Food Sci. Technol. Res., **14** (4), 377–382, 2008

Anticancer and Antimicrobial Activities of β-Phenylethyl Isothiocyanate in *Brassica rapa* L.

Eunyoung HONG[1] and Gun-Hee KIM[*]

[1] *Department of Food and Nutrition, Duksung Women's University, 419 Ssangmun-dong, Dobong-gu 132-714, Seoul, Korea*

Received January 11, 2006; Accepted March 26, 2008

Glucosinolates, precursors of isothiocyanates, are present in cruciferous vegetables such as the turnip (*Brassica rapa* L.). Glucosinolates are usually broken down through hydrolysis catalyzed by myrosinase released from damaged plant cells. Glucosinolates and their breakdown products, in particular isothiocyanates, have long been known to have various pharmaceutical benefits, including anticarcinogenic, antimicrobial and antioxidant properties. In this study, quantitative analyses of isothiocyanates and total glucosinolates in turnip, which was divided into three parts, were performed by UV-spectrometer, GC and GC/MS. Total glucosinolates showed no significant differences among different parts of turnip. However, the amounts of 3-butenyl and 4-pentenyl isothiocyanates in turnip leaf were higher than those in other parts. β-Phenylethyl isothiocyanate, abundant in the peel, showed the highest content in turnip. In addition, β-Phenylethyl isothiocyanate inhibited the growth of human-derived hepatoma cell line (HepG2) in a concentration-dependent manner (IC50 value of 24.5 μM), assessed by the MTT method. β-Phenylethyl isothiocyanate also exhibited antimicrobial activity against food-borne pathogens *Vibrio parahaemolyticus*, *Staphylococcus aureus* and *Bacillus cereus*. In particular, minimum inhibitory concentration (MIC) against *Vibrio parahaemolyticus* was the most efficient, at 100 μg/ml. These results suggest that the major isothiocyanate in turnip is β-phenylethyl isothiocyanate. Furthermore, β-phenylethyl isothiocyanate may have anticancer effects and antimicrobial properties against food-borne pathogens.

Keywords: glucosinolate, β-phenylethyl isothiocyanate, anticancer activity, antimicrobial activity, turnip

Introduction

Cruciferous vegetables, in particular members of the *Brassica* genus such as broccoli, cauliflower, cabbage and turnip, are a rich source of glucosinolates, which are broken down into isothiocyanates by the enzymatic action of plant-specific intestinal flora myrosinase in the body. Turnip (*Brassica rapa* L.) is a biennial cool season crop which matures in two months and can be planted in the spring, late summer or fall for roots or greens. Turnip is a root *Brassica* crop and has been used for human consumption. However, the bioactivities of the turnip have not been thoroughly investigated.

Epidemiological studies suggest that intake of cruciferous vegetables is associated with decreased risks of developing cancers (Gerber *et al.*, 2002; La Vecchia *et al.*, 2001). The underlying mechanism for the reduction of cancer by cruciferous vegetables is not clear. However, these vegetables, such as broccoli, cauliflower, brussel sprouts, cabbage, turnip, and horseradish, contain glucosinolates that are sulfur-containing secondary metabolites derived from protein and non-protein amino acids (Rosa *et al.*, 1997; Fahey *et al.*, 2001). When plant tissue is damaged, the enzyme myrosinase (β-thioglucosidase, thioglucoside glucoside glucohydrolase, EC 3.2.3.1) hydrolyzes glucosinolates (β-d-thioglucosides) into glucose, sulfate, isothiocyanates, nitrile, thiocyanate and so on (Chung *et al.*, 1996; Stoner *et al.*, 1997). The breakdown products of certain glucosinolates have been shown to protect against lung, colon, liver and stomach cancers (Vekerk *et al.*, 1998). In particular, isothiocyanates have important biological properties including anticarcinogenic activity (Fahey *et al.*, 1997; Barrett *et al.*, 1998) and activities that defend the plant against insects, fungi and microbial infections (Chew *et al.*, 1988).

