1 (A). INTRODUCTION

Human beings have relied on natural products as a resource of drugs for thousands of years. Plant-based drugs have formed the basis of traditional medicine systems that have been used for centuries in many countries including India. Today plant-based drugs continue to play an essential role in health care. It has been estimated by the World Health Organization (WHO) that 80% of the population of the world rely mainly on traditional medicines for their primary health care. In addition, plants have a long history of use in the treatment of cancer. It is significant that over 60% of currently used anticancer agents are derived in one way or another from natural sources, including plants, marine organisms and micro-organisms (Kaur et al., 2011).

Cancer is a growing health problem around the world. World Health Organization reported more than 10 million cases of cancer per year worldwide. Although cancer may be experienced by any age group, incidence increases with time, suggesting in many cases there is a prolonged period from the time of initiation to the time of invasive and metastatic cancer. Accordingly, numerous opportunities for intervention are apparent, either through primary prevention at early stages or through therapeutic interventions during later stages of carcinogenesis.

The conventional radiotherapy and chemotherapy with synthetic drugs evoke severe side effects including severe immunosuppression and in majority of cases patient may recover cancer but ultimately die due to infectious diseases and organ failure. Thus from therapeutic point of view, the best strategy is “induce apoptosis in the neoplastic cell line without affecting the normal cells of the body”. In this context, dietary phytochemicals are a potential alternative source of safer chemicals
which are not only anticancerous but are also antioxidants, antidiabetic, antimitogenic and of other physiological benefits. Edible phytochemicals are inexpensive, effective and are readily applicable, acceptable and accessible approach to cancer control and management (Barh, 2008).

*Amorphophallus campanulatus* (Roxb.) Blume (Family: Araceae), commonly known as elephant foot yam, is a tuber crop of south East Asian origin. *A. campanulatus* tubers are traditionally used in inflammations, colic, piles, hemorrhoids, liver diseases and abdominal tumors (Nair, 1993). It is largely cultivated throughout the plains of India for using its corm (bulb) as food (Das et al., 2009). Food plants and culinary herbs and spices are known to contain myriad phytochemicals with medicinal properties (Aggarwal et al., 2004). Many of these phytochemicals act as antioxidants, preventing cellular destruction and abnormalities. Furthermore, the dietary supplementation of phytochemicals augment the cellular antioxidant defense mechanisms and thereby helps to ease the oxidative stress, which have been implicated in the etiology of a wide array of human diseases including cancer. Literature survey reveals that no authentic work has been done so far on antioxidant and anticancer activities of *A. campanulatus* tuber, although the ethnic people use it widely to cure different types of diseases. Thus, the main objective of the present investigation was to evaluate the *in vitro* and *in vivo* antioxidant and anticancer properties of *A. campanulatus* tuber and to identify the phytochemical constituents responsible for its medicinal properties.
1 (B). REVIEW OF LITERATURE

According to the World Health Organization (WHO) cancer is one of the leading causes of death worldwide, which accounted for 7.6 million deaths (around 13%) of the world’s population in 2008. They have furthermore estimated that the worldwide deaths are likely to rise to over 13.1 million in 2030. According to the International Agency for Research on Cancer, one in eight deaths worldwide is due to cancer and it is estimated to be the second leading cause of death in economically developed countries, following heart diseases; and the third leading cause of death in developing countries, following heart diseases and diarrheal diseases (Garcia et al., 2007). Cancer is an abnormal growth of cells caused by multiple changes in gene expression leading to dysregulated balance of cell proliferation and cell death and ultimately evolving into a population of cells that can invade tissues and metastasize to distant sites, causing significant morbidity and, if untreated, death of the host (Ruddon, 2007). Cancers of lung, prostate and stomach are most commonly diagnosed among men worldwide, while breast, cervix uteri, and lung cancers are more prevalent among women (Boyle et al., 2008).

Cancer is caused by both external factors (tobacco, chemicals, radiation, and infectious organisms) and internal factors (inherited mutations, hormones, immune conditions, and mutations that occur from metabolism). These causal factors may act together or in sequence to initiate or promote carcinogenesis. The development of most cancers requires multiple steps that occur over many years (American Cancer Society, 2011b). Studies have shown that the generation of reactive oxygen species may be involved in various carcinogenic processes. Cancer cells can be subject to increased and persistent oxidative stress due to elevated levels of intracellular ROS
generation. Reducing oxidative stress can therefore suppress the proliferation of tumor cells and enhance apoptosis (Sun et al., 2004). One of the plausible ways to prevent reactive oxygen species mediated cellular injury is to augment or fortify endogenous defense capacity against oxidative stress through dietary or pharmacological intake of antioxidants (Fang et al., 2002).

1B.1. Oxidative Stress and Antioxidant Defense

Oxidative stress induced by free radicals is believed to be a primary factor in various degenerative diseases such as cancer, cardiovascular diseases including atherosclerosis and stroke, neurological disorders, renal disorders, liver disorders, hypertension, rheumatoid arthritis, acute respiratory distress syndrome, autoimmune deficiency diseases, inflammation, degenerative disorders associated with aging, diabetes mellitus, cataracts, glomerulonephritis, lupus erythematos, gastric ulcers, hemochromatosis, preeclampsia, Alzheimer’s, Parkinson’s, and Huntington’s diseases (Carocho and Ferreira, 2013). Oxidative stress is a disturbance in the oxidant-antioxidant balance leading to potential cellular damage. Most cells can tolerate a mild degree of oxidative stress, because they have sufficient antioxidant defense capacity and repair systems, which recognize and remove molecules damaged by oxidation. The balance between the production and neutralization of reactive oxygen species (ROS) by antioxidants is very delicate, and if this balance tends to the over production of ROS, the cells start to suffer the consequences of oxidative stress (Wiernsperger, 2003).
Reactive species are unstable and highly reactive structures. They may be free radicals which possess an unpaired electron in their outer orbit and are capable of independent existence. Their half-lives vary from a few nanoseconds for the most reactive compounds to seconds and hours for rather stable radicals. They trigger chain reactions resulting in the oxidation of macromolecules in order to reach a steady state (Veskoukis et al., 2012). They are divided into four main categories based on their central atom, specifically reactive oxygen species (ROS), reactive nitrogen species (RNS), reactive sulfur species, and reactive chloride species.
derived from oxygen, nitrogen, sulfur, and chloride, respectively (Halliwell and Gutteridge, 2007).

Reactive oxygen and nitrogen species are physiologically produced during metabolic processes and especially during electron transport chain reactions (Di Meo and Venditti, 2001). Another internal source of reactive species is peroxisomes, small membrane-enclosed organelles containing enzymes important for oxidation reactions. Furthermore, the enzymes of the P450 complex generate reactive species during the detoxification of xenobiotics, such as drugs. There are also external sources of reactive species related to UV radiation, air pollution, smoking, alcohol consumption and exercise (Halliwell and Gutteridge, 2007).

ROS is the main category of reactive species produced from molecular oxygen (O$_2$) with partial chemical reductions (Figure 1.2 and 1.3). One of the most abundant reactive species is superoxide anion (O$_2^-$), which is generated by O$_2$ following reduction by one electron. When O$_2$ is reduced by two and three electrons, hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (OH) are produced, respectively. Finally, H$_2$O is produced after the full reduction of O$_2$ by four electrons (Simic, 1988). The most harmful reactive species is OH, which is generated by Fenton and Haber–Weiss reactions in the presence of a transition metal, usually iron (Fe) or copper (Cu). Hydrogen peroxide has a relatively long half-life and can be transferred through membrane transport far from the place of its generation. Hydrogen peroxide is not a free radical, but it may have detrimental effects on DNA, proteins, and lipids when its concentration reaches 10 µM (Halliwell and Gutteridge, 2007).
Nitric oxide radical (NO\(_\cdot\)) is the most common RNS. It is derived by the amino acid L-arginine and is highly reactive. It reacts with \(O_2^-\), generating peroxynitrite anion (ONOO\(^-\)), which is converted to peroxynitrous acid and finally to harmful OH\(^-\) and nitrite anion (NO\(_2^-\)) (Halliwell, 2001).

Figure 1.2. Generation of different ROS by energy transfer or sequential univalent reduction of ground-state triplet oxygen

(Adapted from Burton et al., 2011)

Figure 1.3. The principal reactive oxygen species, their potential origins and detoxification pathways.

(NADPH - nicotinamide adenine dinucleotide phosphate).
ROS are potential carcinogens because of their roles in mutagenesis, tumor promotion, and progression (Dröge, 2003). If not regulated properly, the excess ROS can damage lipids, protein or DNA, inhibiting normal function (Perry et al., 2000).

Proteins are one of the major targets of reactive species which induce the formation of carbonyl groups (aldehydes and ketones) in those amino acids that are susceptible to oxidation, such as histidine, arginine, lysine, and proline. The carbonyl groups are not metabolized in proteasomes and lysosomes, but are accumulated (Levine, 2002). Furthermore, the thiol groups (−SH) present in protein molecules are oxidized in thiol radicals (RS). Protein oxidation leads to conformational changes which result in the modification or loss of protein function (Halliwell and Gutteridge, 2007).

Apart from proteins, lipids are vulnerable in reactive species-induced oxidative damage (Halliwell and Chirico, 1993). Polyunsaturated fatty acids (PUFA) are abundant in cellular membranes and are susceptible to oxidation, leading to lipid peroxidation chain reactions (Alessio, 1993). Lipid peroxidation increases the permeability of cellular membranes, resulting in cell death.

Reactive species also affect DNA by causing chain breaks and damaging its repair mechanism (Jenkins, 1988). DNA, and especially guanine, oxidation results in the production of 8-hydroxy-2′-deoxyguanosine. This by-product, if not repaired, induces DNA mutations that may cause aging and carcinogenesis (Radak et al., 1999) (Figure 1.4).

In addition, excessive production of reactive species has been implicated in immune system dysfunction (Schneider and Tiidus, 2007), muscle damage
(Nikolaidis et al., 2007a, Nikolaidis et al., 2007b) and fatigue (Betters et al., 2004). Choi and Ou (2006) also reported that, ROS induced alterations in different signaling pathways may modulate gene expression, cell adhesion, cell metabolism, cell cycle and cell death. These events may induce oxidative DNA damage, which in turn increases chromosomal aberrations associated with cell transformation.

(Agarwal et al., 2005)

Figure 1.4. Mechanisms of oxidative stress-induced cell damage
1B.1.2. Endogenous antioxidants

Antioxidants are substances that delay or prevent the oxidation of cellular oxidizable substrates (Halliwell and Gutteridge, 2007). Antioxidants can be divided into categories according to specific characteristics. They include enzymes such as superoxide dismutase (SOD), catalase, glutathione reductase (GR), and glutathione peroxidase (GPx) and non-enzymatic metabolites such as glutathione, vitamins and polyphenols. Regarding their origin, various antioxidants such as glutathione, catalase and SOD can be synthesized in vivo, whereas others, namely, polyphenols and β-carotene, are obtained from food. Based on their physical properties, antioxidants can be divided into water soluble antioxidants such as glutathione and polyphenols or lipid-soluble antioxidants such as vitamins A and E and lipoic acid (Veskoukis et al., 2012).

