INTRODUCTION

Cancer is the second leading cause of death all over the world. Preventive measures that target the various steps involved in cancer initiation and progression could significantly decrease the incidence and mortality of cancer. Cancer has become a huge burden on various nations of the world [1]. Cancer can attack in many different ways affecting different organs and varies in its degree of lethality. Cancer is a disease that can justifiably receive our full attention in efforts to eradicate it. The words tumor and cancer are often used interchangeably and refers to a clump of abnormal cells. The word cancer refers more to the state of being of a patient having malignant tumors in their body. Tumor cells can also break away from their point of origin and take hold in another organ of the body through the process of metastasis [2]. This can lead to tumor growth in an otherwise healthy organ. Tumors may have a range of responses to therapy and these responses can depend on the type of tumor involved and the treatment methods used [3]. Likewise, potential antitumor agents and established anticancer drugs are often having selective activity towards some tumor types. Lung, breast, cervix, pancreatic and mouth cancers are some of the major forms of cancer with very low survival rates [4].

1.1 Importance of Cancer Research

The rates of survival from cancer in developing countries are exceptionally low due to poor medical infrastructure and lack of trained physicians. Most people do not seek medical attention because of their lack of awareness, stigma and reliance on traditional healers mean [5]. Cancer is the leading cause of death in developed countries and is rising in alarming rates in developing countries. Estimates of the worldwide incidence and mortality from 27 cancers in 2008 have been prepared for 182 countries as part of the GLOBOCAN series published by the International Agency for Research on Cancer recent. Results for 20 world regions, summarizing the global patterns for the eight most common cancers have been made in this report [6]. The people of developing countries are more killed by cancer each year than AIDS, tuberculosis or malaria and it has been confirmed in
2008 that more than 12 million new cases of cancer were diagnosed worldwide. Overall, an estimated 12.7 million new cancer cases and 7.6 million cancer deaths occurred in 2008 with 56% of new cancer cases and 63% of the cancer deaths occurring in the less developed regions of the world. The most commonly diagnosed cancers worldwide are lung, breast, oral cavity and colorectal cancers [7].

1.2 Larynx

The larynx or voice box is the organ containing vocal cords. It is made up of a complex arrangement of muscles, cartilages and ligaments all are lined by epithelium such as stratified squamous epithelium on the vocal chords. The larynx is anatomically divided into the glottis (true vocal cords, anterior and posterior comissures, the supra glottis (epiglottis, arytenoids and aryepiglottic folds) and sub glottis [8].

1.2.1 Laryngeal cancer

Laryngeal cancer reveals that cancers of neck and throat are considered together because they share many similarities in incidence, cancer type, predisposing factors, pathological features, treatment and prognosis. Most laryngeal cancers originate in the glottis, spread by direct extension to adjacent structures by metastasis. They also extend to regional cervical lymph nodes, blood stream by distant metastasis to the lung are most common [9].

1.2.2 Status in India

Laryngeal cancer is the most common malignancy of the head and neck. It is estimated that around 80-95 % of them represents squamous cell carcinoma [10]. Carcinoma of the larynx forms an important group of malignancies as it accounts for approximately 1% of new cancer diagnosis. It is the 14th most common cancer in the world. Sixty percent of the incidence is in developing countries. In India, the incidence is 1.3-8.8 per 1 lakh population in six different regions (ICMR, 1992). In Delhi Cancer Registry Program it is the second most common cancer among males and according to Bombay Cancer Registry Program it is the third most common
cancer [11]. The National Cancer Registry Programme of the Indian Council of Medical Research which collected data from six different parts of the country, both rural and urban areas showed varying figures at different areas. While cancer of the mouth, trachea, bronchus and lungs was the most common form of malignancy in males in 1989 from Bombay, Delhi, and Bhopal, it was the second most common in Madras and third in Bangalore [12].

