphane from Brassica sps.

Liang et al. (2007) [114] developed solid phase extraction column for extraction of sulforaphane from Broccoli. Agrawal et al. (2006) [115] had reported a method for simultaneous
determination of sulforaphane (SFN) and its major metabolites from biological matrices with liquid chromatography mass spectrometry. Matusheski et al. (2001) [117] used preparative liquid chromatography for separation of sulforaphane from Brassica sps.

Cuijuan et al. (2008) [118] developed high speed counter current chromatography for isolation of sulforaphane from broccoli seed meal. Rochfort et al. (2006) [119] developed and validated method for measurement of sulforaphane in Broccoli. Both gas-liquid chromatography (GLC) and high performance liquid chromatography (HPLC) can be used to determine the content of individual glucosinolates. Because of the thermal instability of indoleglucosinolates HPLC is the most suitable method for determination of these compounds. Isothiocyanates and nitriles can be analyzed by GLC. HPLC with UV detection may be used for analysis of oxazolidinethiones and indoles (Verhoeven et al. 1997) [20].

Ugolini et al. (2008) [120] devised the method for simultaneous extraction of oil, proteins and glucosinolates from cruciferous oil seeds. In another separate study, it is reported that when seed sprouted, the concentration of Isothiocyanates decreased so sharply that very low peaks of Isothiocyanates were found in autolysis of seedling meal on fluorescence detector (You et al. 2007) [121]. It was reported that crucifer seed contained approximately 10 times the total glucosinolate concentration than the edible portion of the vegetable (Tookey et al. 1980) [122].

Staack et al. (1998) [123] supported that extraction of isothiocyanates is achieved with weak polar fluids such as methylene chloride, ethyl acetate, etc.

3.2 Testing the effect of phytochemicals for anticancer activity

In India the incidence of non communicable diseases such as cancer, heart disease, diabetes etc occurs as a result of stress and changes in life style. Anticancer activity of the crucifers is attributed mainly due to the presence of glucosinolates. Sulforaphane (SFN) a potent cancer preventive agent is a dietary isothiocyanate and its research has grown in last few years due to its putative beneficial pharmacological effects which include antioxidant, anti-inflammatory and antitumor properties (Brown et al., 2009; Heiss et al., 2001; Payastre et al.,
Waltenberg et al. (1978) [127] reported that inhibition of polycyclic aromatic hydrocarbon induced neoplasia by naturally occurring indoles.

The proliferation of eukaryotic cells is regulated by an intricate network of growth inhibitory and stimulatory signal transduction pathways thus controlling the cell cycle (Cover et al., 1998) [128]. There exist a transcriptional indole signalling pathway that specifically targets cell cycle gene promoter in various cancer cells. I3C or DIM induces a G1 cell cycle arrest accompanied by down regulation of cyclin dependent kinase CDK6 and strong stimulation of p21 gene expression (Hong et al., 2002) [129].

Grana et al. (1995) [130] indicated that sulforaphane could induce G2-M cell cycle arrest thus regulating the cdc2 kinase activity. Sulforaphane and erucin both of which are present in the broccoli sprout were previously shown to disrupt mitotic spindles and cause M phase arrest in mammary carcinoma cells, presumably by inhibiting tubulin polymerization (Tang L et al., 2006) [131].

Singh et al. (2004) [132] studied the synthetic sulforaphane induced G2/M arrest and involves checkpoint kinase 2 mediated phosphorylation of Cdc25C leading to accumulation of inactive cyclin dependant kinase. Xu et al. (2006) [133] demonstrated the inhibition of 7, 12 dimethylbenzanthracene induced skin tumorigenesis in mice by sulforaphane mediated by nuclear factor E2 related factor.


Bosetti et al. (2001) [136] reported that Diindolylmethane (DIM) can cause DNA damage in treated colon cancer cells.