The SOD enzyme catalyzes the dismutation of $O_2^-$ to $H_2O_2$. It exists in mitochondrial form (MnSOD) and in cytoplasmic form (Cu/ZnSOD) that is primarily found in muscle cells (Das et al., 1997). Catalase is present in almost every kind of cell, but its concentration is higher in the erythrocytes and liver (Masters et al., 1986). Its subcellular localization is in peroxisomes, in mitochondria, and in the nucleus. It catalyzes the conversion of $H_2O_2$, which is produced by SOD to $H_2O$ and $O_2$. The antioxidant activity of catalase is of great significance as it prevents the conversion of $H_2O_2$ to the very harmful $OH$ (Cutler, 1984). GPx, which requires selenium as a cofactor, is present in the cytoplasm and mitochondria and is an alternative route of $H_2O_2$ degradation. Specifically, $H_2O_2$ is converted to $H_2O$ and $O_2$ and oxidizes GSH (reduced form of glutathione) to GSSG (oxidized form of glutathione).
Glutathione is considered one of the most important antioxidant metabolites and is the first line of defense against reactive species. At rest, glutathione is usually present in the reduced state. GSH is a tripeptide consisting of glutamic acid, cysteine, and glycine. It is the most abundant low-molecular-weight thiol-containing compound in biological fluids and tissues of mammals. In eukaryotic cells, 90% of the intracellular GSH pool resides in the cytoplasm, and the remaining 10% is found in the mitochondria, endoplasmic reticulum, and the nucleus. However, the biosynthesis of GSH appears to occur exclusively in the cytoplasm. (Barycki, 2007). GSH possesses potent antioxidant properties, maintaining the intracellular redox homeostasis due to the thiol group of cysteine which serves as a substrate of GPx. Glutathione is important for the regeneration of antioxidant vitamins E and C and contributes to xenobiotic detoxification (May et al., 1996; Halliwell and Gutteridge, 2007). In physiological conditions, GSH is in a dynamic equilibrium with GSSG. However, in the context of oxidative stress, GSH works with GPx to efficiently remove intracellular \( \text{H}_2\text{O}_2 \) (Figure 1.5). This process protects biomolecules from oxidative modifications, and GSH is converted to GSSG. GR reduces GSSG to GSH using NADPH as an electron donor, thus replenishing the GSH pool. (Barycki, 2007).
Figure 1.5. Role of antioxidants in prevention of diseases caused by free radicals

Generation of ROS and reactive nitrogen species (RNS) is initiated by respiratory burst, which is set off by various physiological and environmental factors. The fabrication of an assortment of ROS and RNS from the molecular O\textsubscript{2} and L-arginine, respectively, carried on by different enzymes like MPO (myloperoxidase), NADPH oxidase, SOD (superoxide dismutase) and NOS (nitric oxide synthase) leads to diverse cellular phenomena, namely, damage of DNA-repair proteins and caspases, lipid peroxidation, DNA damage followed by mutation and NF-κB activation. All these phenomena give rise to wide range of diseases. Antioxidants inhibits the generations of the free radicals by scavenging both the mother and the daughter products and also by inducing the increase of SOD, CAT, GST and GSH, resulting the obstruction of various disease formation.
1B.1.3. Exogenous antioxidants

The *in vivo* system of free radical’s detoxification may not be capable to neutralising all the free radicals produced in the body as well as those derived from the environment, and there is therefore a need for an external source of antioxidants to neutralise the free radical load in the body. A large number of antioxidants, both nutritive and nonnutritive, occur in foods. Besides β-carotene, vitamin C and vitamin E (which are nutrients), a number of carotenoids, phenols and flavonoids also occur naturally in foods and can act as antioxidants. β-carotene is an excellent scavenger of singlet oxygen. Vitamin C interacts directly with radicals like $\mathrm{O}_2^-$ and $\mathrm{OH}^-$ (hydroxyl). Vitamin C and vitamin E prevent formation of nitrosamine, which is carcinogenic. Vitamin E also protects selenium against reduction and protects polyunsaturated fatty acids (PUFA) in the membrane against oxidative damage (Rao, 2003).

Polyphenolic compounds constitute a crucial category of antioxidant metabolites. They are plant secondary metabolites which have at least one aromatic ring in their molecule and usually exist in the form of glycosides. More than 8,000 different polyphenolic compounds have been described. They are subdivided into nonflavonoids (e.g., hydrobenzoic acids, hydroxycinnamic acids, and stilbenes) and flavonoids (e.g., flavonols, flavanals, isoflavones, and anthocyanins). Flavonoids are composed of more than 4,000 different species that have two aromatic benzene rings linked through three carbons forming an oxygenated heterocycle. Plant polyphenols possess antioxidant properties as they inactivate free radicals by offering a hydrogen atom and an electron. They are converted into relatively harmless free radicals, which may react with other free radicals and inactivate them. Furthermore, they act
as metal chelators, mainly Fe and Cu, thus not allowing them to initiate Fenton and Haber–Weiss reactions (Nijveldt et al., 2001). In addition, they inhibit the activity of enzymes related to reactive species production, such as xanthine oxidase, cyclooxygenase, and lipoxygenase. It has been demonstrated that consumption of polyphenols results in the prevention of cardiovascular diseases and cancer. Polyphenolic compounds, especially flavonoids are abundantly present in vegetables, legumes fruits, oil and wine. (Rice-Evans et al., 1996; Hertog et al., 1997; Cotelle, 2001).

### Table 1.1. List of selected natural substances and their major active compounds with proven antioxidant activity

<table>
<thead>
<tr>
<th>Natural sources</th>
<th>Major active compounds</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fruits</strong></td>
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<td></td>
</tr>
<tr>
<td><em>Punica granatum</em> (Pomegranate)</td>
<td>Ellagic acid, Ellagitannins, Anthocyanins, Punicic acid, Flavonoids, Anthocyanidins, Flavones</td>
<td>Karasu et al., 2012</td>
</tr>
<tr>
<td><em>Vitis vinifera</em> (Grapes)</td>
<td>Resveratrol, Anthocyanins, Catechins</td>
<td>Bunea et al., 2012</td>
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<tr>
<td><em>Psidium guajava</em> (Common Guava)</td>
<td>Carotenoids, Polyphenols</td>
<td>Mai et al., 2007</td>
</tr>
<tr>
<td><em>Malus domestica</em> (Apple)</td>
<td>Quercetin, Catechin, Phloridzin, Chlorogenic acid</td>
<td>Boyer and Liu, 2004</td>
</tr>
<tr>
<td><em>Rubus idaeus</em> (Raspberry)</td>
<td>Ellagic acid, Phenolic compounds, Vitamin C</td>
<td>Liu et al., 2002</td>
</tr>
</tbody>
</table>
Table 1.1. (Cont.) List of selected natural substances and their major active compounds with proven antioxidant activity

<table>
<thead>
<tr>
<th>Natural sources</th>
<th>Major active compounds</th>
<th>References</th>
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</thead>
<tbody>
<tr>
<td><strong>Vegetables</strong></td>
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<tr>
<td><em>Solanum lycopersicum</em></td>
<td>Lycopene</td>
<td>Palozza et al., 2012</td>
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<td>(Tomato)</td>
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<tr>
<td><em>Allium sativum</em> (Garlic)</td>
<td>Allicin, Ajoene, Selenium, Quercetin</td>
<td>Meriga et al., 2012</td>
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<tr>
<td><em>Momordica charantia</em></td>
<td>Gallic acid, Gentisic acid, Catechin</td>
<td>Santos et al., 2010; Horaz et al., 2005</td>
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<td>(Bitter melons)</td>
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<tr>
<td><em>Moringa oleifera</em> (Leaf)</td>
<td>Polyphenols, Anthocyanin, Thiocarbamates</td>
<td>Luqman et al., 2012</td>
</tr>
<tr>
<td><em>Solanum tuberosum</em></td>
<td>Phenolic compounds</td>
<td>Kanatt et al., 2005</td>
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<td>(Potato peel)</td>
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<tr>
<td><strong>Cereals</strong></td>
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<tr>
<td><em>Oryza sativa</em> (Rice)</td>
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<td>Chung and Woo, 2001</td>
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<tr>
<td><em>Avena sativa</em> (Oat)</td>
<td>Phenolic acids</td>
<td>Emmons et al., 1999</td>
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<tr>
<td><em>Fagopyrum esculentum</em></td>
<td>Flavonoids</td>
<td>Oomah and Mazza, 1996</td>
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<td>(Buckwheat)</td>
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<td><strong>Herbs and Spices</strong></td>
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<tr>
<td><em>Rosmarinus officinalis</em></td>
<td>Carnosoic acid</td>
<td>Kim et al., 2011</td>
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<td>(Rosemary)</td>
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<tr>
<td><em>Salvia officinalis</em></td>
<td>Carnosol, Carnosic acid, Rosmanol, Apigenin</td>
<td>Walch et al., 2011</td>
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<tr>
<td>(Sage)</td>
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<td><em>Piper nigrum</em> (Black pepper)</td>
<td>Piperine, Arbutin, Magnoflorine</td>
<td>Singh et al., 2008</td>
</tr>
<tr>
<td><em>Thymus zygis</em> (Thyme)</td>
<td>Thymol, Carvacrol, Terpinene</td>
<td>Youdim et al., 2002</td>
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<tr>
<td><em>Syzygiun aromaticum</em></td>
<td>Eugenol, Eugenyl acetate</td>
<td>Lee and Shibamoto, 2001</td>
</tr>
<tr>
<td>(Clove bud)</td>
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<td></td>
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</tbody>
</table>
Table 1.1. (Cont.) List of selected natural substances and their major active compounds with proven antioxidant activity

<table>
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<th>Roots</th>
<th>Major active compounds</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cassia sieberiana</em></td>
<td>Polyphenolic compounds</td>
<td>Nartey et al., 2012</td>
</tr>
<tr>
<td><em>Daucus carota</em> L. (Carrot)</td>
<td>Carotenoids</td>
<td>Mech-Nowak et al., 2012</td>
</tr>
<tr>
<td><em>Zingiber officinale</em></td>
<td>Quercetin, Catechin,</td>
<td>Rahman et al., 2011</td>
</tr>
<tr>
<td>(Ginger root)</td>
<td>Kaempferol</td>
<td></td>
</tr>
<tr>
<td><em>Glycyrrhiza uralensis</em></td>
<td>Glycyrrhizin</td>
<td>Tanaka et al., 2008</td>
</tr>
<tr>
<td>(Licorice root)</td>
<td>Ellagic acid derivatives</td>
<td>Tanaka et al., 2003</td>
</tr>
</tbody>
</table>

1B.2. Role of reactive species in the development of cancer

Reactive species, which mainly include reactive oxygen species (ROS), such as superoxide radical, hydrogen peroxide, singlet oxygen and hydroxyl radical are well known to be cytotoxic and have been implicated in the etiology of a wide array of human diseases, including cancer. Various carcinogens may also partly exert their effect by generating ROS during their metabolism. Oxidative damage to cellular DNA can lead to mutations and may, therefore, play an important role in the initiation and progression of multistage carcinogenesis (Figure 1.6.). Oxidative DNA damage is a major source of the mutation load in living organisms, with more than one hundred oxidative DNA adducts (purine, pyrimidine, and the deoxyribose backbone) having been identified. The estimated frequency of oxidative DNA damage in human cells is 104 lesions/cell/day. Being highly reactive, the hydroxyl radical is the predominant ROS that targets DNA. Hydrogen peroxide, a precursor to
hydroxyl radical, is less reactive and more readily diffusible and thus more likely to be involved in the formation of oxidized bases through Fenton and Haber-Weiss reactions. ROS induced DNA damage can result in single or double strand breakage, base modifications, deoxyribose modification, and DNA cross-linking. Cell death, DNA mutation, replication errors, and genomic instability can occur if the oxidative DNA damage is not repaired prior to DNA replication (Waris and Ahsan, 2006; Klaunig et al., 2010).