Table 1.1 Percentage of Cancer deaths among men and women in India

<table>
<thead>
<tr>
<th>Sl no</th>
<th>Types of cancer</th>
<th>Cancer death %</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Men</td>
</tr>
<tr>
<td>1</td>
<td>Lip, mouth, oral pharynx</td>
<td>19.7</td>
</tr>
<tr>
<td>2</td>
<td>Stomach</td>
<td>10.6</td>
</tr>
<tr>
<td>3</td>
<td>Larynx, trachea</td>
<td>9.7</td>
</tr>
<tr>
<td>4</td>
<td>Liver</td>
<td>6.7</td>
</tr>
<tr>
<td>5</td>
<td>Colorectal</td>
<td>2.8</td>
</tr>
<tr>
<td>6</td>
<td>Breast</td>
<td>----</td>
</tr>
<tr>
<td>7</td>
<td>Cervical</td>
<td>-----</td>
</tr>
<tr>
<td>8</td>
<td>Prostate</td>
<td>3.0</td>
</tr>
<tr>
<td>9</td>
<td>Bone</td>
<td>3.2</td>
</tr>
<tr>
<td>10</td>
<td>Lymphoid hemopoietic</td>
<td>5.8</td>
</tr>
<tr>
<td>11</td>
<td>Oesophagus</td>
<td>6.0</td>
</tr>
<tr>
<td>12</td>
<td>Eye and Brain</td>
<td>5.3</td>
</tr>
</tbody>
</table>

1.3 Phytochemicals

The term "phytochemical" is used to refer to compounds produced by living beings organisms as secondary metabolites [13]. The first reason is that nature has a proven track record of yielding various phytochemicals that are useful as treatments or cures for several diseases. As an extension of this, natural products can provide a clue about what an effective drug might look like. A synthetic derivative might even have higher potency than the natural product. Overall
nature provides investigators with a library of chemical leads to the research in the different plant molecules for development of various drug leads in the treatment of cancer.

1.3.1 Rationale for studying natural products

There are several principal reasons for studying natural product sources in a search for antitumor agents. The first reason is that nature has a proven track record of yielding chemicals that are useful as treatments or cures for several diseases. Natural products can provide a clue about what an effective drug might look like. A simple derivatized natural product might retain the bioactivity of the original parent molecule but possibly without high toxicity. A synthetic derivative might even have higher potency than the natural product. Overall nature provides investigators with a library of chemical leads from which to choose a useful compound. Modern strategies for drug discovery emphasise on availability of some simple and inexpensive biological assays to evaluate medicinal potential of plant species [14]. Present study demonstrates a modern strategy with combination of local and modern knowledge for evaluation of biological activity of medicinal plant species.

1.3.2 Research using phytochemicals

In recent times a high level of awareness/interest has been created among general people worldwide about the protective effects of fruit and vegetables against cancer risk [15]. But, it should be clear that no protective effects have actually been firmly established. Although, a recent publication of the International Agency for Research on Cancer (IARC) has suggested to increase or maintain fruit and vegetable intake to improve nutrition for reducing the burden of cancer and other chronic diseases [16]. However different studies have generally agreed that consumption of a diet rich in vegetables, fruits and other plant food constituents low in animal fats, along with maintaining a physically active healthy weight can reduce the risk of cancer and other chronic diseases [17].
1.4 Dietary agents as cancer fighters

Dietary chemopreventive compounds offer great potential in the fight against cancer by inhibiting the carcinogenesis process through the regulation of cell defensive and cell-death machineries. An issue of utmost importance is whether the journey of normal cell to full blown malignancy can be slowed by one or more dietary agents. Additionally, many of these dietary agents appear to exhibit some degree of specificity for neoplastic cells while sparing normal cells [18]. The last decade has seen an extraordinary increase in our understanding of mechanism of development of cancer [19]. The chemopreventive agents when administered as an adjunct to radiation therapy or chemotherapy may improve their efficacy by increasing tumor response, decreasing toxicity and sensitize cancer cells to chemo radiation when patients become unresponsive to standard therapy [20]. This could in fact improve quality of life and possibly increase the survival time of patients. Diet can affect the overall process of carcinogenesis by different mechanisms. In particular, the use of dietary chemoprevention strategies has gained significant interest. Research investigating the use of diet derived chemoprevention compounds may have significant impact on qualifying or changing recommendations for high-risk cancer.

