1. Introduction

Discovering curative and remedial property in plants is an old thought. People employ plant-based products as their primary medicine because of their therapeutic and health promoting properties (Stockwell, 1988). Currently, there is a renewed global interest in the study and use of plants because such investigations provide important new lead on novel and active molecules of therapeutic importance. Traditional plant-based remedies are finding increasing application as a source of direct therapeutic agent. Presently, plants are the original source materials for as many as 40% of pharmaceuticals currently used around the world. These drugs either contain plant-derived materials, or materials synthesized from agents originally derived from plants.

Plant-derived medicines are believed to be risk-free, milder and superior to chemically synthesized drugs for human health. Human body recognizes components that occur in plants and has sophisticated mechanism for metabolizing such plant materials. The bioactive compounds naturally available in plants may have lower potency than allopathic (synthesized) drugs. However, as they are traditionally consumed in significant amounts through diet, they may provide long term physiological benefits without any detrimental side effects (Espin et al, 2007).

In recent years, there is a tremendous interest in the possible role of nutrition for prevention of diseases. The concept of foods that combine both nutritional and medicinal benefits called Functional Foods and Nutraceuticals (Tsao and Akhtar, 2005) are especially popular these days. Until now, only certain essential nutrients such as vitamins have been recognized as important for maintenance of optimal health. However, increasing evidences have shown that minor components of food such as phytochemicals play significant role in the reduction of incidence of many chronic diseases. Therefore, these food phytochemicals are considered necessary to promote good health. Consumption of foods rich in phytochemicals and other bioactive food components have been clearly linked to the prevention and reduction of cancer (Steinmetz and Potter, 1991), cardiovascular diseases (Duthie and Brown, 1994) and to improvement in immune system (German and Dillard, 1998).
Historically, dietary intake of certain vegetables and fruits has been believed to bring health benefits. In fact, early medicines revolved largely around the prescription of specific food concoctions for certain ailments. Cruciferous vegetables are cultivated primarily for the medicinal purpose and are used therapeutically to cure several diseases. Until recently, these attributes of vegetables are based more upon beliefs than scientific evidences. However, over the past decades, a number of studies have examined the effect of consumption of cruciferous vegetables on health and diseases (Talalay and Fahey, 2001).

Cruciferous vegetables are characterized by the presence of a group of secondary metabolites called glucosinolates (GLs). They are glucose and sulfur-containing organic anions, which are hydrolyzed into isothiocyanates (ITCs) by myrosinase (β-thioglucosidase glucohydrolase; EC 3.2.3.1) during physical damage to vegetables by cutting, chewing or cooking. GLs can be found in roots, seeds, leaves and stem of a plant and their contents vary among cultivars and in different parts of the plant (Ciska et al., 2000). GLs play a key role in plant defense, growth regulation and plant seedlings (Minorsky, 2001). The typical odor and taste of cruciferous plants are mainly due to GLs and their hydrolyzed products such as ITCs. Other than GLs, polyphenolic compounds are also found in cruciferous vegetables.

Antioxidants, especially derived from cruciferous vegetables require special attention. Free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced during metabolic processes and are indispensable as mediators in many normal cellular processes. However, when produced excessively and/or when there is an inadequate antioxidant protection, it can lead to oxidative stress. Cellular damage induced by oxidative stress has been implicated in the etiology of several human diseases as well as process of aging (Halliwell and Gutteridge, 1997). Antioxidants act as free radical scavengers and alleviate free radical, mediated cellular damage in the body (Nice, 1997).

Polyphenolics are perhaps the largest group of phytochemicals with proven antioxidant properties. Polyphenolics have become the focus of current nutritional and therapeutic interest largely due to their disease preventing and health promoting effects. The antioxidant activity of dietary polyphenolics is considered to be superior to that of essential vitamins and is attributed to their high redox potential, which allows them to
interrupt free radical-mediated reactions by donating hydrogen from their phenolic hydroxyl groups (Parr and Bolwell, 2000). Apart from radical scavenging property, polyphenolics can chelate metal ions and therefore, decrease metal catalyzed radical reactions such as lipid peroxidation. Polyphenolics from cruciferous vegetables have many potential applications, especially in relation to human health, both in terms of prevention of disease and therapy (Kaur and Kapoor, 2001).