*To whom correspondence should be addressed.
Email: ghkim@duksung.ac.kr

The objective of this study was to determine the qualitative and quantitative levels of glucosinolates, isothiocyanates, and soluble sugars in different parts of turnip. In addition, the study investigated the anticancer and antimicrobial activities of β-phenylethyl isothiocyanate.

Materials and Methods

Plant materials and chemicals Turnip was purchased from Gang hwa (Incheon, Korea). Turnip was cut and separated as follows. The peel of the root, consisting of the epidermis, cortex and vascular cambium, was prepared by careful slicing using a well-sharpened knife. The cutting surface was between the vascular cambium and internal parenchyma. Phosphate buffer (pH 7.0) was prepared following Gomori (1974). All other reagents of laboratory grade were purchased from Junsei Chemical (Japan). Standards of 3-butenyl isothiocyanate, 4-pentenyl isothiocyanate, and β-phenylethyl isothiocyanate were purchased from Asahi Kasei Chemicals (Japan).

Preparation of crude myrosinase Myrosinase was prepared from radish purchased from a local market in Dobong-gu, Seoul. Chilled radish roots were homogenized in a blender and filtrated with two layers of gauze. One volume of the resultant juice was added to 1.5 times volume chilled acetone and stored at 0-4°C for 5 min. The mixture was centrifuged at 3000 rpm for 15 min at 4°C. The precipitate was freeze dried and powdered. The acetone powder (crude enzyme including myrosinase) was stored at -20°C until use.

Sample preparation and quantitative analysis of soluble sugars A 10 g sample was weighed and homogenized with 30 ml of distilled water, and the resulting mixture was shaken at 120 rpm for 30 min. The mixture was centrifuged at 15000 rpm for 22 min at 4°C. The supernatant was then filtrated with Sep-pac (C18) and membrane filter (0.45 μm). The samples were used for quantitative analysis of soluble sugars by HPLC (Younglin instrument, Korea). The HPLC system consisted of a CTS-30 oven, RI detectors linked with SP-930D pump to the column (6.5 × 300 mm) fitted with a Sugar-Pac I (Waters, USA) at 70°C. Distilled water purified by Millipore Milli Q plus filtration system was used as a mobile phase at a flow rate of 0.5 ml/min.

Sample preparation of total glucosinolates and isothiocyanates A 50 g sample of divided turnip was boiled in 100 ml of 80% ethanol in a flask for 15 min in a water bath and then homogenized in a blender. This step was repeated twice using the same procedure. The extracts were filtrated and concentrated to 25 ml using an evaporator (EYELA, Tokyo, Japan) at 40°C. Concentrated samples were centrifuged at 3000 rpm at 4-5°C for 15 min. After centrifugation, the supernatant was made up to 50 ml with distilled water and 25 ml of this solution was passed through an anion exchange column (5 ml of Dower 1-X, Cl-form, 50/100 mesh, Lancaster, England). The column was washed with 50 ml water until glucose was not detected by Molish reagent. The ion exchange resin was transferred to a 50-ml Erlemmeyer flask which contained 5 ml of methylene chloride (CH_2Cl_2), 50 mg of crude myrosinase, 1 ml of 10 mM ascorbic acid, and 5 ml of 0.1 mol/l sodium phosphate buffer (pH7.0). The flask was shaken gently on a shaker for 18 h at room temperature. The concentrated samples were centrifuged at 3000 rpm at 4°C for 15 min. The methylene chloride layer (bottom part of centrifuge tube) was used for isothiocyanate analysis using gas chromatography (GC) and the water layer (upper part of centrifuge tube) was used to determine total glucosinolates.

Measurement of total glucosinolates and isothiocyanates Total glucosinolates were determined using a UV-visible spectrophotometer (Model Genesis 10vis, USA) at 505 nm by the thymol method (Brzezinski *et al.*, 1984). Sample solution, thymol reagent and H_2SO_4 were added and reacted in a boiling water bath for 35 min. A glucose standard calibration curve was used to analyze the concentration of total glucosinolates. The conditions of GC and gas chromatography/mass spectrometry (GC/MS) analysis of isothiocyanates are shown in Table 1.