1.5 Cruciferous vegetables

One group of vegetables that has been widely regarded as potentially cancer protective’s are vegetables of the cruciferae family. Cruciferous vegetables are the major source of glucosinolates in the diet which distinguishes them from other vegetables. Brassica vegetables, including all cabbage-like vegetables, are a genus of the family Cruciferae and contribute most to our intake of glucosinolates [21]. Different cruciferous vegetables contain different profiles of glucosinolates, which result in different profiles of isothiocyanates or indoles when they are chopped or chewed. Brassica oleracea is a rich source of glucoraphanin, the precursor of sulforaphane, and glucobrassicin, the precursor of indole-3-carbinol. In the 1960s interest emerged in the possibility that certain aromatic and indolic glucosinolates hydrolysis products might influence carcinogenesis [22]. Cruciferous vegetables play an important role against the risk of cancer. The Cruciferae (syn. Brassicaceae) are the family of plants that include a wide variety of familiar members of the species Brassica oleracea (e.g.,
broccoli, cabbage, cauliflower, parsnip, kale, kohlrabi, brussels sprouts, turnip, rutabaga) and many other plants which are commonly consumed in various parts of the world such as oriental cabbage, arugula, watercress, radish, daikon, wasabi, various mustards, horseradish, etc [23]. The role of cruciferous vegetables to protect against cancer is attributed to the fact that they are the unique source of glucosinolates (β-thiogluco-side-N-hydroxsulfates) in our diet.

1.5.1 Glucosinolates

Glucosinolate content accounts for 1% of dry weight of the Brassica vegetables and it plays important role in plants such as in allelopathy (suppression of growth of neighbouring plants) and in protection against predators including nematocidal, microbicidal, antifungal and insecticidal activities [24]. At least 120 different glucosinolates have been identified. Glucorabasssicin and glucoraphanin are generally found in high concentrations in Brassica oleracea, broccoli, Chinese cabbage, radish and watercress contain high amount of gluconasturtii whereas sinigrin is found in high concentrations in brussels sprouts, green cabbage and cauliflower [25]. As mentioned earlier, hydrolysis of glucosinolates by myrosinase produces various types of isothiocyanates. Hydrolysis of glucoraphanin results in sulforaphane, hydrolysis of sinigrin results in allyl isothiocyanate, gluconasturtii produces phenethyl isothiocyanate (PEITC) and glucobrassicin produces indole-3-carbinol [26].

<table>
<thead>
<tr>
<th>Glucosinolate</th>
<th>Isothiocyanate or Indole</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucoraphanin</td>
<td>Sulforaphane</td>
<td>Brassica oleracea, Broccoli, Brussels sprouts</td>
</tr>
<tr>
<td>Glucobrassicin</td>
<td>Indole-3 carbinol</td>
<td>Broccoli, cauliflower, cabbage</td>
</tr>
<tr>
<td>Gluconasturtii</td>
<td>Phenethyl isothiocyanate</td>
<td>Watercress</td>
</tr>
<tr>
<td>Glucotropaeolin</td>
<td>Benzylisothiocyanate</td>
<td>Cabbage, garden cress</td>
</tr>
<tr>
<td>Sinigrin</td>
<td>Allyl isothiocyanate</td>
<td>Horse radish, Chinese cabbage, mustard</td>
</tr>
</tbody>
</table>

Table 1.2 Different glucosinolates and its hydrolysis products
The glucosinolate hydrolysis products consist of equimolar amounts of an aglucon, glucose and sulphate. The aglucons are unstable and undergo further reactions to form for instance thiocyanates, nitriles or isothiocyanates. The nature of the hydrolysis products depends primarily on the side chain of the glucosinolate, besides the conditions of the hydrolysis and the presence of any cofactors. The glucosinolate precursor to SFN, glucoraphanin, is abundant in cabbage, broccoli and kale [27, 28]. Hydrolysis of glucoraphanin to its aglycon product SFN requires the activity of myrosinase enzymes released from the plant during consumption and other myrosinase enzymes present in our gut.