Dashwood (1988) [137] reported Indole 3 carbinol from Brassica vegetables as an anticancer agent. Many researchers reported that Brassica vegetables contain an enriched amount of glucosinolates and its hydrolysis products such as sulforaphane and Indole 3 carbinol were found to possess effective anticancer properties (Gasper et al., 2007; Elliot and Stowe, 1971) [138,139]. The hydrolysis-derived products from glucoraphanin decreased the growth of several
other tumor cells, viz. FL (murine erythroleukemic cells), Jurkat (human T-lymphoid cells), HeLa (human cervix carcinoma cells), H9 (human T-lymphoid cells) and H3-TI-1 cells obtained by transfection of HeLa with a LTR-HIV-1-CAT plasmid). 2-Phenethyl isothiocyanate (PEITC) and its mercapturic acid pathway metabolites inhibited the growth of HL60 (human leukaemia 60) cells in vitro (Zhang et al., 2003) [140].

2.3 Studies on mechanism of action

Vegetables of the Brassicaceae family, in particular those of the Brassica genus (broccoli, cabbage, cauliflower, Brassica oleracea, mustard, etc) received much attention, because of their anticancer activity in vitro and in vivo (Fimognari et al., 2002) [141]. Glucosinolates found in Brassica vegetables are known to inhibit the growth of cancer cells and to induce apoptosis but the mechanisms are still only partially understood (Denoyelle et al., 2003) [142]. A reduction in cell growth and an induction in cell death are two major means to inhibit tumor growth (Firestone et al., 2003) [143].

Apoptosis, a form of programmed cell death, plays a critical role in both development and tissue homeostasis. It involves the concerted action of a number of intracellular signalling pathways, including members of the caspase family of cysteine proteases stored in most cells as zymogens or procaspases. Additional evidence also suggests that SFN suppresses tumor development during the “post-initiation” phase of cancer via induction of cell cycle arrest and apoptosis. Apoptosis is one of the important pathways through which chemo preventive and chemotherapeutic agents inhibit the growth of cancer cells (Pan et al. 2008) [144].

Tumor necrosis factor related apoptosis inducing ligand (TRAIL) is known to induce apoptosis in several cell lines by SFN (Matsui et al., 2007; Jin et al., 2007) [145,146]. Pappa et al. (2006) [147] compared the growth inhibition profiles and mechanisms of apoptosis induction in human colon cancer cell lines by isothiocyanates and indoles from Brassicaceae. Pledger- Tracy et al. (2007) [148] studied the induction of apoptosis in human breast cancer cell by sulforaphane.
Jakubikova et al. (2005) [149] reported that sulforaphane treatment inoculate the MEK/JRK signals thus promoting cell cycle arrest and cell death in CaCo2 cells. Isothiocyanates are principally metabolized through the mercapturic acid pathway in vivo, giving rise to dithiocarbamates which are biologically similar to their parent glucosinolates.

Tang et al. (2006) [150] have evaluated the antiproliferative activity of broccoli sprout extracts and explored the underlying mechanism of action using human bladder carcinoma UM-UC-3 cells and proved that isothiocyanates could induce apoptosis and cell cycle arrest in the cells.

Several investigations have attempted to characterize the pathways involved in the apoptotic responses in cancer cells exposed to indole derivatives, which are found in cruciferous vegetables. These studies found that such compounds have anti-carcinogenic activities and may affect many biochemical pathways (Costello et al., 1997; Esteller et al., 2007) [151,152].

Varieties of genes are involved in cell metabolism, cell cycle regulation and apoptosis. Both pro apoptotic (Bax, Bak, Bid, Noxa, etc.) and anti-apoptotic (Bcl-XL, Mcl-1, Bcl-w etc) proteins have been reported to be key regulators of apoptosis. Genes transcriptionally up regulated by p53 are implicated in promoting apoptosis, which includes the Bcl-2 family members (e.g. Bax, Bak) and Noxa gene proteins. The p53-dependent apoptotic pathway can lead to the cellular protein cleavage (e.g. PARP), DNA damage and cell death (Zhivotovsky, 1999) [153].