However, presence of polyphenolics alone cannot explain the protective effects of cruciferous plants against oxidative stress related chronic diseases. GLs and ITCs are also found to be strong antioxidants, not due to their direct radical scavenging capability, but by virtue of their ability to neutralize carcinogenic and toxic agents through induction of phase II detoxification enzymes (Zhang and Talalay, 1998). Thus, the study of cruciferous plants as potential antioxidants and their probable mechanism of inhibitory actions are of significant interest.

Cruciferous plants have a long history of being used as important constituents that limit growth of pathogenic microorganisms (Forter, 1940). Extracts from cruciferous plant have become increasingly popular as an alternative source of natural preservatives, as they are widely cultivated, effective and safe for human consumption (Delaquis and Mazza, 1995). There has been a renewed interest in plant antimicrobials due to emergence of drug-resistant pathogens (Okeke et al, 2005). The increase in the outbreaks of food-borne diseases caused by pathogenic and spoilage microorganisms in foods is also of concern (Perreten et al, 1998). Mainstream medicine is increasingly receptive to the use of antimicrobial and other drugs derived from plants, as traditional antibiotics have become ineffective due to emergence of drug resistance among pathogens. The antimicrobial compounds from plants may inhibit bacteria by a mechanism that is different from currently used antibiotics and may be of value in the treatment of resistant microbial strains.

Many plants belonging to Cruciferae family have been shown to possess inhibitory activity against different pathogens, especially bacterial pathogens (Hu et al, 2004; Hashem and Saleh, 1999). Among the chemical components present in crucifers that contribute significantly to health-related properties, are the degradation products of GLs, whose antimicrobial properties have been reported since 1937 (Walker et al, 1937).
Only a few of cruciferous plants have been explored for their apparent antimicrobial activity so far and this provides opportunities for the study of others.

Chemoprevention is an active cancer preventive strategy to inhibit, delay or reverse human carcinogenesis, using naturally occurring or synthetic chemical agents (Morse and Stoner, 1993). The multifactorial nature of the carcinogenic process provides numerous pathways that could be manipulated by these agents in order to inhibit or delay tumor development (Surh, 1999). It is estimated that more than two-thirds of human cancers could be averted by lifestyle modifications including dietary changes. Epidemiological studies have shown that consumption of fruits and vegetables has been associated with the reduced risk of several types of cancers (Potter and Steinmetz, 1996). Despite the substantial amount of research, an understanding of the identity of the food components that prevent cancer is not comprehensive.

Among vegetables with chemopreventive property, members of *Cruciferae* family appear to be most effective in providing protection against risk of several cancers (Hecht, 1999). The ability of cruciferous vegetables to protect against neoplastic diseases has been credited to ITCs, rather than to their parent GLs (Zhang and Talalay, 1994; Hecht, 2000). Two sets of putative mechanisms have been identified that help to explain the chemopreventive effects of ITCs. The first involves modulation of enzymes that are required for the activation or detoxification of many carcinogens (Barcelo et al, 1996). In particular, ITCs have been shown to induce the activity of phase II enzymes (e.g., glutathione S-transferase, quinone reductase and glucuronosyl transferases) and/or inhibit phase I enzymes (Zhang et al, 1992). The second set of putative mechanism involves suppression of tumor development following initiation of pre-cancerous cells. Possible mechanism of suppression includes deletion of initiated cells from genetically-damaged tissues by apoptosis, so that clonal expansion of lesion is aborted (Bonnesen et al, 2001; Chiao et al, 2002). However, the exact mechanism by which cruciferous plants serve as cancer chemopreventive agents in humans are not yet fully understood.