1.5.2 Myrosinase

Like other dark green vegetables, many cruciferous vegetables are rich in folate and chlorophyll. One of the unique things about cruciferous vegetables is that they are rich sources of glucosinolates, sulphur-containing compounds that give them their pungent aromas and spicy (some say bitter) taste. Chopping or chewing cruciferous vegetables releases myrosinase, an enzyme that breaks down glucosinolates into biologically active compounds, such as indoles and isothiocyanates. Myrosinase released by chopping or chewing cruciferous vegetables breaks down glucosinolates to isothiocyanates, releasing glucose and sulphate in the process. “R” designates side chains of other elements in the molecule [29].

\[ \begin{align*}
\text{Glucosinolate} & \quad \overset{\text{Myrosinase}}{\longrightarrow} \quad \text{Isothiocyanate} \\
R & \quad N = C = S \\
C = \text{carbon}, \ S = \text{sulfur}, \ N = \text{nitrogen}
\end{align*} \]

**Figure 1.1  Mechanism of action of Myrosinase**
1.5.3 Isothiocyanates

Isothiocyanates are derived from the hydrolysis (breakdown) of glucosinolate compounds found in cruciferous vegetables. Cruciferous vegetables contain a variety of glucosinolates each of which forms a different isothiocyanate when hydrolyzed. Isothiocyanates such as sulforaphane may help prevent cancer by promoting the elimination of potential carcinogens from the body and by enhancing the transcription of tumor suppressor proteins [30]. Epidemiological studies provide some evidence that human exposure to isothiocyanates through cruciferous vegetable consumption may decrease cancer risk but the protective effects may be influenced by individual genetic variation in the metabolism and elimination of isothiocyanates from the body. Isothiocyanates act by inhibition of cell proliferation and induction of apoptosis. The major isothiocyanates with the strongest anticancer effects are sulforaphane, phenylethylisothiocyanate, benzylisothiocyanate and 3-phenylpropylisothiocyanate [31]. Brassica oleracea and Broccoli is a good source of glucoraphanin, the glucosinolate precursor of sulforaphane (SFN) and sinigrin the glucosinolate precursor of allylisothiocyanate (AITC). Watercress is a rich source of gluconasturtiin, the precursor of phenethyl isothiocyanate (PEITC), while garden cress is rich in glucotropaeolin, the precursor of benzyl isothiocyanate (BITC) [32, 33].

1.5.4 Molecular targets/Anticancer properties of Isothiocyanates

Two important and well studied compounds in cruciferous vegetables are sulforaphane (SFN) and indole-3-carbinol (I3C). Sulforaphane, an isothiocyanate present in Brassica members is found to have antioxidant properties and is capable of stimulating detoxifying enzymes in the body and therefore a powerful cancer preventive agent. Sulforaphane [1-isothiocyanato-4- (methylsulfinyl)-butane] which has been identified in crucifers as a product of enzymatic or acid hydrolysis of the corresponding x-(methylsulfinyl)- alkyl-glucosinolate (glucoraphanin). This isothiocyanate can decrease the risk of developing different cancers such as breast cancer, gastric cancer and skin cancer. The mechanisms of SFN chemoprevention have been well studied and
reveal diverse responses depending upon the stage of carcinogenesis [34]. SFN can function by blocking initiation via inhibiting phase 1 enzymes that convert procarcinogens to proximate or ultimate carcinogens and by inducing phase 2 enzymes that detoxify carcinogens and facilitate their excretion from the body [35]. Once cancer is initiated SFN can act via several mechanisms that modulate cell growth and cell death signals to suppress cancer progression. SFN is found to intervene at different stages for chemoprevention during cancer development especially during initiation and post-initiation stages [36]. Also, there is a large body of research that has examined SFN effects on many other cancers such as breast, hepatic, bladder, osteosarcoma, glioblastoma, leukemia, pancreatic, and melanoma [37]. The structures of glucoraphanin and SFN are shown in Figure 1.2.

![Figure 1.2 Hydrolysis of glucoraphanin to sulforaphane by myrosinase](image)

1.5.5 Evidence for the anti-cancer activity of crucifers

Cell culture and small animal studies have found some anticancer effects of substances isolated or derived from cruciferous vegetables. An extensive review of epidemiologic studies published prior to 1996 reported that the majority (67%) of 87 case-control studies found an inverse association between some type of cruciferous vegetable intake and cancer risk [38]. At that time, the inverse association appeared to be most consistent for cancers of the lung and digestive tract. The results of such retrospective
case-control studies are more likely to be distorted by bias in the selection of participants and dietary recall than prospective cohort studies which collect dietary information from people over years before they are diagnosed with cancer [39]. In the past decade, results of prospective cohort studies and studies taking into account individual genetic variation suggest that the relationship between cruciferous vegetable intake and the risk of several types of cancer is more complex than previously thought.