Wolter et al. (1997) [154] reported sulforaphane induced HT 29 cells death is not associated with a change in p53 protein expression but is accompanied by an over expression of bax one of the bcl-2 gene family acting as a promoter of cell death. Phenethyl ITC induces p53 transactivation in mouse epidermal cells in a dose and time dependent manner (Huang et al., 1998) [155].

Granville et al. (1998) [156] induces the opening of the mitochondrial permeability transition pore thus mediating the release of cytochrome C finally activating the cascade. Yu et al. (1998) [157] evidenced the caspase 3 involvement by the presence of a proteolytic fragment of caspase 3 in treated cells.
Jackson et al. (1998) [158] indicated that sulforaphane could inhibit the induction of growth arrest and apoptosis. Smith et al. (1998) [159] indicated that sulforaphane could reduce the occurrence of aberrant crypt foci on rat model. Several studies show that 3-3’ diindolylmethane (DIM) metabolised product reduce the incidence for different classes of reproductive tumors also support that it induces apoptosis (Chen et al., 1998; Leong et al., 2001; Chen et al., 2001) [160-162].

The major apoptotic signal transduction cascades associated with programmed cell death include the proteins of Bcl-2 family. The members of this group of proteins either promote cell survival (e.g., Bcl-2 and Bcl-XL) or induce programmed cell death (Hoeppner et al., 2001) [163]. Bcl-2 is thought to inhibit apoptosis by forming heterodimers with Bax, the down regulation of Bcl-2. Bax translocation from cytosol into mitochondria has been known as a critical event that occurs during apoptotic processes (Bedner et al., 2000; Jia et al., 2001) [164,165]. The down regulation of Bcl-2 may be one of the mechanism by which I3C induces apoptosis in cancer cells (Chinni et al., 2001) [166]. Chen et al. (1996) [167] studied the molecular mechanism of c-Jun N terminal kinase mediated apoptosis induced by anticarcinogenic isothiocyanates. Favreu et al. (1995) [168] induced the increased expression of these enzymes by isothiocyanates through activation of MEKK1 and JNK pathway.

Krul et al. (2002) [169] indicated that glucosinolate sinigrin degraded to AEITC (allylethyl isothiocyanate) induced apoptosis of aberrant colonic crypt cells induced by dimethylhydrazine induced cancer. Multiple apoptotic stimuli trigger the activation of proteases called caspases which in turn initiate and execute the apoptotic program. In a previous study, the SFN activated the caspase-8-dependent death receptor pathway coinciding with the activation of the mitochondrial pathway (Fimognari et al., 2006) [170]. Kim et al. (2004) [171] stated that resistant A549 cells are sensitised on co treatment with genistein by studying the elevated expression levels of bcl-2 (antiapoptotic) protein.

2.4 Studies on other biological activities of Brassica oleracea

Consumption of vegetables and fruits may help to reduce the risk of many age related degenerative diseases and cancer. Green leafy vegetables, fruits, wheat germ, nuts and vegetable
oils are excellent sources of antioxidant components (Borek, 1991; Xianquan et al., 2005) [172,173]. Vegetables contain a group of natural antioxidants that possess not only high antioxidant activity but also good antioxidant quality. It also contains phenolic antioxidants that may account for its specific protective effects (Chen & Ahn 1998) [174].

Cruciferous vegetables have relatively abundant sources of antioxidant substances with potential anticarcinogenic activity. These vegetables are known to be rich in antioxidant substances such as ascorbic acid, β carotene and carotenoids (Donaldson, 2004) [175]. Tsuda et al. (1994) [176] reported that anthocyanidins is found to have rich antioxidant activity. Wills et al. (1984) [177] studied the nutrient composition of Chinese vegetables and found that carotenes present in various types of cabbage are α-, β–carotenes and cryptoxanthin. Tee et al. (1997) [178] reported that mustard cabbage had highest content of vitamin C followed by white and green cabbage.