Studies show that although all the four bases are modified by ROS, mutations are usually related to modification of GC base pairs, while that of AT base pair rarely leads to mutations. The majority of mutations induced by ROS appear to involve modification of guanine, causing G→T transversions. These mutations are usually base pair substitutions, whereas base deletions and insertions are less frequent. If it relates to critical genes such as oncogenes or tumor suppressor genes, initiation/progression can result (Ames et al., 1993; Retèl et al., 1993).

The most extensively studied and most abundant oxidative DNA lesion produced is 8-hydroxydeoxyguanosine (8-OHdG), which is mutagenic in bacterial and mammalian cells (Cheng et al. 1992). Numerous studies have demonstrated that 8-OHdG levels are elevated in various human cancers, including esophageal squamous cell carcinoma and hepatocellular carcinoma (Diakowska et al. 2007; Tanaka et al. 2008), and in animal models of tumors (Gottschling et al. 2001; Muguruma et al. 2007). 8-OHdG in its stable conformation can pair with both cytosine and adenine. If the A:G mismatch is not repaired, a G:C to T:A transversion will occur, commonly found in mutated oncogenes and tumor suppressor genes (Cheng et al. 1992; Grollman and Moriya 1993). During DNA replication, ROS can
also react with dGTP in the nucleotide pool to form OH8dG. Therefore, in addition to the G:C to T:A caused by 8-OHdG in the DNA template, it is postulated that during DNA replication, OH8dG in the nucleotide pool can be incorporated into DNA opposite dC or dA on the template strand, resulting in A:T to C:G transversions (Cheng et al. 1992; Klaunig et al., 2010).

The changes in DNA such as base modification, rearrangement of DNA sequence, miscoding of DNA lesion, gene duplication and the activation of oncogenes may be involved in the initiation of various cancers. Mutation studies have suggested that chronic oxidative stress, particularly from chronic inflammation, is associated with carcinogenesis. For example, ulcerative colitis has long been linked with high incidence of colorectal cancer; and chronic gastritis, such as from infection with Helicobacter pylori, has been associated with a high incidence of gastric cancer. Likewise, the carcinoma of hepatic cells is often associated with chronic infection by hepatitis B or C viruses or ingestion of aflatoxins. Oxidative stress induced by these viruses represents one of the intracellular events that cause the genesis of hepatocellular carcinoma. G→T transition has been shown to be one of the more common types of mutation produced by aflatoxin lesion and ROS damage to DNA. ROS can promote many aspects of tumor development and progression, which can be classified into the following biological processes: (a) cellular proliferation, (b) evasion of apoptosis or anoikis, (c) tissue invasion and metastasis, and (d) angiogenesis. Elevated levels of ROS and down regulation of ROS scavengers and antioxidant enzymes are associated with various cancers. Indeed, ROS can act at several steps in multistage carcinogenesis (Waris and Ahsan, 2006; Klaunig et al., 2010; Sosa et al., 2013).
Figure 1.6. Pathways illustrating the sources of reactive oxygen species and its role in the development of cancer

1B.3. Cancer – an overview

Cancer is a general term applied for a series of malignant diseases that may affect different parts of body. These diseases are characterized by a rapid and uncontrolled formation of abnormal cells, which may mass together to form a growth or tumor, or proliferate throughout the body, initiating abnormal growth at other sites. If the process is not arrested, it may progress until it causes the death of the organism. The majority of deaths (about 90%) associate with cancer are due to the metastasis of the original tumor cells to sites distant from the initial or primary tumor. Cancers are capable of spreading throughout the body by two mechanisms: invasion and metastasis. Invasion refers to the direct migration and penetration by
cancer cells into neighboring tissues. Metastasis refers to the ability of cancer cells to penetrate into lymphatic and blood vessels, circulate through the bloodstream and then invade normal tissues elsewhere in the body. The capability for invasion and metastasis enables cancer cells to escape the primary tumor mass and colonize new terrain in the body where, at least initially, nutrients and space are not limiting (Brooks et al., 2010).

The main forms of treatment for cancer in humans are surgery, radiation and drugs (cancer chemotherapeutic agents). Since chemotherapy, radiation, etc. cause severe toxicity, recent pharmacological researches revolve around the urgency to evolve suitable chemotherapeutic agents for the treatment of tumors (benign and malignant) without having toxic effects. In recent years, a lot of effort has been applied to the synthesis of potential anticancer drugs. Many hundreds of chemical variants of known class of cancer chemotherapeutic agents have been synthesized but have a more side effects. A successful anticancer drug should kill or incapacitate cancer cells without causing excessive damage to normal cells (Sakarkar and Deshmukh, 2011; Sharma and Govind, 2009).

1B.3.1. Causes of cancer

Modern medicine attributes most cases of cancer to changes in DNA that reduce or eliminate the normal controls over cellular growth, maturation, and programmed cell death. These changes are more likely to occur in people with certain genetic backgrounds and in persons infected by chronic viruses (e.g., viral hepatitis may lead to liver cancer; HIV may lead to lymphoma). The ultimate cause, regardless of genetic propensity or viruses that may influence the risk of the cancer, is often exposure to carcinogenic chemicals (including those found in nature) and/or
to radiation (including natural cosmic and earthly radiation), coupled with a failure of the immune system to eliminate the cancer cells at an early stage in their multiplication. The immunological weakness might arise years after the exposure to chemicals or radiation. Other factors such as tobacco smoking, alcohol consumption, excess use of caffeine and other drugs, sunshine, infections from such oncogenic virus like cervical papilloma viruses, adenoviruses Karposis sarcoma (HSV) or exposure to asbestos. These obviously are implicated as causal agents of mammalian cancers (Sakarkar and Deshmukh, 2011).

Factors involved in the causation of cancer include the following (Cassidy et al., 2002):

(a). Genetic factors

The majority of recognized carcinogens cause genetic mutations. Changes in gene expression in somatic cells, mostly due to mutation, are thought to be the basis for malignant transformation; there may be one or more, rare, dominantly inherited susceptibilities to every type of cancer. The contribution made by these highly penetrant, dominant susceptibilities to the total incidence of cancer has been estimated at 2–5% of fatal cancers. Genetic variation in susceptibility to cancer may also arise because of genetic polymorphism affecting the absorption, transport, metabolic activation, or detoxification of environmental carcinogens.

(c). Alcohol

The main effect of alcohol is a joint effect with tobacco smoking in cancers of the oral cavity, pharynx, larynx, and oesophagus. Alcohol alone is implicated in cirrhosis (liver cancer) and may contribute to some cancers of the breast and large bowel.
(b). Smoking

Tobacco smoking is the largest single avoidable cause of premature death and the most important known carcinogen. Recent cohort studies show that smoking for 30 years or more increases the risk of colon cancer, with about 25% of cases being attributable to smoking. In addition, passive smoking may account for a small proportion of the cancer burden. In men from developed countries, the tobacco burden has been estimated as 32% of all annual incident cases, whereas in those from developing countries, it has been estimated as 19%. In regions where men have smoked for several decades, 30–40% of all cancers are attributable to tobacco. In women from developed countries, 6% of all annual incident cases are accounted for by tobacco, in contrast with 2% in those from developing countries.

(d). Diet

High intake of vegetables and fruits shows a consistent inverse relationship with cancer of the larynx, lung, oesophagus and stomach, and there is weaker evidence that this is the case also for cancer of the mouth and pharynx, pancreas, and cervix. High levels of vegetable consumption are associated with a reduced risk of colon cancer whereas high levels of meat consumption appear to increase the risk of colon cancer. Obesity in adult life is considered to be the main factor in endometrial cancer, probably increases the risk of post-menopausal breast cancer, and is associated with cancer of the kidney. Regular physical activity is consistently associated with a reduced risk of colon cancer.

Low levels of consumption of fruits and vegetables, high levels of meat consumption, obesity and lack of regular physical activity tend to be aspects of a lifestyle more typical of developed than of developing countries. In developing
countries it has been estimated that 33–50% of nasopharyngeal cancer cases could be prevented by avoiding regular consumption of salt fish. While the contamination of foods with aflatoxins increases the risk of hepatocellular cancer. Generalized dietary deficiencies are associated with increased risk of oesophageal cancer in areas of high incidence in developing countries. In randomized controlled trials in Linxian, China, a combination of carotene, tocopherol, and selenium reduced mortality from cancer of all types and mortality from stomach cancer in particular.

(e). Infections

16% of the worldwide incidence of cancer is due to infection. For developed countries, the proportion is 9%, and for developing countries, 21%. Human papilloma virus (HPV) of any type accounts for 82% of cervical cancers in developed countries and 91% in developing countries. The human papilloma viruses occur in 70 different types. The strongest evidence for carcinogenicity is for HPV types 16 and 18. 81% of cases of liver cancer are attributable to chronic infection with hepatitis B or hepatitis C.

Strong evidence supports a causal relationship between chronic infection with the bacterium *Helicobacter pylori* and the development of gastric adenocarcinoma, and there is some evidence for gastric lymphoma. 60% of cases of gastric cancer in developed countries, and 53% in developing countries, may be attributable to *Helicobacter pylori*.

Epstein–Barr virus may account for up to 60% of Hodgkin’s disease in developed countries, and 80% in developing countries. The virus accounts for over 90% of cases of Burkitt’s lymphoma in sub-Saharan Africa, just over 80% in north
Africa and the Middle East, just under 50% in Latin America and the Caribbean, and less than a quarter of cases elsewhere.

Other infections considered to be carcinogenic include:

- *Schistosomiasis haematobium* and bladder cancer (attributable proportion in developing countries 8%, in developed countries 0%).
- Human T-cell lymphotrophic virus and acute T-cell leukaemia/lymphoma (attributable proportion worldwide 1%).
- HIV and Kaposi’s sarcoma.
- HIV and non-Hodgkin’s lymphomas.
- *Opisthorchis viverrini* and *Clonorchis sinensis* and cholangiocarcinoma.

(f). **Environmental factors**

The incidence of many types of cancer varies greatly between geographical areas. There are changes of rates following migration between areas of contrasting incidence, changes in incidence over time, and variation within populations according to socio-economic status. Thus environmental factors appear to have a major role in the aetiology of most types of cancer, accounting for over 80% of human cancer.

(g). **Solar exposure**

The 1996 Harvard Report on Cancer Prevention concluded that over 90% of malignant melanoma is attributable to solar radiation. Malignant melanoma accounted for just over 1% of the world cancer burden in 1985. Uncertainties remain, even though it is widely assumed that exposure to solar radiation also accounts for the great majority of cases of basal cell and squamous cell carcinoma.
Other exposures account for 5% or less of the cancer burden. Occupational exposures have been linked with lung, bladder and haematopoietic malignancies. Breast cancer has consistently been associated with early age at menarche, late age at first birth and late age at menopause with relative risks of the order of 2.0 or less.

Although most types of cancers are more common in urban than in rural areas, few causal links with environmental pollutants have been firmly established. It has been estimated that 1% of lung cancer deaths in the US are attributable to air pollution. While exposure to ionizing radiation at doses of 500–2000 millisievert (mSv) is known to be carcinogenic, exposures of this magnitude are unusual—about 1% of the deaths of the Japanese atomic bomb survivors could be attributed to radiation. The average per capita dose from all sources of ionizing radiation is about 3.4 mSv per year, of which about 88% is from natural sources and the remainder primarily from medical exposures. Extrapolation from data on people exposed to doses of 500 mSv or more suggests that 1–3% of all cancers might be attributable to radiation arising largely from natural sources. No clear association with exposure to extremely low frequency magnetic fields has been established. In addition, some pharmaceutical agents such as immunosuppressive agents, anti-neoplastic drugs, and hormonal preparations are also human carcinogens.