1.6 Red Cabbage

Red Cabbage (Brassica oleraceae) contained similar amounts of glucoraphanin and glucobrassicin but in addition, appreciable amounts of glucoiberin, progoitrin, sinigrin, gluconapin and glucosinolates while neo-glucobrassicin occurred at trace levels only [40]. Brassica oleraceae is rich in phenolic flavanoids, acylated anthocyanins, vitamin C and A in the form of caroteneoids which are stable to pH, temperature, light and acidic gastric digestion conditions. It is also a very good source of fiber, manganese, folate, molybdenum, vitamin B_6, potassium, thiamine (vitamin B_1), and calcium [41]. Polyphenols rank at the top of the list for phytonutrient antioxidants in cabbage. Brassica oleraceae is even more unique among the cabbage in providing about 30 milligrams of the red pigment polyphenols called anthocyanins. Anthocyanins, a group of polyphenols present in fruits and vegetables are the most abundant antioxidants in our diet and reported anti-inflammatory agent [42]. Brassica oleraceae is a rich source of anthocyanins mainly acylated anthocyanins: cyanidin 3, 5-diglucoside, cyanidin 3-sophoroside-5-glucoside and cyanidin3-sophoroside-glucoside acylated with sinapic acid [43]. Brassica oleraceae ranked in WH Foods rating system as an excellent source of vitamin C and a good source of vitamin A (which comes from its concentration of carotenoids such as beta-carotene). But in terms of antioxidants in the newer, phytonutrient category cabbage is impressive even among cruciferous vegetables [44]. Brassica oleraceae colour can be used as a natural food colour. The Brassica oleraceae anthocyanins transition from purple-red to pink-red to blue-green between pH levels of 3 and 6 respectively. This Brassica oleraceae colour can be widely used in wines, beverages, fruit sauces, candies and cakes [45].
Several epidemiological studies have suggested associations between the consumption of anthocyanins rich foods and prevention of chronic diseases like cancer and heart disease. The anti-inflammatory effect of *Brassica oleracea* is due to the omega 3 fatty acid in the form of α-linoleic acid (AHA) [46, 47].

**The Scientific classification of Brassica oleracea**

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>SubKingdom</td>
<td>Angiosperms</td>
</tr>
<tr>
<td>Division</td>
<td>Eudicots</td>
</tr>
<tr>
<td>Class</td>
<td>Rosids</td>
</tr>
<tr>
<td>Order</td>
<td>Brassicales</td>
</tr>
<tr>
<td>Family</td>
<td>Brassicaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Brassica</td>
</tr>
<tr>
<td>Species</td>
<td>B. oleracea</td>
</tr>
</tbody>
</table>
1.7 Anticancer agents using plant foods

Present study demonstrates a modern strategy with combination of local and modern knowledge for evaluation of biological activity of medicinal plant species. The main steps of this strategy are as following as screening of various plant species for their phytochemical activity including preparation of plant extract, characterisation of phytochemical using HPLC, LC-MS etc. Screening and assessing the biological activity using cell culture assays and its signalling mechanism using real time PCR, blotting techniques and basic in silico approaches. Drug binding with receptor are been developed to study the binding effect of various ligands with the expected biological receptor. Binding affinity was also calculated thus optimising the various interactions between the receptor and ligand.

1.8 Human Laryngeal Epidermoid carcinoma cell line HEp-2

Moore, Sabachewsky and Toolan had established HEp-2 cell line in 1952. They established from tumors that had been produced in irradiated cortionised weanling rats after injection with epidermoid carcinoma tissue from the larynx of a 56 yr old male [48]. A hardy cell line HEp-2 resists temperature nutritional and environmental changes without a loss of viability. It has supported growth of 10 to 14 arboviruses and measles virus and it has been used for experimental studies of tumor production in rats, hamsters, mice and embryonated eggs [49, 50].This cell line lacks a Y chromosome and it has a number of markers associated with HeLa cells. Leibovitz 15 medium with 10 % heat inactivated foetal bovine serum is used to grow HEp-2 cells. Plating efficiency of these cells is normal. The origin of these cells is confirmed as human by immunofluorescence and antibody tests. It supports the growth of a broad range of virus including Adenovirus, Coxsackie B, Herpes simplex virus, Poliovirus, Respiratory syncytial virus and vesicular stomatitis. HEp-2 cell line has a high proliferation rate and a 23 hours cell cycle [51].
1.9 Studies on mechanism of action