Colditz et al. (1985) [179] reported that cabbage, chinese kale, cauliflower contains the highest levels of several anti oxidising agents including vitamin C, carotenoids and polyphenols. Anthocyanins content in Brassica oleraceae is observed with elevated antioxidant activity. Broccoli is reported to have moderate antioxidant activity with elevated tocopherols and flavanoids.

GSH also plays an important role in multidrug resistance either through its spontaneous reactions or through its function as a coenzyme in glutathione S-transferases (GST) reacting with the drug (Song et al., 2004) [180]. Glutathione (GSH) is a glutamylated-L-cysteine glycine, a tripeptide that functions as an important intracellular radical scavenger, thus protecting cells against reactive oxygen species (ROS) and toxins. Kawamota et al. (2000) [181] stated that sulforaphane induced the expression of GSTP1 in rat liver epithelial cells.

Xu and Thornalley (2001) [182] stated that GSH involves in the metabolism of phenethyl isothiocyanate and its cysteine conjugate to human leukemia cells in vitro. Rowan et al. (2001) [183] studied the expression of GST A in human adenocarcinoma cells. Tumour cells with elevated GSH are resistant to apoptosis thereby decreasing the permeability of anticancer drugs (Bittencourt et al. 1998) [184].
Shan et al. (2006) [185] proposed the antioxidant activity in crucifers elicited due to the induction of phase II detoxification enzymes. The chemo protective effect of sulforaphane was thought to be due solely to its ability to behave as an inducer of phase II detoxification enzymes.

Indoles thereby also affect estradiol metabolism which is P450 dependent and may reduce the risk of estrogen-dependent diseases such as mammary cancer. In subsequent research, however, sulforaphane was also shown to inhibit the CYP2E1 isoenzymes of the cytochrome P450, thus emerging as an inhibitor of Phase I enzymes. Barcelo (1996) [186] reported that sulforaphane could inhibit the CYP2E1 isozyme of cytochrome 450 thus emerging as an inhibitor of Phase I enzymes.

The classic chemoprevention mechanism for SFN involves induction of phase 2 enzymes thereby facilitating detoxification of carcinogens and other genotoxic stresses. In a study of Kore et al. (1993) [187] stated that no significant induction of phase I and phase 2 enzymes were detected when 3-methylsulfinylpropyl isothiocyanates was tested at doses approximate to those found in human diet. However, isothiocyanates, indoles and brassicas may have beneficial effects. At high but realistic consumption levels, indoles and brassicas did show positive effects on health in human studies.

Liu et al. (2005) [188] have found that modulating effects of indoles on the induction of phase 1 enzymes and phase 2 enzymes of xenobiotic metabolism. Indoles, unlike isothiocyanates induce the phase I enzyme estradiol 2-hydroxylase. Kim et al. (1994) [189] proved that prolonged exposure to I3C reportedly increased the size and number of glutathione S-transferase-placental form (GST-P)- positive liver foci after 24 weeks, in rats given diethyl nitrosamine (DEN) or DEN plus two other initiating agents.

Besides modulators of biotransformation enzymes isothiocyanates are seen as suppressing agent. Suppressing agents act during the promotion phase of the neoplastic process via prevention of the evolution of the neoplastic process in cells (Elson, 1994) [190]. The majority of chemoprevention studies have focused on the ability of SFN to act “pre-initiation” as a potent phase 2 enzyme inducer via Keap1-Nrf2 signalling and antioxidant response element (ARE)-driven gene expression (Singh et al., 2004) [191].
Quattrochi et al. (1994) [192] reported that signal transduction is initiated due to elevated expression of phase 2 enzymes thus promoting apoptosis in cancer cells. Yu et al. (1997) [193] suggest that e-Jun N-terminal kinase 1 (JNK1) is involved in the regulation of phase 2 detoxifying enzyme gene expression.