Cell culture HepG2 cells were cultured in complete MEM (containing 10% FSB, 100 U/ml penicillin) in 75 cm^2 culture flasks at 37°C in 5% CO_2 unless otherwise stated (Shen *et al.*, 1999). HepG2 cells were incubated with various concentrations of β-phenylethyl isothiocyanate (5, 10, 25, 50, 75, 100 μmol/l) in the medium for 24 h at 37°C in an incubator. β-Phenylethyl isothiocyanate, lipid-soluble samples, were dissolved in medium containing 1% DMSO, then diluted to each concentration. Control samples were composed of medium with the same concentrations of DMSO without test compounds.

Assessment of cell viability Cell viability was determined using the MTT (3-(4,5-dimethylthiazol-2-yl-2,5-diphenyl tetra-sodium bromide) viability assay (Hanen *et al.*, 1989). Viability was measured by the colorimetric change using a plate reader at 540 nm (ELISA, Tokyo, Japan). IC50 (effective concentration 50%) is the concentration required for 50% inhibition in vitro.

Determination of antimicrobial activity by the disc diffusion method Antibacterial activity was investigated by disc diffusion test (Kim *et al.*, 1995). Four representative food poisoning bacterial strains (*Vibrio parahaemolyticus* (KCCM 11965), *Salmonella choleraesuis* (KCCM 40050), *Staphylococcus aureus* (KCCM 40935), and *Bacillus cereus* (KCCM 41034)) were purchased from the Korean Culture Center of Microorganisms. Two-layer plates made with 1% lower agar

Table 1. GC and GC/MS condition for determination of isothiocyanates.

	GC	GC/MS
Instrument	Agilent GC 4890	Agilent GC 6890 Agilent MSD 5973
Column	DB-5, 30 m, ID 0.53 mm, 0.5 μm	Ultra 2 5% phenyl Methyl siloxane (19091B-005 : 0.20 mm x 50 m)
Carrier gas	N_2, 1 ml/min	H_2, 1 ml/min
Split	30:1	10:1
Detector	FID	FID
Inlet temperature	280°C	280°C
Detector	280°C	280°C
Oven temperature	80°C 1 min 80°C ~180°C (8°C /min) 180°C ~255°C (30°C /min)	80°C 5 min 80°C ~180°C (8°C /min) 180°C ~255°C (30°C /min)
Injection volume	2 μl	1 μl

(Nutrient agar, Difco Co., USA) previously poured into Petri dishes and 0.8% upper agar (Nutrient agar, Difco Co.) were inoculated with individual microorganisms. β-Phenylethyl isothiocyanate was dissolved in ethanol and diluted to each concentration. Sterile filter papers were impregnated with 30 μl extracts (Aventec, 8 mm) and placed onto the culture medium. A disc impregnated with sterile water and placed in the middle of an agar plate was used for control. After 24 h of incubation at 37°C, the diameter of the clear zone around the disc was measured. All tests were performed in triplicate.

Determination of minimum inhibitory concentration (MIC) β-Phenylethyl isothiocyanate of different concentrations in ethanol (0.1 ml) were added to 10 ml of bacterial culture containing 0.1 ml bacterial strain. The optical density at 600 nm was measured at 12 h intervals for 72 h. Controls were treated with ethanol without β-phenylethyl isothiocyanate. Antimicrobial activity was observed as the minimum inhibitory concentration (MIC), defined as the lowest concentration at which the growth of strains cannot be detected through the measurement of absorbance.

Results and Discussion

Quantitative analyses of soluble sugar Levels of soluble sugar in different parts of turnip are shown in Fig. 1. Levels of glucose and fructose were higher than sucrose.