Desirable mechanisms of action are those which might enable a drug to target tumor cells selectively or specifically. For example, the target for isolation might be a compound which will damage DNA in a cell. Since tumor cells tend to replicate more frequently and therefore replicate their genomes frequently, DNA damaging agents are somewhat selective to tumor cells. Mechanism based bio assays are based on the identification of certain mechanisms of action that are believed to be potentially useful in treating tumors. There are many possible mechanisms for general cytotoxicity but the mechanisms of action of the historically most successful cancer drugs are more limited in number.

1.9.1 Cell cycle arrest

The important characteristic of cancer is hyper proliferation due to loss of cell cycle regulatory mechanisms. The cycle of increase in components (growth) and division, followed by growth and division of these daughter cells, etc is called the cell cycle [52, 53]. The two most obvious features of the cell cycle are the synthesis and duplication of nuclear DNA before division and the process of cellular division itself mitosis. These two components of the cell cycle are usually indicated in shorthand as the “S phase” and “mitosis” or “M”. There is increasing interest in measurement of cells undergoing programmed “self-destruction” via apoptosis. During apoptosis, the nuclear DNA is fragmented. The fragments can be removed from cells by one of a number of staining protocols, making apoptotic cells visible as a peak below the G1 of DNA content. Usually, this peak is approximately Gaussian in shape and can be quantified using the “overlapped peak” multicycle fitting option [54]. Flow cytometry can detect changes in chromosomes when a population of cells with a DNA content which is not a multiple of DNA Index 1.0 is observed, as this requires that either the numbers or the composition of chromosome(s) have been altered. The G1 phase is a synthetic growth phase for many RNA and protein molecules that will be needed for DNA synthesis and cell growth before division. The G2 phase is a time for repair of any DNA damage which has occurred during the preceding cell
cycle phases and for the reorganization of the DNA structure which must take place before the DNA can be divided equally between daughters during mitosis.

1.9.2. Apoptosis

Apoptosis or programmed cell death is a highly regulated process that allows a cell to self-degrade in order for the body to eliminate unwanted or dysfunctional cells. During apoptosis, the genome of the cell will fracture, the cell will shrink and part of the cell will disintegrate into smaller apoptotic bodies. Unlike necrosis, where the cell dies by swelling and bursting its content in the area, which causes an inflammatory response, apoptosis is a very clean and controlled process where the content of the cell is kept strictly within the cell membrane as it is degraded [55]. The apoptotic cell will be phagocytised by macrophages before the cell’s contents have a chance to leak into the neighbourhood. Therefore, apoptosis can prevent unnecessary inflammatory response. Apoptosis is essential for embryonic development and the maintenance of homeostasis in multicellular organisms. Characteristic apoptotic features include cell shrinkage, membrane blebbing, chromatin condensation and formation of a DNA ladder with multiple fragments caused by internucleosomal DNA cleavage finally ending with the engulfment by macrophages or neighbouring cells thereby avoiding an inflammatory response in surrounding tissues. Cells can undergo apoptosis via two different pathways the intrinsic or mitochondrial-mediated pathway and the extrinsic or death receptor-mediated pathway. The intrinsic pathway is usually activated by the loss of growth factor signals or in response to many different damaging influences for example DNA damage, oxidative stress, hypoxia, or chemotherapeutic drugs. The extrinsic pathway is initiated by binding of the transmembrane death receptors such as Fas, tumor necrosis factor (TNF) receptor, DR3, DR4, or DR5 with their specific ligands. These cell surface receptors are activated when cross-linked by their ligands. It induces caspase-8 thus promoting apoptosis [56, 57].