1B.3.2. Molecular Basis of Cancer

Cancer cells are characterized by multiple structural, molecular and behavioral features. According to a recent description, ‘six essential alterations in cell physiology collectively dictate malignant growth: self sufficiency in growth signals, insensitivity to growth-inhibitory (antigrowth) signals, evasion of
programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis and tissue invasion and metastasis’ (Vineis et al, 2010). During development, these processes are regulated by positional cues, cell-cell interactions, and signals from the local microenvironment. The deregulation of the pathways that govern the normal developmental processes of cellular proliferation, apoptosis, differentiation, migration and invasion and even metabolism during pre and postnatal development, contribute to the cancer phenotype. 85% of all human cancers originate from epithelial cells and are termed carcinomas or adenocarcinomas. Cancers arising from cells in the blood, lymph nodes, connective tissue or bone marrow are termed leukemia, lymphoma, sarcoma or myeloma respectively. It could be almost any organ or part of the body where cancers can arise, based on which they are commonly identified and named as lung, colon, breast, cervical, ovarian, prostate etc (DeVita, 2008).

In normal tissues of multicellular organisms the function of the single cell is tightly controlled due to inner and surrounding constraints, to maintain tissue homeostasis and function. During malignant transformation the cancer cell will gradually become more autonomous and develop into an 'asocial citizen' of its tissue, growing in an uncontrolled manner at the expense of the function of the normal tissue. More specifically, the cancer cell has obtained relaxed control of several normal cell regulatory' mechanisms (Grander, 1998). According to the modern understanding of cancer, it is a disease that is primarily associated with genetic and epigenetic alterations by specific mutations in specific key regulatory genes. The alterations in carcinogenesis involve the activation of tumor-promoting oncogenes and the inactivation of growth-inhibiting tumor suppressor genes (Ishii et
al., 2010). Oncogenes, when activated by genetic alterations such as amplification, small mutations or translocations, show a dominant gain of function and encode proteins that are involved in the control of cell proliferation, cell death or both. The functional inactivation of tumor suppressor genes through mutations results in the loss or altered expression of proteins encoded by them and contributes to the neoplastic phenotype (Cleaver, 1994). There are some genes that can be assigned to both categories, acting to promote or inhibit tumorigenesis depending on the timing or context in which they function. For example; Tumor growth factor beta (TGF-β), it is a growth-inhibitory cytokine, but growth inhibition is only one of its many effects including cell proliferation, differentiation, migration, and apoptosis (DeVita, 2008). Mutations can be caused by environmental factors such as radiation, environmental carcinogens, carcinogenic or mutagenic chemicals, such as those present in tobacco, infectious agents etc. Hereditary cancers occur when a person inherits mutations that occurred in the germ line cells of the parents. About 90-95% of human cancers are sporadic while some types of breast and ovarian cancers, hereditary nonpolyposis colorectal cancer, retinoblastoma etc are hereditary (Hanahan and Weinberg, 2000). In addition to these genetic factors, the genesis and growth of tumor cells are also influenced by various epigenetic factors such as gene silencing by DNA hypermethylation, gene activation by hypomethylation, genomic imprinting, histone modifications etc (Feinberg and Tycko, 2004).

1B.3.3. Cancer Prevention and Therapy

It is estimated that more than half of all new cancers and cancer deaths worldwide are potentially preventable. Cancers related to tobacco use, heavy use of alcohol, use of exogenous hormones, obesity and exposure to environmental
carcinogens can be effectively prevented through a combination of education and social policies that discourage unhealthy practices. Certain cancers that are related to infectious agents such as hepatitis B virus (HBV), human immunodeficiency virus (HIV), human papilloma virus (HPV) and Helicobacter pylori could be prevented through known interventions such as vaccines, antibiotics, improved sanitation and education. Some cancers (colorectal and cervix) can be avoided by detection and removal of pre-cancerous lesions through regular screening examinations at an early stage.

Current cancer treatment modalities includes surgery, radiation therapy, chemotherapy and for some cancer types, hormone therapy and immunotherapy. These therapies have gained a considerable clinical success over the past many years and these treatment modalities are life extending for many patients, they are rarely curative for disseminated cancers. For instance, surgery and radiotherapy are quite effective in the treatment of localized tumors, but they usually play a palliative role in the treatment of disseminate diseases (Marchand et al., 1995; Locher et al., 2010).

(a). Surgery

Selective killing or removal of the cancer cells without affecting the rest of the body is the goal of cancer treatment and the therapeutic mainstays of cancer remain as surgery, radiotherapy and chemotherapy. Surgical interventions can be used for diagnosis, treatment of precancerous lesions or for removal of normal organs which are at an elevated risk of developing cancers. As long as the growth of the tumor remains localized, it can usually be treated and cured by surgical removal of the tumor and surrounding tissue, but the tendency of cancers to invade adjacent
tissue or to spread to distant sites by metastasis makes complete surgical excision of the cancer usually impossible (Ozgediz et al., 2008).

(b). Radiotherapy

Radiotherapy involves the use of ionizing radiation to kill cancer cells and shrink tumors and also developing as a clinically essential part of cancer therapy for the majority of solid malignant neoplasm including brain tumors. It has been proved to be a fundamental tool available in the battlefield against cancer, offering a clear survival benefit in most cases. However, numerous studies have associated tumor irradiation with enhanced aggressive phenotype of the remaining cancer cells. A cell population manages to survive after the exposure, because it either receives sub lethal doses or it successfully utilizes the repair mechanisms. The biology of irradiated cells is altered leading to up-regulation of genes that favor cell survival, invasion and angiogenesis (Kargiotis et al., 2010).

(c). Chemotherapy

Chemotherapy remains as the treatment modality of choice for most advanced cancers or as an adjunct to other treatment modalities like surgery and radiotherapy, but severe toxic effects toward normal tissues also limit its use (Baxevanis et al., 2009). It involves the use of cytotoxic agents which are transported by the bloodstream to different parts of the body to destroy cancer cells. The first uses of chemotherapy to control cancer were reported in the 1940s, and in the decades since, treatment of patients with broadly toxic chemicals have represented a mainstay of medical oncology, in spite of the frequent severe side effects associated with such treatments. Many chemotherapeutic agents used to treat
malignant diseases damage lymphocytes and consequently suppress cell-mediated immunity (Bagyukova et al., 2010).

(d). Immunotherapy

Historically, the first successful immunotherapy to treat cancer involved the use of toxins from *Streptococcus erysipelatis* and *Bacillus prodigious* by William Coley in the 1890's (Coley, 1991). Toll-like receptor agonists have been shown to boost immune responses toward tumors. Also, a wide array of cell based immune therapies utilizing T cells, NK cells and DC cells have been established. Furthermore, a rapidly expanding repertoire of monoclonal antibodies is being developed to treat tumors and many of the available antibodies have demonstrated impressive clinical responses (Borghaei et al., 2009). More recently, the development of vaccines to tumor-causing hepatitis B virus and papilloma virus are contributing significantly to preventing cancer in a large portion of the human population (Blumberg, 1997; Rogers et al., 2008). Many groups have attempted to activate a patient's own (autologous) tumor-reactive T cells and tumor-infiltrating lymphocytes (TILs) by culturing them with IL-2 and other cytokines and re-injecting these cells to treat cancer. Such methods are designed to increase the number of reactive T cells and provide long-term immune protection with minimal autoimmune responsiveness (Morgan et al, 2006; Oble et al., 2009). Some success has also been observed using genetically engineered T cells in which an antigen specific antigen receptor has been introduced by retrovirus. DC cells have been widely studied as potential vectors that can be loaded with peptide antigens for presentation to T cells and stimulate adaptive immunity (Disis et al., 2009).
(e). Complementary and Alternative Medicine

Chemotherapy, being a major treatment modality used for the control of advanced stages of malignancies and as a prophylactic against possible metastasis, exhibits severe toxicity on normal tissues. Because of the serious side effects of chemotherapy and radiation therapy, many cancer patients seek alternative and/or complementary methods of treatment (Pandey and Madhuri, 2006; Madhuri and Pandey, 2009). Recently, complementary and alternative medicine (CAM) is becoming a popular treatment for various cancers. Among the CAMs, herbal medicine is one of the methods used in cancer therapy (Cassileth, 1999). CAM has been defined as a group of diverse medical and healthcare systems, practices and products that are not presently considered to be part of conventional medicine. In the last three decades, the use of CAM has increased in popularity in both the worldwide general population and in patients with cancer (Yildirim, 2010). The goals of CAM are to increase the efficacy of conventional cancer treatment programs, reduce symptoms, and improve quality of life for patients with cancer (Levine, 2010).

1B.3.4. Natural products as anti-cancer agents

Natural products have afforded a rich source of compounds that have found many applications in the fields of medicine, pharmacy and biology and are being used to treat a wide variety of clinical conditions, with relatively little knowledge of their modes of action. Within the sphere of cancer, a number of important new commercialized drugs have been obtained from natural sources, by structural modification of natural compounds, or by the synthesis of new compounds and by designing as natural compound as model (Gordaliza, 2007). Currently, numerous scientific studies support herbal medicine as a potent anticancer drug. However,
herbal remedies are yet to be integrated into mainstream medicine mainly due to lack of experimental and clinical studies on their safety, efficacy, quality control and pharmacological mechanisms. Careful *in vitro* and *in vivo* studies will be essential and necessary to evaluate their efficacy and safety before clinical trials can be contemplated (Buchanan et al., 2005; Kwon et al., 2009).

### Table 1.2. List of selected plants, vegetables and fruits with proven anti-cancer activity

<table>
<thead>
<tr>
<th>Natural sources</th>
<th>Major anti-cancer compounds</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Medicinal plants</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aloe barbadensis</em> (Aloe vera)</td>
<td>Aloe-emodin, Emodin</td>
<td>Chiu et al., 2009</td>
</tr>
<tr>
<td><em>Berberis amurensis</em> (Berberis)</td>
<td>Berbamine,</td>
<td>Wei et al., 2009</td>
</tr>
<tr>
<td><em>Taxus brevifolia</em> (Pacific Yew)</td>
<td>Paclitaxel</td>
<td>Kingston., 2007</td>
</tr>
<tr>
<td><em>Aglaia foveolata</em></td>
<td>Silvestrol</td>
<td>Kim et al., 2007</td>
</tr>
<tr>
<td><em>Catharanthus roseus</em> (Vinca)</td>
<td>Vinblastine, Vincristine , Alstonine, Ajmalicine.</td>
<td>Van Der Heijden et al., 2004</td>
</tr>
<tr>
<td><strong>Vegetables</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Allium sativum</em> (Garlic)</td>
<td>Diallyl sulfide, Diallyl disulfide , Diallyl trisulfide</td>
<td>Choi and Park, 2012</td>
</tr>
<tr>
<td><em>Glycine max</em> (Soyabeans)</td>
<td>Genistein</td>
<td>Li et al., 2012</td>
</tr>
<tr>
<td><em>Brassica oleracea</em> (Cabbage)</td>
<td>Indole-3-carbinol</td>
<td>Wu et al., 2010</td>
</tr>
<tr>
<td><em>Solanum lycopersicum</em> (Tomato)</td>
<td>Lycopene</td>
<td>Tang et al., 2009</td>
</tr>
<tr>
<td><em>Vicia faba</em> (Fava bean)</td>
<td>Diadzein, Genistein</td>
<td>Kaufman et al., 1997</td>
</tr>
</tbody>
</table>
Table 1.2. (Cont.) List of selected plants, vegetables and fruits with proven anti-cancer activity