Determination of glucosinolates and isothiocyanates by GC and GC/MS The total glucosinolate contents of food samples can be measured by determining the quantity of glucose released after treatment with the enzyme; however, this does not take into account endogenous glucose. Alternatively, glucosinolates can be extracted, followed by selective clean-up that eliminates free glucose and other interfering compounds, after which the controlled enzymatic release of bound glucose is possible (Vekerk *et al.*, 1998). Figure 2 shows total glucosinolate content in different parts of turnip. The total glucosinolate content of turnip ranged from 147 to 151 μmol/100 g. Total glucosinolate contents in different parts of turnip were comparatively similar.

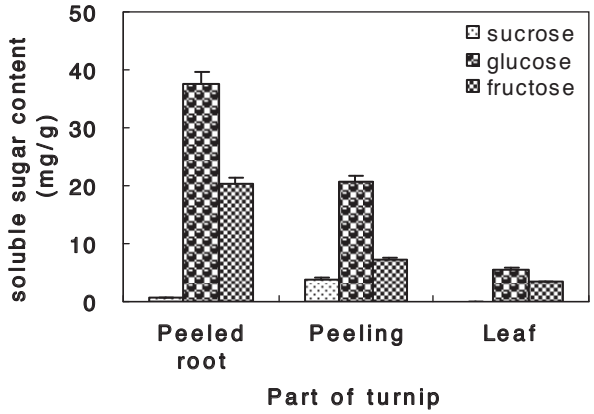

Fig. 1. Levels of soluble sugar in different parts of turnip.

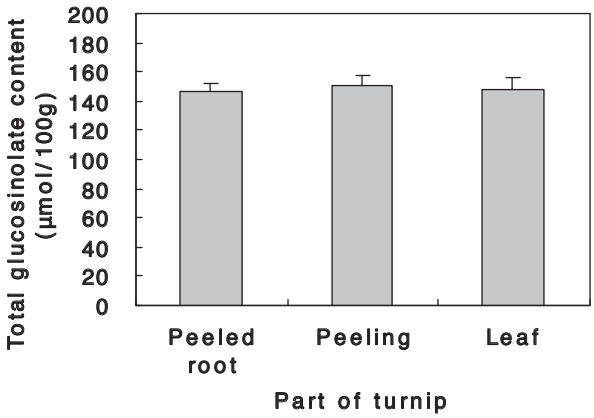

Fig. 2. Levels of total glucosinolates in different parts of turnip.

The major isothiocyanates were identified as 3-butenly, 4-pentenyl, β-phenylethyl isothiocyanate in turnip by GC/MS and GC (Fig. 3). Isothiocyanates have been shown to possess anticarcinogenic properties (Zhang *et al.*, 1994; Grubbs *et al.*, 1995) and to induce apoptosis in various cancer cell lines (Huang *et al.*, 1998; Ge *et al.*, 1999; Bonnesen *et al.*, 2001; Yang *et al.*, 2002; Nachschon-Kedmi *et al.*, 2003). Besides enhancing protection of cells against chemical carcinogens, it is well documented that exposure of cells to low micromolar concentrations of isothiocyanates leads to increased resistance to oxidative damage (Gao *et al.*, 2001). Isothiocyanates are recognized as the major inhibitor of microbial activity (Rosa *et al.*, 1997). The major isothiocyanate contained in turnip was shown to be β-phenylethyl isothiocyanate (37381 μmol/100 g dw) followed by 4-pentenyl isothiocyanate (5357 μmol/100 gdw) and 3-butenyl isothiocyanate (1858 μmol/100 gdw). The levels of 3-butenyl and 4-pentenyl isothiocyanate were much higher in the leaf than in other parts. Turnip peel showed the highest level of β-phenylethyl isothiocyanate.

Anticancer activity by β-phenylethyl isothiocyanate Various epidemiological studies have indicated that incidence of cancer is closely associated with diet (Cummings *et al.*, 1998). People who consume higher amount of fruits and vegetables have a lower risk of various types of cancers. Studies have demonstrated that fruits and vegetables contain naturally occurring anticarcinogenic compounds (Suganuma *et al.*, 1999) such as glucosinolates in cruciferous vegetables (Stoewsand *et al.*, 1995). Isothiocyanates and indoles are two major autolytic breakdown products of glucosinolates, and both exhibit anti-cancer properties (Zhang *et al.*, 1994).