*Raphanus sativus* Linn, is a root vegetable of *Brassicaceae* (*Cruciferae*) family and commonly seen as a small-rooted, short season vegetable. The most popular part for eating is napiform taproot, although the entire plant is edible and aerial part can be used as a green leafy vegetable. It is widely grown in India for its culinary and medicinal purposes. Medicinal uses of *R. sativus* have been documented in India since the tenth
R. sativus is used widely throughout East Asia as fresh, soaked, brined, fermented and dried products (Carlson et al., 1985). It contains three main GLs: 4-(methylsulfinyl) butyl glucosinolate (glucoraphanin) and 4-(methylsulfinyl) but-3-enyl glucosinolate (glucoraphenin), which are present in seed (Daxenbichler et al., 1991), and 4-(methylthio)-3-butenyl glucosinolate (glucoraphasatin), which is present in root (Carlson et al., 1985). R. sativus possesses some interesting properties such as (i) sulfurous and pungent flavor (Ishii, 1991) and (ii) capacity to keep nematodes in soil under control (Brown et al., 1991). These properties can be attributed to glucoraphasatin and its corresponding isothiocyanate, 4-(methylthio)-3-butenyl isothiocyanate (MTBITC).

Pharmacological studies have shown that R. sativus root decreased blood glucose level in diabetic rats (Chaturvedi and Akala, 2001), alleviated hyperlipidemic changes in the colon mucosa of rats fed with fat rich diet (Sipos et al., 2002) and prevented formation of calculus in the urinary tract of experimental animals (Vargas et al., 1999). R. sativus root extract has shown antimutagenic activity in an UV-induced E. coli B/r WP2 mutation assay and MTBITC is identified as the principal antimutagenic principle in it (Nakamura et al., 2001). R. sativus root and sprout extracts exhibit antioxidant properties and stimulate bile flow in experimental animals (Takaya et al., 2003; Barillari et al., 2006). R. sativus sprout extract inhibits cell proliferation and induces apoptosis in cancer cells (Papi et al., 2008; Barillari et al., 2008). R. sativus has shown antimicrobial activity against Escherichia coli, Pseudomonas pyocyaneus, Salmonella typhi, Staphylococcus aureus, Bacillus subtilis, Saccharomyces cerevisiae, and Aspergillus oryzae (Abdou et al., 1972; Esaki and Onozaki, 1982; Khan et al., 1985). R. sativus leaves display spasmogenic effect in guinea – pig ileum and colon (Ghayur and Gilani 2005).

Although R. sativus is a common Indian vegetable having a great curative potential, not much research has been conducted nationally to explore its prospective
medicinal and pharmaceutical values. As of yet, there has been very limited information on the phytochemical profile and biological activity of different parts of *R. sativus* such as root, stem and leaves. In fact, in most of those literature, there is an apparent lack of data about *R. sativus* grown in India except by (i) Khan *et al* (1985), who have reported the antibacterial activity of roots, flowers and pods against bacteria such as *Staphylococcus aureus* and *Bacillus subtilis* and (ii) Shishu *et al* (2003), who have investigated the anti-genotoxic effect of sulphoraphene (glucoraphenin ITC), an isothiocyanates isolated from *R. sativus* seeds against food mutagen in Ames Salmonella/Reversion assay.

Thus the objective of the current research is the study of the phytochemical profile and biological activity of *R. sativus* of Indian origin. This is achieved by breaking down the problem into following sub-objectives:

- Assessment of antioxidant and free radical scavenging activity of the different parts of *R. sativus* such as root, stem and leaves.
- Evaluation of *R. sativus* antimicrobial action against various human pathogenic microorganisms including drug-resistant and food-borne pathogens.
- Determination of *R. sativus* ability to protect the cells against chemically-induced oxidative damage (cytoprotective effect).
- Investigation of *R. sativus* ability to induce cellular and molecular changes leading to the induction of cell death in the human cancer cell lines (chemopreventive effect).