<table>
<thead>
<tr>
<th>Natural sources</th>
<th>Major anti-cancer compounds</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fruits</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Citrus reticulate</em> (Mandarin orange)</td>
<td>Tangeretin, Nobiletin, Hesperetin, hesperidin, Naringenin, Naringin</td>
<td>Meiyanto et al., 2012</td>
</tr>
<tr>
<td><em>Vitis vinifera</em> (Grapes)</td>
<td>Resveratrol, Piceatannol</td>
<td>Kita et al., 2012</td>
</tr>
<tr>
<td><em>Citrullus lanatus</em> (Watermelon)</td>
<td>Lycopene</td>
<td>Ilic et al., 2011</td>
</tr>
<tr>
<td><em>Ananas comosus</em> (Pine apple)</td>
<td>Bromelain</td>
<td>Hale et al., 2010</td>
</tr>
<tr>
<td><em>Malus domestica</em> (Apple)</td>
<td>Ursolic acid</td>
<td>Gayathri et al., 2009</td>
</tr>
<tr>
<td><strong>Roots/rhizome</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Curcuma longa</em> (Turmeric)</td>
<td>Curcumin, Tumerone</td>
<td>Manikandan et al., 2012</td>
</tr>
<tr>
<td><em>Zingiber officinale</em> (Ginger)</td>
<td>Curcumin, gingerenone A, Gingeols, 6-shogaol, 10-gingerol, enone-diarylheptanoid</td>
<td>Peng et al., 2012</td>
</tr>
<tr>
<td><em>Wikstroemia indica</em> (Indian stringbush)</td>
<td>Daphnoretin</td>
<td>Lu et al., 2011</td>
</tr>
<tr>
<td><em>Typhonium flagelliforme</em> (Rodent tuber)</td>
<td>Pheophorbide-a, Pheophorbide-a', Pyropheophorbide-a, Methyl pyropheophorbide-a</td>
<td>Lai et al., 2010</td>
</tr>
<tr>
<td><em>Rheum rhabarbarum</em> (Rhubarb)</td>
<td>Emodin</td>
<td>Huang et al., 2009</td>
</tr>
</tbody>
</table>

1B.3.5. Diet and Cancer Prevention

It has been estimated that more than two-third of human cancers could be prevented through appropriate lifestyle modification. Doll and Peto (1981) have reported that 10–70% (average 35%) of human cancer mortality is attributable to
diet. Their observations, which are based on statistical and epidemiological data, mainly concerned dietary factors that increase the risk of cancer. Wide arrays of substances derived from the diet have been found to stimulate the development, growth and spread of tumors in experimental animals, and to transform normal cells into malignant ones. These are regarded as suspected human carcinogens.

A number of studies support the hypothesis that a diet high in fat and low in fibers and carbohydrates increases the risk of colorectal and others types of cancer (Sandler et al., 1993; Doll and Peto, 1981). A considerable amount of evidence from both animal experiments and human epidemiological studies suggests that high-fat and high-caloric diets increase the risk of cancer (Weindruch et al., 1991), but there is also accumulating evidence from population as well as laboratory studies to support an inverse relationship between regular consumption of fruits and vegetables and the risk of cancers. Vegetables and fruits are excellent sources of cancer preventive substances. Many population-based studies have highlighted the ability of macronutrients and micronutrients in vegetables and fruit to reduce the risk of cancer. Recently, attention has been focused on phytochemicals - non-nutritive components in the plant based diet that possess cancer preventive properties.

There are a number of potentially cancer preventive ingredients in foods that can act at various stages in tumor initiation and promotion (Figure 1.7) (Caragay, 1992). β-carotene, vitamin A and synthetic retinoids have attracted a lot of attention as possible chemopreventive agents (Omenn et al., 1994). There is also experimental evidence supporting the role of vitamin D in preventing colorectal cancer. Vitamin D has been reported to regulate cellular proliferation and differentiation and to inhibit angiogenesis (Feskanich et al., 2004). Similarly, The National Cancer
Institute (NCI) has determined that more than 35 plant based foods and 1000 individual phytochemicals possess cancer-preventive activity in cell culture and animal models. Well-studied food sources and representative phytochemicals include garlic (diallyl sulfide), soybeans (genistein), turmeric (curcumin), tomatoes (lycopene), grapes (resveratrol), green tea (epigallocatechin-3-gallate [EGCG]), and cruciferous vegetables (such as broccoli, cabbage, and Brussels sprouts; indole-3-carbinol, sulforaphane) (Surh, 2003). However, the repertoire of chemopreventive phytochemicals is vast, and foods, dietary supplements, and traditional herbal medicines with previously undocumented anticancer activities are continually being identified.
Figure 1.7. Dietary phytochemicals that block or suppress multistage carcinogenesis.

Phytochemicals can interfere with different steps of carcinogenesis. Some chemopreventive phytochemicals inhibit metabolic activation of the procarcinogens to their ultimate electrophilic species, or their subsequent interaction with DNA. These agents therefore block tumor initiation (blocking agents). Other phytochemicals suppress the later steps (promotion and progression) of multistage carcinogenesis (suppressing agents). Some phytochemicals can act as both blocking and suppressing agents. [(-) sign indicates the inhibition]. (Adapted from Surh, 2003).
1.4. Colorectal cancer

The colon and rectum are parts of the digestive system, also called the gastrointestinal, or GI, system. The digestive system processes food for energy and rids the body of solid waste (fecal matter or stool). After food is chewed and swallowed, it travels through the esophagus to the stomach. There it is partially broken down and sent to the small intestine, where digestion continues and most of the nutrients are absorbed. The small intestine joins the large intestine in the lower right abdomen. The small and large intestine are sometimes called the small and large bowel. The first and longest part of the large intestine is the colon, a muscular tube about 5 feet long. Water and mineral nutrients are absorbed from the food matter in the colon and the waste (feces) left from this process passes into the rectum, the final 6 inches of the large intestine, and is then expelled from the anus (American Cancer Society, 2011a).

Colon cancer, also called colorectal cancer or bowel cancer, is ranked as the third most commonly diagnosed cancer and the third leading cause of cancer death in both men and women in the Western world (American Cancer Society, 2011a). The incidence rates of colorectal cancer continue to increase in economically transitioning countries including most parts of Asia where the overall risk was formerly low. This increase may reflect the adoption of Western lifestyles and behaviors (Center et al., 2009).

Colorectal cancer usually develops slowly over a period of 10 to 15 years (Kelloff et al., 2004). Before a cancer develops, a growth of tissue or tumor usually begins as a non-cancerous polyp on the inner lining of the colon or rectum. Tumor is an abnormal tissue and can be benign (not cancer) or malignant (cancer). A polyp is
a benign, non-cancerous tumor. Some polyps can change into cancer but not all do. The chance of changing into a cancer depends upon the kind of polyp:

- **Adenomatous polyps (adenomas)** are polyps that have the potential to change into cancer. Because of this, adenomas are called a pre-cancerous condition.
- **Hyperplastic polyps and inflammatory polyps**, in general, are not pre-cancerous (Levine and Ahnen, 2006).

Another kind of pre-cancerous condition is called *dysplasia*. Dysplasia is an area in the lining of the colon or rectum where the cells look abnormal (but not like true cancer cells) when viewed under a microscope. These cells can change into cancer over time. Dysplasia is usually seen in people who have had diseases such as ulcerative colitis or Crohn's disease for many years. Both ulcerative colitis and Crohn's disease cause chronic inflammation of the colon.

If cancer forms within a polyp, it can eventually begin to grow into the wall of the colon or rectum. Cancers that have invaded the wall can also penetrate blood or lymph vessels, which are thin channels that carry away cellular waste and fluid. Cancer cells typically spread first into nearby lymph nodes, which are bean-shaped structures that help fight infections. Cancerous cells can also be carried in blood vessels to the liver or lungs, or can spread in the abdominal cavity to other areas, such as the ovary. The process through which cancer cells travel to distant parts of the body through blood or lymphatic vessels is called metastasis (American Cancer Society, 2011a).
1B.4.1. Types of cancer in the colon and rectum

Several types of cancer can start in the colon or rectum.

- **Adenocarcinomas:** More than 95% of colorectal cancers are a type of cancer known as *adenocarcinomas*. These cancers start in cells that form glands that make mucus to lubricate the inside of the colon and rectum.

  Other, less common types of tumors may also start in the colon and rectum. These include:

- **Gastrointestinal carcinoid tumors:** These tumors start from specialized hormone-producing cells of the intestine.

- **Gastrointestinal stromal tumors (GISTs):** These tumors start from specialized cells in the wall of the colon called the *interstitial cells of Cajal*. Some are benign (noncancerous); others are malignant (cancerous). These
tumors can be found anywhere in the digestive tract, but they are unusual in the colon.

- **Lymphomas**: These are cancers of immune system cells that typically start in lymph nodes, but they may also start in the colon, rectum, or other organs (Stewart et al., 2006).

### 1B.4.2. Colorectal Cancer Risk Factors

There are many known factors that increase or decrease the risk of colorectal cancer; some of these factors are modifiable and others are not. Nonmodifiable risk factors include a personal or family history of colorectal cancer or adenomatous polyps, and a personal history of chronic inflammatory bowel disease. Modifiable risk factors that have been associated with an increased risk of colorectal cancer include physical inactivity, obesity, high consumption of red or processed meats, smoking, and moderate-to-heavy alcohol consumption. A recent study found that about one-quarter of colorectal cancer cases could be avoided by following a healthy lifestyle, i.e., maintaining a healthy abdominal weight, being physically active at least 30 minutes per day, eating a healthy diet, not smoking, and not drinking excessive amounts of alcohol (Kirkegaard, 2010).

(a). **Family history**

People with a first-degree relative (parent, sibling, or offspring) who has had colorectal cancer have 2 to 3 times the risk of developing the disease compared to individuals with no family history; if the relative was diagnosed at a young age or if there is more than one affected relative, risk increases to 3 to 6 times that of the general population (Butterworth et al., 2006; Johns and Houlston, 2001). About 20%
of all colorectal cancer patients have a close relative who was diagnosed with the disease (Lynch and de la Chapelle, 2003).

(b). Inherited syndromes

About 5% to 10% of people who develop colorectal cancer have inherited gene changes that cause the disease (Lynch and de la Chapelle, 2003). The two most common inherited syndromes linked with colorectal cancers are hereditary non-polyposis colorectal cancer (HNPCC) and familial adenomatous polyposis (FAP).

Hereditary non-polyposis colon cancer (HNPCC): HNPCC, also known as Lynch syndrome, accounts for about 3% to 5% of all colorectal cancers. HNPCC can be caused by inherited changes in a number of different genes that normally help repair DNA damage. This syndrome also develops when people are relatively young. The lifetime risk of colorectal cancer in people with this condition may be as high as 80%.

Familial adenomatous polyposis (FAP): Familial adenomatous polyposis (FAP) is the second most common predisposing genetic syndrome; for these individuals, lifetime risk of colorectal cancer approaches 100% without intervention. About 1% of all colorectal cancers are due to FAP. It is caused by mutations in the adenomatous polyposis coli (APC) gene that a person inherits from his or her parents. The APC gene is a tumor suppressor gene - it normally helps keep cell growth in check. In people who have inherited changes in the APC gene, this "brake" on cell growth is turned off, causing hundreds of polyps to form in the colon. People with this disease typically develop hundreds or thousands of polyps in their colon and rectum, usually in their teens or early adulthood. Cancer usually develops in one or more of these polyps as early as age twenty. By age forty, almost
all people with this disorder will have developed cancer if preventive surgery (removing the colon) is not done (Jasperson et al., 2010).

(c). Personal medical history

People who have a chronic inflammatory bowel disease have an increased risk of developing colorectal cancer which increases with extent and duration of the disease (Bernstein et al., 2001). This includes conditions such as ulcerative colitis and Crohn disease, in which the colon is inflamed over a long period of time. It is estimated that 18% of patients with a thirty year history of ulcerative colitis will develop colorectal cancer (Eaden et al., 2001).