The p53 protein is a transcription factor encoded by the TP53 gene. After its discovery in 1979, p53 has been extensively studied, mainly because of its role as tumor suppressor [58]. There is now evidence for additional functions of p53 in processes such as glycolysis, autophagy and regulation of oxidative stress [59]. p53 controls the transcription of many different genes in response to stress stimuli like DNA damage, oncogene activation,
nutrient deprivation, telomere erosion or hypoxia [60]. This results in cell cycle arrest, DNA repair, apoptosis, autophagy, senescence, cell migration or angiogenesis [61]. Following DNA damage p53 regulates basic cell cycle processes including DNA repair, cell-cycle arrest, programmed cell death and senescence in order to eliminate potential harmful cells. Research in the last years has shown that in addition to p53 itself, two homologs named p63 and p73 also play an important role in apoptosis [62]. p53 represents the most well characterized of tumor suppressors with a clear role in the induction of apoptosis or cellular arrest in response to stresses such as DNA damage. As such, this gene is frequently mutated in cancers, thereby inactivating the protective proapoptotic role of p53 and contributing to the drug-resistant phenotype. Although it is clear that p53 is able to induce apoptosis, the precise mechanism remains unclear, with both transcriptional-dependent and independent mechanisms being attributed to its ability to induce cell death [63]. In keeping with its role as a transcription factor, p53 is able to regulate the expression of a number of genes that have a direct role in regulating cellular sensitivity to apoptosis via both the death receptor signalling pathway and mitochondria-mediated events. These include Bax, Noxa, a BH3- containing proapoptotic protein that interacts with Bcl-2, p53AIP1 (p53 apoptosis-inducing protein) [64], Fas, DR5, and Pidd, a novel death domain containing protein [65].

The Bcl-2 family of proteins plays a central role in controlling the mitochondrial pathway. More than 20 members of this family have been identified to date in humans, including suppressors (Bcl-2, Bcl-xL, Mcl-1, Bfl-1/A1, Bcl-W, and Bcl-G) and promoters (Bax, Bak, Bok, Bad, Bid, Bik, Bim, Bcl-Xs, Krk, Mtd, Nip3, Nix, Nora, and Bcl-B) of apoptosis [66]. In addition to cytochrome c, mitochondria release a large number of other polypeptides, including AIF, Endo G, second mitochondrial activator of caspases (Smac/Diablo), and HtrA2/Omi from the intermembrane space. Smac/Diablo and Omi/HtrA2 promote caspase activation through neutralizing the inhibitory effects of inhibitor of apoptosis proteins (IAPs). Antiapototic Bcl-2 members sequester proapoptotic Bcl-2 members by binding to their BH3 domains and thereby ultimately prevent Bax or Bak activation/ oligomerization and consequently inhibit mitochondrial proapoptotic event. Over expression of Bcl-2 or Bcl-XL potently inhibits apoptosis in response to many cytotoxic insults, among others by suppressing the generation of ROS and consequently blocking the release of cytochrome c [67]. Besides eliciting its antiapototic effects
on the mitochondrial level by indirectly controlling the activation of the apoptosome, Bcl-2 also appears to inhibit apoptotic pathways that are independent of Apaf-1/caspase-9 and which might depend on caspase-7 as a central effector [68], Apaf-1 homologue that can be directly controlled by Bcl-2/Bcl-X<sub>L</sub> [69].

Figure 1.4 Diagrammatic representation of Apoptosis process
Figure 1.5 Diagrammatic representation of Extrinsic and Intrinsic pathway of Apoptosis

The production of reactive oxygen species (ROS) has been postulated to be a key mechanism by which SFN induces apoptosis. Conjugation of SFN with GSH a necessary step in SFN metabolism depletes the intracellular concentration of GSH and potentially lowers the
oxidative stress threshold of the cell. In their experiments SFN treatment increases mitochondrial ROS production and induces apoptosis as indicated by release of cytochrome C via both death-receptor and mitochondrial caspase cascades. Research study reported that cell cycle arrest response were blocked by addition of antioxidants NAC or GSH, indicating that generation of ROS was indispensable for growth arrest under the assay conditions used [70]. Generally, high doses of SFN are needed in order to induce ROS production. Mitochondrial ROS generation and disruption of the mitochondrial membrane potential have both been shown to induce the formation of acidic vesicular organelles and autophagy in human prostate cells. This response has unique morphological effects and, interestingly, has the ability to inhibit mitochondrial cytochrome C release and apoptosis [71, 72]. All of these effects were reversed with administration of the antioxidant N-acetylcysteine and overexpression of catalase [73]. Therefore, ROS production after ITCs exposure has the ability to influence cell death in cancer cells.