Brassica vegetables have a goitrogenic potential. The goitrogenic effects have been ascribed to hydrolysis products of glucosinolates, in particular thiocyanate ion and 5-vinyloxazolidine-2-thione (goitrin). The mechanism of goitrogenicity seems to be different between thiocyanate ion and goitrin. The thiocyanate ion would compete with iodine for uptake by the thyroid gland. Thus its goitrogenicity depends upon the iodine content of the diet (Stoewsand, 1995) [194].

Isothiocyanates have important biological properties that defend the plant against insects, fungi and microbial infection (Iwu et al., 1991; Korber 1995) [195,196]. SFN, an isothiocyanate compound found abundantly in Brassica oleracea and other cruciferous vegetables, inhibits the growth of the bacterial pathogen Helicobacter pylori [85]. β- Phenyl ethyl isothiocyanate was found not to have antimicrobial activity against Salmonella choleraesuis.

β-Phenyl ethyl isothiocyanate had very strong activity (> inhibition zone; i.z. of sample 20 mm) against Vibrio parahaemolyticus and Staphylococcus aureus and clear activity (i.z. of sample 10-15 mm > i.z. of control) against Bacillus cereus at 1000 mg/l. β-Phenyl ethyl isothiocyanate at 100 μg/ml inhibited the growth of food-borne pathogens, exhibiting antimicrobial effects at low concentration. β- Phenyl ethyl isothiocyanate inhibited Vibrio parahaemolyticus most effectively (Hong et al., 2008) [197].

2.5 Docking study

Ligand protein interaction serves as an essential way to analyse the binding interaction between the molecules in oncogenesis. Many researchers studied the interaction of SFN with histone deacetylase inhibitors and disease proteins such as leishmaniasis (Sahoo et al., 2009; Bhoi et al., 2012) [198,199]. The interaction of ITCs with apoptotic proteins have not been
reported so far. Thus the insilico analysis of SFN and Indole 3 carbinol has been done using various insilico docking tools.
OVERVIEW OF THESIS

The objective of the study is to extract Red cabbage (*Brassica oleraceae var rubra*) and to study the anticancer activity and its mechanism of action in Human epithelial carcinoma cells HEp-2.

Chapter 1 deals with preparation of crude extract of *Brassica oleraceae* and to study its anticancer activity in Human epithelial carcinoma cells HEp-2.

Chapter 2 deals with separation and purification of crude extract of *Brassica oleraceae* and to study the anticancer activity of different fractions on Human epithelial carcinoma cells.

Chapter 3 deals with mechanism of action of purified extract of *Brassica oleraceae* on Human Epithelial carcinoma cells HEp-2 by studying the regulation of various apoptotic proteins such as p53 (tumor suppressor), bax (pro apoptotic), bcl-2 (anti apoptotic) and caspase -3.

Chapter 4 deals with study of various other biological activities of purified extract of *Brassica oleraceae* such as antioxidant and antimicrobial activities.

Chapter 5 deals with insilico analysis of interaction between apoptotic proteins and isothiocyanates isolated from *Brassica oleraceae*. 
OBJECTIVES

The objective of this work is to study the anticancer activity of purified extract of Brassica oleraceae and its mechanism of action on Human Epithelial carcinoma cells HEp-2.

- To prepare the crude extract of Brassica oleraceae by maceration.
- To purify the crude extract of Brassica oleraceae by solid phase extraction and analysis using HPLC.
- To assess the cytotoxicity of different fractions (1, 2 and 3) on HEp-2 cells and Vero cells.
- To determine the effect of purified extract of Brassica oleraceae on cell cycle progression by Flow cytometry.
- To determine the apoptotic protein and mRNA expression in HEp-2 cells treated with purified extract of Brassica oleraceae.
- To determine antioxidant activity in HEp-2 cells treated with purified extract of Brassica oleraceae.
- To determine the activity of Phase 2 enzymes in HEp-2 cells treated with purified extract of Brassica oleraceae.
- To analyse the interaction of various apoptotic proteins such as p53, bax, bcl-2 and caspases with Isothiocyanates using insilico docking studies.