People who have had one or more adenomatous polyps also have an increased risk of colorectal cancer. This is especially true if the polyps were large or if there was more than one (Schatzkin et al., 1994).

Many studies have found an association between diabetes and increased risk of colorectal cancer (Larsson et al., 2005a; Campbell et al., 2010). Though adult onset (Type 2) diabetes and colorectal cancer share similar risk factors, including physical inactivity and obesity, a positive association between diabetes and colorectal cancer has been found after accounting for physical activity, body mass index, and waist circumference (Larsson et al., 2005b).

(d). Other risk factors

Physical inactivity: One of the most consistently reported relationships between colon cancer risk and behavior is the protective effect of physical activity (Wolin et al., 2009). Epidemiologic studies proved that high levels of physical activity
decrease the risk of colon cancer among men and women by possibly as much as 50% (Chan and Giovannucci, 2010).

**Overweight and obesity:** Being overweight or obese is associated with a higher risk of colorectal cancer, with stronger associations more consistently observed in men than in women (Huxley et al., 2009). Overweight and obesity increase the risk of colorectal cancer independent of physical activity (Larsson and Wolk, 2007). Abdominal obesity (measured by waist size) may be a more important risk factor for colon cancer than overall obesity in both men and women (Wang et al., 2008).

**Diet:** A diet that is high in red meats and processed meats can increase colorectal cancer risk. Cooking meats at very high temperatures (frying, broiling or grilling) creates chemicals that might increase the cancer risk (Chao et al., 2005; Cross et al., 2010). But the diets high in vegetables and fruits have been linked with a decreased risk of colorectal cancer (McCullough et al., 2003; Terry et al., 2001).

**Smoking:** Long-term smokers are more likely to develop and die from colorectal cancer than non-smokers. Smoking is a well-known cause of lung cancer, but some of the cancer-causing substances are swallowed and can cause digestive system cancers, like colorectal cancer. In November 2009, the International Agency for Research on Cancer reported that there is now sufficient evidence to conclude that tobacco smoking causes colorectal cancer (Secretan et al., 2009).

**Alcohol:** Colorectal cancer has been linked to even moderate alcohol use. Individuals who have a lifetime average of 2 to 4 alcoholic drinks per day have a 23% higher risk of colorectal cancer than those who consume less than one drink per day (Ferrari et al., 2007).
1B.4.3. Animal models of chemical carcinogenesis

The development of well-characterized preclinical models of colorectal carcinogenesis has played a critical role in the process of screening and selection of agents deserving further clinical study. The study of experimental colon carcinogenesis in rodents has had a remarkably long history, dating back almost 80 years (Krebs, 1928). Frequently used chemical carcinogens include dimethylhydrazine (DMH), azoxymethane (AOM), methoxymethane (MAM) and N-methyl-N-nitro-N-nitrosoguanidine (MNNG) (Narisawa et al., 1971; Thurnherr et al., 1973).

DMH, a metabolic precursor of MAM, was used in several early studies to induce tumors in rats (Schauer et al., 1969; Thurnherr et al., 1975). Repetitive treatment with this methylating agent was reported to produce colon tumors in rodents that exhibit many of the pathological features associated with the human disease (Shamsuddin et al., 1981; Ward, 1974). Thus, DMH has provided cancer researchers with a reproducible experimental system for studying ‘sporadic’ (non-familial) forms of CRC (LaMont et al., 1978).

DMH and its metabolite, AOM, are procarcinogens that require metabolic activation to form DNA-reactive products (Figure 1.9). Metabolism of these compounds involves multiple xenobiotic-metabolizing enzymes, which proceeds through several N-oxidation and hydroxylation steps, including the formation of MAM following hydroxylation of AOM. The reactive metabolite, MAM, readily yields a methylidiazonium ion, which can alkylate macromolecules in the liver and colon, including the addition of methyl groups at the $O^6$ or $N^7$ position of guanine ($O^6$-methyl-deoxyguanosine and $N^7$-methyl-deoxyguanosine). MAM is a substrate
of the NAD$^+$-dependent dehydrogenase present in the colon and liver. A direct role for the alcohol inducible cytochrome P-450 isoform, CYP2E1, in activation of AOM and MAM has also been established. These metabolites of CYP2E1 are transported to the colon via the bloodstream. The ability of AOM and DMH to target the colonic mucosa is probably due to the relative stability of the hydroxylated metabolite, MAM; with a half-life of ~12 h, it is the time sufficient for MAM to distribute to the colon. Further activation of blood-borne metabolites may then proceed via non-P450-dependent mechanisms, including possible oxidation of MAM, directly within the colon (Rosenberg et al., 2009).

Figure 1.9. Metabolic activation of DMH.
**1B.4.4. Chemoprevention of colon cancer**

The observations that certain chemicals, some of them natural dietary constituents and some not, can decrease tumorigenesis in animals and, by epidemiological implication, in humans have led to the idea that the intake of certain chemicals can either prevent cancer or slow its progression (Abbruzzese and Lippman, 2004). Chemoprevention refers to the administration of natural or synthetic compounds to block, reverse, delay or prevent the development of invasive neoplasms. The ultimate goal of implementing a chemopreventive intervention in the general or alternatively in an at-risk population is to slow the onset of cancer or to decrease the incidence rate of the specific cancer being targeted (Wattenberg, 1997). Cancer chemopreventive agents can be classified as antimutagens and carcinogen-blocking agents, antiproliferatives, or antioxidants, however, there is some overlap among agents in these categories, i.e., they may have more than one mechanism of action (Kelloff et al., 1994).

**1B.4.5. Mechanisms of chemoprevention**

Carcinogenesis is generally recognized as a multistep process in which distinct molecular and cellular alterations occur. From the study of experimentally induced carcinogenesis in rodents, tumor development is considered to consist of several separate, but closely linked, stages- tumor initiation, promotion and progression (Figure 1.10).
Initiation is a rapid and irreversible process that involves a chain of extracellular and intracellular events. These include the initial uptake of or exposure to a carcinogenic agent, its distribution and transport to organs and tissues where metabolic activation and detoxification can occur, and the covalent interaction of reactive species with target-cell DNA, leading to genotoxic damage. In contrast to initiation, tumor promotion is considered to be a relatively lengthy and reversible process in which actively proliferating preneoplastic cells accumulate. Progression, the final stage of neoplastic transformation, involves the growth of a tumor with invasive and metastatic potential (Surh, 2003).

According to the conventional classification, chemopreventive agents are subdivided into two main categories - blocking agents and suppressing agents (Wattenberg, 1985). Blocking agents prevent carcinogens from reaching the target sites, from undergoing metabolic activation or from subsequently interacting with crucial cellular macromolecules (for example, DNA, RNA and proteins).
Suppressing agents, on the other hand, inhibit the malignant transformation of initiated cells, in either the promotion or the progression stage (Figure 1.7). Chemopreventive phytochemicals can block or reverse the premalignant stage (initiation and promotion) of multistep carcinogenesis. They can also halt or at least retard the development and progression of precancerous cells into malignant ones. Therefore, the ability of any single chemopreventive phytochemical to prevent tumor development should be recognized as the outcome of the combination of several distinct sets of intracellular effects, rather than a single biological response. The cellular and molecular events affected or regulated by these chemopreventive phytochemicals include carcinogen activation/detoxification by xenobiotic metabolizing enzymes; DNA repair; cell-cycle progression; cell proliferation, differentiation and apoptosis; expression and functional activation of oncogenes or tumor-suppressor genes; angiogenesis and metastasis; and hormonal and growth-factor activity (Surh, 2003).

(a). **Cellular signalling molecules as targets**

Many of the molecular alterations that are associated with carcinogenesis occur in cell-signalling pathways that regulate cell proliferation and differentiation. One of the central components of the intracellular signalling network that maintains homeostasis is the family of proline-directed serine/threonine kinases - the mitogen-activated protein kinases (MAPKs). Abnormal or improper activation or silencing of the MAPK pathway or its downstream transcription factors can result in uncontrolled cell growth, leading to malignant transformation. Some phytochemicals ‘switch on’ or ‘turn off’ the specific signalling molecule(s), depending on the nature of the signalling cascade they target, preventing abnormal cell proliferation and
growth. Cell-signalling kinases other than MAPKs, such as protein kinase C (PKC) and phosphatidylinositol 3-kinase (PI3K), are also important targets of certain chemopreventive phytochemicals. These upstream kinases activate a distinct set of transcription factors, including nuclear factor κB (NF-κB) and activator protein 1 (AP1) (Surh, 2003).

Numerous intracellular signal-transduction pathways converge with the activation of the transcription factors NF-κB and AP1, which act independently or coordinately to regulate target-gene expression. Aberrant activation of NF-κB has been associated with protection against apoptosis and stimulation of proliferation in malignant cells (Beg and Baltimore, 1996; Wang et al., 1998), and over expression of NF-κB is causally linked to the phenotypic changes that are characteristic of neoplastic transformation (Visconti et al., 1997). Many chemopreventive phytochemicals that are derived from the diet have been shown to suppress constitutive NF-κB activation in malignant cells or NF-κB activation induced by the external tumor promoter phorbol 12-myristate 13-acetate (PMA) or tumor-necrosis factor-α (TNF-α) (Manson et al., 2000; Bharti and Aggarwal, 2002; Bremner and Heinrich, 2002).

AP1 is another transcription factor that regulates expression of genes that are involved in cellular adaptation, differentiation and proliferation. Functional activation of AP1 is associated with malignant transformation as well as tumor promotion. As NF-κB and AP1 are ubiquitous eukaryotic transcription factors that mediate pleiotropic effects of both external and internal stimuli in the cellular-signalling cascades, they are prime targets of diverse classes of chemopreventive
phytochemicals (Dong et al., 1994; Dong et al., 1995; Dong et al., 1997; Huang et al., 1998).

Curcumin, [6]-gingerol, capsaicin, epigallocatechin gallate (EGCG.), genistein, resveratrol, caffeic acid phenethyl ester (CAPE), sulphoraphane, silymarin, apigenin, emodin, quercetin, anethole etc. are reported to suppress the activation of NF-κB and AP1, which might contribute to their chemopreventive and/or cytostatic effects (Bharti and Aggarwal., 2002; Surh, 2003).

(b). NRF–KEAP1 complex

Other than suppressing tumor promotion or progression, another important approach to chemoprevention is to block the DNA damage caused by carcinogenic insult - the initiation stage of carcinogenesis. Toxic xenobiotic chemicals, including carcinogens, are detoxified by phase II enzymes - such as glutathione-S-transferase (GST) and NAD(P)H: quinone oxidoreductase (NQO). The phase II enzyme induction system is an important component of the cellular stress response in which a diverse array of electrophilic and oxidative toxicants can be removed from the cell before they are able to damage the DNA. Antioxidants exert their protective effects not only by scavenging ROS, but also by inducing de novo expression of genes that encode detoxifying/defensive proteins, including phase II enzymes. Many xenobiotics activate stress-response genes in a manner similar to that achieved by antioxidants. These genes encode enzymes such as glutathione peroxidase (GPx), gamma-glutamylcysteine synthetase (γ-GCS), GST, NQO and heme oxygenase-1 (HO-1). The 5′-flanking regions of these genes contain a common cis-element, known as the Antioxidant-Responsive Element (ARE) (Hayes and McMahon, 2001).
**NRF:** During oxidative stress or other types of toxic insult that are induced by xenobiotic chemicals, certain members of the helix–loop–helix bZIP family of transcription factors - particularly the nuclear factor-erythroid 2p45 (NF-E2)-related factors (NRF1 and NRF2) - heterodimerize and bind to the ARE sequence to activate transcription (Itoh et al., 1997).