1.10 GSTs as regulators

Glutathione S-transferases (GSTs) are a family of Phase 2 detoxification enzymes that function to protect cellular macromolecules from attack by reactive electrophiles. GSTs are divided into two distinct super-family members: the membrane bound microsomal and cytosolic family members. Cytosolic GSTs are subject to significant genetic polymorphisms in human populations. In general, the substrate specificity for the different isoenzymes overlaps but specifically each class has varying degrees of reactivity for different substrates. The effect that GST genotype has on cancer development and chemoprevention is complex. In the context of high cruciferous vegetable intake, evidence is mounting in favour of a GST null genotype providing a protective effect against lung, colon, and breast cancers [74, 75]. GSTs are a promising target because its expression is enhanced in many tumors and high levels are sometimes correlated with poor prognosis. In addition, GSTP1-1 is frequently elevated in drug-resistant tumors.
1.10.1 GST/GSH detoxification

GSTs catalyse the conjugation of glutathione (GSH) to a wide variety of endogenous and exogenous electrophilic compounds. Glutathione conjugation is the first step in the mercapturic acid pathway that leads to the elimination of toxic compounds. GSTs have evolved with GSH and are abundant throughout most life forms [76]. Traditional chemotherapeutics are cytotoxins that target dividing cells. The therapeutic index is compromised because normal tissues, such as bone marrow, the gastrointestinal mucosa and hair follicles receive exposure equivalent to the tumor. An attractive treatment approach is provided by prodrug therapy. Prodrugs are rationally designed inactive agents that are converted to cytotoxins preferentially and specifically in target tissues by enzymes that are specifically elevated in the tumor. This strategy allows for an increased delivery of active agent to the tumor tissue while minimizing the toxicity toward normal tissues [77, 78]. ITC metabolizing genes such as GSTs play a significant role in determining the detoxifying ability of an organism. Most have attempted to exploit the capacity of GSTs to catalyse GSH conjugation to electrophilic intermediates and subsequent metabolism for activation [79]. Hence established agents as well as novel agents can be synthesized as inactive compounds via GSH conjugation through a sulfone linkage.

1.11 Isothiocyanate compounds appears promising agent against Cancer

The discovery was made using a “gene chip” that allows researchers to monitor the complex interactions of thousands of proteins on a whole genome rather than one at time. Using gene chip technology, researchers at various universities have identified the blueprint of genes and enzymes in the body that enable sulforaphane, a compound found in cruciferous vegetables to prevent cancer and remove toxins from cells [80]. Many scientists are working to include ITCs as a adjuvant in cancer chemotherapy. Both compounds such as SFN with various chemotherapeutics like 5 fluorouracil, Paclitaxel etc potentiates the body’s response against the therapy [81].
1.12 Molecular Docking

Bioinformatics is seen as an emerging field with the potential to significantly improve how drugs are found, brought to the clinical trials and eventually released to the marketplace. The Protein-Ligand interaction plays a significant role in structural based drug designing. They are essential components of all biological processes such as oncogenesis [82]. This aspect is utilised in insilico analysis of various phytomolecules on cancer research. Computer – Aided Drug Design (CADD) is a specialized discipline that uses computational methods to simulate drug – receptor interactions. CADD methods are heavily dependent on bioinformatics tools, applications and databases. Computational Biology has the potential not only of speeding up the drug discovery process thus reducing the costs but also of changing the way drugs are designed. Rational Drug Design (RDD) helps to facilitate and speedup the drug designing process which involves variety of methods to identify novel compounds. One such method is the docking of the drug molecule with the receptor (target). The site of drug action, which is ultimately responsible for the pharmaceutical effect, is a receptor [83]. Docking is the process by which two molecules fit together in 3D space. Docking allows the scientist to virtually screen a database of compounds and predict the strongest binders based on various scoring functions.