**KEAP1:** A cytosolic actin-binding protein called Kelch-like ECH-associated protein 1 (KEAP1) is a negative regulator of NRF, suppresses the transcriptional activity of NRF2 by retaining the transcription factor in the cytoplasm and hampering its nuclear translocation. The KEAP1–NRF2 complex is an intracellular sensor that recognizes redox signalling by detecting electrophiles or ROS (Itoh et al., 1999). Many phase II gene inducers are able to generate ROS, or else can be readily converted - nonenzymatically, via redox cycling - or metabolized to electrophilic intermediates in the body. Phase II enzyme inducers mimic pro oxidants and electrophiles, although most of them are antioxidants by nature. It is plausible that these reactive species interact with thiol groups of KEAP1 and oxidize or covalently modify the cysteine residues within KEAP1 and also, possibly, NRF2. This would cause KEAP1 to release NRF2, so it could translocate to the nucleus and activate transcription of phase II enzymes. In this context, the cysteine residues in KEAP1 could serve as a molecular sensor of intracellular redox status, ensuring the proper and timely expression of genes that are involved in cellular antioxidant defense or detoxification of electrophilic toxicants (Dinkova-Kostova et al., 2001; Dinkova-Kostova et al., 2002; Wolf, 2001; Na and Surh, 2003; Surh, 2003).
(c). β-Catenin

β-Catenin is another important target of chemopreventive phytochemicals. β-Catenin is a multifunctional protein that was originally identified as a component of the cell–cell adhesion machinery. It binds with the cytosolic tail of E-cadherin and connects actin filaments through α-catenin to form the cytoskeleton (Kemler, 1993; Aberle et al., 1996). β-Catenin can also function as a transcription factor, and nuclear translocation of β-catenin has been associated with various human cancers (Morin, 1999).

Several dietary phytochemicals have been shown to down regulate the β-catenin-mediated signalling pathway as part of their molecular mechanism of chemoprevention. Curcumin reduced the cellular levels of β-catenin through caspase-mediated cleavage of the protein (Jaiswal et al., 2002). Down regulation of β-catenin expression by resveratrol was observed in a human colon cancer cell line (Joe et al., 2002). COX inhibitors have also been found to suppress β-catenin signalling and β-catenin–TCF/LEF transcriptional activity. As up regulation of COX2 promotes tumorigenesis, and β-catenin is found to regulate COX2 expression, modulation of β-catenin signalling could be another molecular target for chemoprevention by dietary phytochemicals (McEntee et al., 1999; Dihlmann et al., 2001; Mori et al., 2002; Surh, 2003).
<table>
<thead>
<tr>
<th>Name of the natural product</th>
<th>Experimental model</th>
<th>References</th>
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<tbody>
<tr>
<td>Caffeic acid (Phenolic constituent of honey)</td>
<td>HCT 15 colon cancer cells</td>
<td>Jaganathan, 2012</td>
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<tr>
<td><em>Garcinia mangostana</em> xanthones extract.</td>
<td>HCT 116 - human colorectal carcinoma cells</td>
<td>Aisha et al., 2012</td>
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<tr>
<td>Hesperetin (Citrus flavanoid)</td>
<td>DMH-induced colon carcinogenesis in rats</td>
<td>Aranganathan and Nalini, 2013</td>
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<tr>
<td>Kenaf (<em>Hibiscus cannabinus</em>)</td>
<td>AOM-induced colon carcinogenesis in rats</td>
<td>Ghafar et al., 2012</td>
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<td>Lycopene (Carotenoid component of tomato)</td>
<td>HT-29 human colon cancer cells</td>
<td>Teodoro et al., 2012</td>
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<td>Paeoniflorin (Paeony root)</td>
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<td>COLO 320DM and HT-29 cells</td>
<td>Hsu et al., 2012</td>
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<td>DMH-induced colon carcinogenesis in rats</td>
<td>Sangeetha et al., 2012</td>
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<td>Wang et al., 2011</td>
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<td><em>Curcuma mangga</em> rhizomes</td>
<td>HCT 116 and HT-29 cells</td>
<td>Malek et al., 2011</td>
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<td>Gallic acid (Plant polyphenol)</td>
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<td>Giftson Senapathy et al., 2011</td>
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<td>Morin (Flavonoid)</td>
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<td>Yadav et al., 2010</td>
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<td>Resveratrol (Polyphenolic compound)</td>
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<td>Oregano (<em>Origanum vulgare</em> L.)</td>
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<td>Srihari et al., 2008</td>
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<td><em>Uncaria rhynchophylla</em></td>
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<td>DMH-induced colon carcinogenesis in rats</td>
<td>Kamaleeswari and Nalini, 2006</td>
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<td>Ginger (<em>Zingiber officinale</em> Rosc)</td>
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<td>Manju and Nalini, 2006</td>
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<td>Neem leaf (<em>Azadirachta indica</em>)</td>
<td>AOM-induced colon carcinogenesis in rats</td>
<td>Arakaki et al., 2006</td>
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<tr>
<td>Indole-3-carbinol (Cruciferous vegetables)</td>
<td>AOM-induced colon carcinogenesis in rats</td>
<td>Suzui et al., 2005</td>
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**1B.5. Hepatocellular carcinoma**

Liver is the largest organ in the human body that performs a multitude of functions. It helps in the maintenance, performance and regulation of homeostasis in the body and is involved in almost all the biochemical pathways pertaining to growth, immunity, nutrition, metabolism and reproduction. (Ahsan et al., 2009). Unfortunately the liver is often abused by environmental and biological toxins, poor eating habits, consumption of alcohol and drugs, and viruses which can damage and weaken the liver. These factors eventually lead to hepatitis, cirrhosis, alcoholic liver disease and hepatocellular carcinoma (Gitlin, 1996).
Hepatocellular carcinoma (HCC) is one of the most frequent tumor types worldwide. It is the fifth most common cancer and the third leading cause of cancer death (El-Serag and Rudolph, 2007). More than 700,000 people are diagnosed with this cancer each year throughout the world and accounting for more than 600,000 deaths (American Cancer Society, 2012). Generally, HCC is more frequent in men than in women and the incidence increases with age (Levrero, 2006).

There are multiple etiological agents that are associated with the development of HCC, the most frequent being chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, and long-term exposure to the mycotoxin and aflatoxin B1. HBV is recognized as a major etiological factor in the development of diseases such as fatty liver (steatosis), cirrhosis, hepatocellular adenoma and HCC. The risk of HCC in chronic HBV carriers is more than 100 times greater than in uninfected individuals (Beasley et al., 1981; Ito et al., 2010). Like other cancers, HCC is a multistep process, involving many genetic alterations, which eventually lead to malignant transformation of hepatocytes (Levrero, 2006).

(Farazi and DePinho, 2006)

**Figure 1.11. Histopathological progression and molecular features of HCC**
After hepatic injury incurred by any one of several factors (hepatitis B virus (HBV), hepatitis C virus (HCV), alcohol and aflatoxin B1), there is necrosis followed by hepatocyte proliferation. Continuous cycles of this destructive–regenerative process foster a chronic liver disease condition that culminates in liver cirrhosis. Chronic hepatitis leads to cirrhosis within 15-40 years. Mostly, HCC develops among 70%-90% of cirrhotic patients, while only 10% of HCC patients have a non-cirrhotic liver, or even have inflammatory lesions. Cirrhosis is characterized by abnormal liver nodule formation surrounded by collagen deposition and scarring of the liver. Subsequently, hyperplastic nodules are observed, followed by dysplastic nodules and ultimately hepatocellular carcinoma (HCC), which can be further classified into well differentiated, moderately differentiated and poorly differentiated tumors - the last of which represents the most malignant form of primary HCC (Levrero, 2006; Farazi and DePinho, 2006).

1B.5.1. Risk factors that lead to HCC

(a). **Hepatitis B virus (HBV) infection:** This DNA virus is the most frequent etiology of liver cancer. There is strong epidemiological evidence correlating HCC to HBV infection. This was shown by positive results in HCC patients for both HB surface antigen (HBs Ag) and HB core antibodies (HBc antibodies) or both together (Abe et al., 1998).

(b). **Hepatitis C virus (HCV) infection:** HCV is a member of the Flaviviridae family of enveloped, positive-stranded RNA viruses, genus Hepacivirus. It is a completely cytoplasmic-replicating virus that induces oncogenic transformation (Tellinghuisen and Rice, 2002). Chronic HCV infection mostly leads to hepatic cirrhosis before developing HCC (Donato et al., 1997). Generally, the prevalence of HCV infection
is accepted to be a major morbidity factor in hepatic carcinogenesis. An increasing body of evidence suggests that HCV has a direct pathway in promoting malignant hepatocyte transformation. However, it is established that many viral proteins are implicated in malignant transformation and HCC development. Of these proteins, core proteins, NS3, NS4, were shown to have transformation potential in tissue culture (Sakamuro et al., 1995; Ray et al., 1996; Gale et al., 1999; Park et al., 2000). These viral proteins, in addition to the viral RNA, interact with many host-cell factors, while still regulating the viral life cycle. They modulate host-cell activities such as cell signaling, transcription, transformation, apoptosis, membrane rearrangement, vesicular trafficking and protein translation. This ultimately misleads the host transcription factors, disturbing cell mitosis and protein synthesis, leading to carcinogenesis (Levrero, 2006).

(c). *Diabetes mellitus*: Liver cirrhosis, which is a functional liver damage (characterized by a decrease in serum albumin level below 4 g/dL and increased prothrombin time), is always higher in HCC patients with diabetes, than among those without a history of diabetes. Thus, there is a positive correlation between the history of diabetes mellitus and HCC (Lagiou et al., 2000).

(d). *Hereditary hemochromatosis*: Hereditary hemochromatosis is an autosomal recessive condition characterized by excessive iron deposition in hepatocytes due to an increased intestinal absorption. Thus, liver disease is the commonest cause of death in patients with hereditary hemochromatosis (Fracanzani et al., 2001). Among hemochromatotic patients, 6% of men and 1.5% of women are at absolute risk of liver cancer (Elmberg et al., 2003).
(e). **Exposure to chemical carcinogens:** Environmental pollutants such as aflatoxin B, a product of mold commonly contaminating badly stored foods, as well as insecticides, were reported to be classical sources for hepatocarcinogenesis. Other known chemical carcinogens are chlorination byproducts in drinking water. Uncontrolled water chlorination converts many organic traces in water into dangerous intermediates, such as di- and tri-chloroacetic acids, which are experimentally known to induce HCC. Many other chemical contaminants, such as solvents, food additives, drugs and hormones are also thought to contribute to HCC (Abdel-Hamid, 2009).

(f). **Alcoholism:** Alcohol is a very common source for steatohepatitis (fatty liver), cirrhosis and eventually HCC (Donato et al., 2002). In developed countries, alcohol drinking seems to be the most common source for HCC. Alcohol either directly initiates HCC after its oxidation into acetaldehyde, which is genotoxic, or indirectly through the development of cirrhosis (London et al., 1996).

(g). **Congenital disorders:** Alpha-1-antitrypsin is an acute-phase protein that is produced by liver cells. Hereditary deficiency of this protein is mostly due to the synthesis of abnormal alpha-1-antitrypsin by the hepatocytes and that cannot be released into the plasma. Accumulation of the protein in hepatocytes can lead to liver damage. This can trigger hepatitis in neonates, end-stage liver disease, cirrhosis and HCC in adults (Kok et al., 2007).
1B.5.2. Molecular pathways and their possible relationship to HCC

(a). *Irregular expression of β-catenin:* β-catenin is a nuclear protein that regulates the cell cycle. Its irregular expression, resulting from β-catenin gene mutations, is implicated in HCC (Abdel-Hamid, 2009).

(b). *Up-regulation of many growth factors:* Insulin-like growth factor (IGF), insulin receptor substrate 1, hepatocyte growth factor (HGF) and transforming growth factor β (TGF-β) have been implicated in the development of HCC (Moradpour and Wands, 2002).

(c). *Transformation from pre-neoplastic to HCC nodules:* HCC is a highly vascular tumor, always accompanied by neo-vascularization. Thus, over expression of angiogenic factors, vascular endothelial growth factor (VEGF) and angiopoietin-2, is another pathway for HCC genesis (Yamaguchi et al., 1998; Mitsuhashi et al., 2003).

(d). *Mutations in transcription factors controlling the cell cycle:* Transcription factors such as phospho-retinoblastoma (pRb), p53, TGF-β and β-catenin participate in hepatocellular carcinogenesis. Mutations in these factors deprive the cell control over the cell cycle, leading to uncontrolled mitosis and cancer (Moradpour and Blum, 2005).

1B.5.3. NDEA - (N-nitroso diethyl amine) induced HCC

*N*-nitrosodiethylamine is a potent carcinogenic dialkyl nitrosoamine present in tobacco smoke, water, cheddar cheese, cured and fried meals and in a number of alcoholic beverages and has been considered to be a potent hepatocarcinogen producing reproducible hepatocellular carcinoma after repeated administration in experimental animals (Travis et al., 1991; Rajeshkumar and Kuttan, 2000). It can
induce carcinoma in all animal species as well as humans, and has been used as an experimental carcinogen in many studies (Loeppky, 1999).

NDEA becomes metabolically active in the liver by the action of cytochrome p450 enzymes to produce reactive electrophiles. The generation of reactive oxygen species (ROS) in the liver is recognized as an important contributor in NDEA induced carcinogenic effects (Telliez et al., 1995; Shaarawy et al., 2009). ROS are continuously generated in vivo as a result of NDEA administration causing oxidative stress that seriously damaged the biological systems by injuring tissues, altering biochemical compounds, causing chromosomal instability, eroding cell membranes and mutation, which are involved in all steps of carcinogenesis, i.e. initiation, promotion and progression (Karbownik et al., 2001).

The conventional therapies of hepatocarcinoma including chemotherapy, radiation, surgical resection and ablation give little hope for restoration of health because of poor diagnosis and serious side effects. Liver transplantation is considered to be the most effective treatment for patients with hepatocarcinoma. However, low availability of organs limits the offer of this option to all candidates, and the high risk of tumor recurrence after transplantation further compromises its efficiency. Therefore, developing more effective and less toxic anti-cancer agents, including natural products, is necessary to prevent or retard the process of hepatocarcinogenesis (Zhang et al., 2012; Tabone and Pellicano, 2006). In this regard, several herbal drugs have been investigated for their chemopreventive potential against hepatocellular carcinoma in rats.
Table 1.4. Selected plants with chemopreventive activity against hepatocellular carcinoma

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<tr>
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<th>Experimental model</th>
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<td>Alocasia macrorrhiza</td>
<td>Hepatocellular carcinoma cell lines SMMC-7721</td>
<td>Fang et al., 2012</td>
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<td>Beetroot juice</td>
<td>NDEA induced hepatocellular carcinoma in rats</td>
<td>Krajka-Kuźniak et al., 2012</td>
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<td>Abrus precatorius</td>
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<td>Acacia nilotica</td>
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<td>Singh et al., 2009</td>
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<td>Gynandropsis gynandra</td>
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<td>Sivasenan and Begum, 2007</td>
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<td>Lygodium flexuosum</td>
<td>NDEA induced hepatocellular carcinoma in rats</td>
<td>Wills et al., 2006</td>
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<tr>
<td>Emblica officinalis</td>
<td>NDEA induced hepatocellular carcinoma in rats</td>
<td>Jeena et al., 1999</td>
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1B.6. Phytomedicines

Medicinal plants have been used in traditional health care systems since prehistoric times and are still the most important health care source for the vast majority of the population around the world. It is estimated that 70-80% of people worldwide rely on traditional herbal medicine to meet their primary health care needs (Uprety et al., 2012). Herbal, botanical or phytomedicines are medicinal products containing active ingredients of exclusively plant origin. These medicines may be consumed as comminuted powders or as decoctions. Herbal medicines exclude products that consist primarily of chemically defined constituents. The use of herbal medicines presents unique clinical and pharmacological challenges that are not encountered with conventional single-compound medicines. These medicines are
usually complex mixtures of many bioactive compounds and conventional “indications and uses” criteria devised for single compound entities may not be applicable to this system in a significant number of ways. Compared to single-agent pharmaceuticals, phytomedicines may differ in the different mechanisms of action of bioactive constituents, in their dose-response relationships, and in the synergistic/combinatorial effects of the many bioactive compounds found in herbal extracts (Yong and Loh, 2004).

India is the largest producer of medicinal plants and is rightly called the "Botanical garden of the World". Medical information referred in the old Indian literatures includes several medicinal herbs, which have been in the use for thousands of years, in one form or the other, under the indigenous system of medicine. In India, 45,000 plant species have been identified, out of which about 15-20 thousand plants are of good medicinal value. However, traditional communities use only about 7000-7500 plants for medicinal purposes. The Siddha system of medicine uses about 600, Ayurveda 700, Unani 700 and modern medicine about 30 medicinal plants for treating a variety of diseases in man and animal (Madhuri and Pandey, 2008).

Traditional medicines all over the world are nowadays being reevaluated by extensive research on different plant species with reference to their therapeutic principles. The ability of certain plant secondary metabolites such as polyphenolic compounds to act as scavengers of free radicals, besides their antioxidant and antimicrobial properties, is raising the possibility of their food and pharmaceutical applications. Despite progress in conventional chemistry and pharmacology in producing effective drugs, the plant kingdom might provide a useful source of new
medicines and pharmaceutical entities or, alternatively, may be used as simple dietary adjuncts to existing therapies (Sing et al., 2003).

1B.7. Selection of plant for the present study

In choosing medicinal plants for scientific evaluation of their biological activities and validation of ethnopharmacological usage, some criteria such as

- Evidence of ethnopharmacological usage by the native population
- The ailment(s) which the plant(s) is used to cure
- The availability of the plant in its natural habitat
- Mode of preparation and administration by traditional healers etc. are usually considered.

Based on these contexts, in the present investigation, *Amorphophallus campanulatus* tuber was selected for its pharmacological evaluations. Warrier et al. (1994) reported that *A. campanulatus* corm has been used traditionally for the treatment of liver diseases, abdominal pain, tumors, piles, enlargement of spleen, asthma and rheumatism. Besides, the boiled/dried corm of *A. campanulatus* is used by the tribes of Andra Pradesh (India) for obesity, abdominal tumors, internal as well as external piles, enlargement of spleen and liver, tuberculosis, vertigo, general weakness etc. (Raghavan, 2011). In light of the above reports, in the present study, *A. campanulatus* tuber was chosen for the scientific validation of some of its traditional and tribal claims.
1B.8. *Amorphophallus campanulatus* (Roxb.) Blume

1B.8.1. Plant profile

**Botanical information**: *Amorphophallus campanulatus* (Roxb.) Blume is a monocot belonging to the family Araceae and subfamily Aroideae.

**Habitat**: Native to tropical Asia; cultivated throughout India.

**Vernacular names**
- English: Elephant-foot Yam.
- Malayalam: Chena, Kattuchena, Kattuchenai, Cena Karana.
- Sanskrit: Suurana, Kanduula, Arshoghna, Kand-ayak, Kandala.
- Urdu: Zamin-qand, Zamikand.
- Tamil: Chenaikkizhangu, Kaathukarunai (wild var.).
- Hindi: Suranakanda, Zamikanda.

1B.8.2. Properties and action of *A. campanulatus* tuber

The Corm is prescribed in bronchitis, asthma, abdominal pain, emesis, dysentery, enlargement of spleen, piles, elephantiasis, diseases due to vitiated blood and rheumatic swellings. Moreover, the tuber is widely used in many ayurvedic preparations and is anodyne, anti-inflammatory, anti-haemorrhoidal, haemostatic, expectorant, carminative, digestive, appetizer, stomachic, anthelmintic, liver tonic, aphrodisiac and emmenagogue. It is also used traditionally for arthralgia, elephantiasis, tumors, inflammations, hemorrhoids, hemorrhages, vomiting, cough, bronchitis, asthma, anorexia, dyspepsia, flatulence, colic, constipation, helminthiasis, hepatopathy, splenopathy, amenorrhea, dysmenorrhoea, seminal weakness, fatigue, anemia and general debility. The corm contains an active diastatic enzyme amylase, betulinic acid, triacontane, lupeol, stigmasterol, β-sitosterol and its palmitate and glucose, galactose, rhamnose and xylose (Nair, 1993; Khare, 2007; The Ayurvedic Pharmacopoeia of India, 2009).
Figure 1.12. *Amorphophallus campanulatus* (Roxb.) Bl. plant

Figure 1.13. *A. campanulatus* tuber
Recent researches on A. campanulatus tuber

Jain et al. (2009) reported that the ethanolic extract of A. campanulatus tuber have potent hepatoprotective action against carbon tetrachloride induced hepatic damage in rats. Further, the ethanolic extract of the corm has been reported to possess antibacterial, antifungal and cytotoxic activities (Khan et al., 2007). A significant antioxidant and antitumor activity against 7,12-Dimethylbenz(a)anthracene (DMBA) induced mammary tumor in rats has also been reported for the ethanolic extract of the tuber (Jagatheesh et al., 2010). Whereas, the methanolic extract of tuber showed a significant analgesic activity in mice (Shilpi et al., 2005). In addition, the petroleum ether extract of the tuber is reported to have central nervous system depressant activity (Das et al., 2009b).

The biologically active constituents isolated from the corm of A. campanulatus include Amblyone, Salviasperanol and 3, 5- diacetyl tambulin, Amblyone - a triterpenoid and Salviasperanol - a diterpenoid, showed significant antibacterial activity against Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, Escherichia coli, Shigella dysenteriae, Shigella sonnei, Shigella flexneri, Pseudomonas aeruginosa and Salmonella typhi (Khan et al., 2008a; Khan et al., 2009). 3, 5- diacetyl tambulin, a flavanoid isolated from the tuber also showed good antibacterial activity (Khan et al., 2008b). In addition, a water-soluble polysaccharide isolated from the aqueous extract of the corm of Amorphophallus campanulatus was found to contain D-galactose, D-glucose, 4-O-acyl-D-methyl galacturonate, and L-arabinose (Das et al., 2009a).
Objectives of the study

The followings were the major objectives of the present investigation:

1. Phytochemical screening and in vitro antioxidant evaluation of the n-hexane and methanolic extract of *Amorphophallus campanulatus* tuber.
2. *In vivo* antioxidant evaluation of the most promising extract of the *A. campanulatus* tuber in a preventive and curative experimental model of thioacetamide (TAA) induced oxidative stress in rats.
3. Dose response study of the chemopreventive action of *Amorphophallus campanulatus* tuber extract on 1, 2-dimethylhydrazine (DMH) induced colon carcinogenesis in Wistar rats.
4. Validation of the effective dose of *Amorphophallus campanulatus* tuber extract in a long term preclinical model of DMH induced colon carcinogenesis.
5. Evaluation of the chemopreventive action of *Amorphophallus campanulatus* tuber extract on *N*-nitrosodiethylamine (NDEA) induced hepatocellular carcinoma in rats.
7. Identification of phytochemical constituents of the crude extract and active fraction of *Amorphophallus campanulatus* tuber using LCMS analysis.