HepG2 cells treated with β-phenylethyl isothiocyanate showed a concentration-dependant decrease in cell viability at 24 h with an IC50 of 24.6 μM (Fig. 4.).

Numerous investigations have shown that isothiocyanates are inhibitors of phase I enzymes and potent inducers of phase II detoxification enzymes. In addition, previous reports have shown that isothiocyanate-mediated apoptosis in vivo is associated with the removal of chemically-induced cancer cells in rodent models; however, little is known about the mechanism by which this is achieved (McDanell *et al.*, 1989; Loft *et al.*, 1992; Zhang *et al.*, 1994; Verhoeven *et al.*, 1997).

Antimicrobial effect of β-phenylethyl isothiocyanate The antimicrobial activities of β-phenylethyl isothiocyanate against *Vibrio parahaemolyticus*, *Staphylococcus aureus*, *Bacillus cereus*, and *Salmonella choleraesuis* obtained by disc diffusion method are shown in Table 4. In this study, β-phenylethyl isothiocyanate was found not to have antimicrobial activity against *Salmonella choleraesuis*. β-Phenylethyl isothiocyanate had very strong activity (> inhibition zone; i.z. of sample 20 mm) against *Vibrio parahaemolyticus* and *Staphylococcus aureus* and clear activity (i.z. of sample 10-15 mm > i.z. of control) against *Bacillus cereus* at 1000 mg/l.

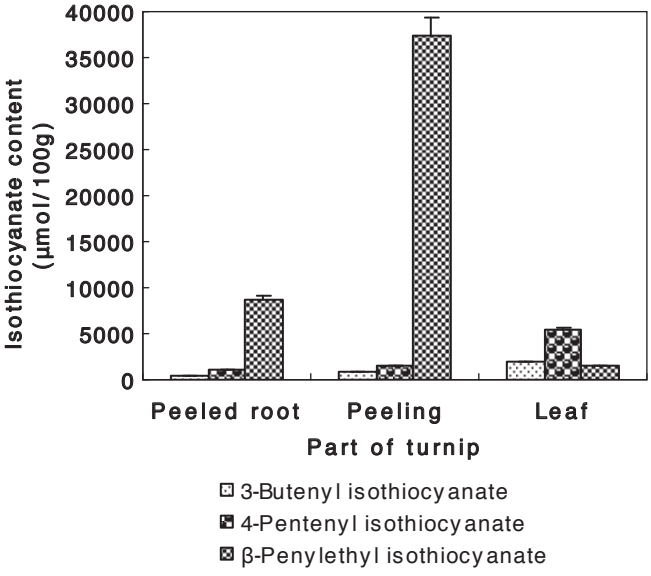

Fig. 3. Levels of 3-butenyl, 4-pentenyl and β-phenylethyl isothiocyanate in different parts of turnip. Values in figure should be simplified as 5000 to 5 mmol/100 g.

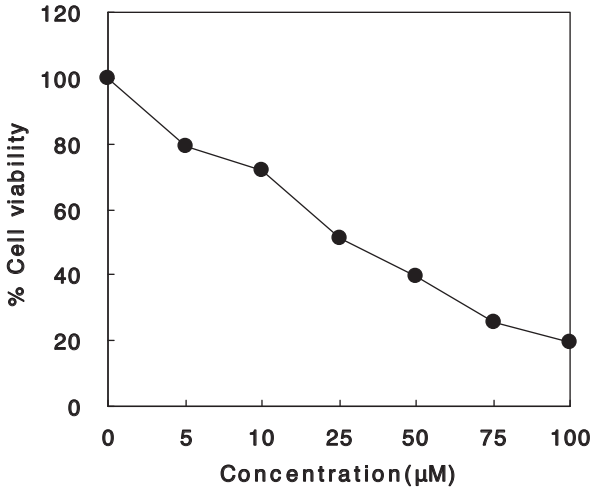

Fig. 4. Concentration dependant effects of β-phenylethyl isothiocyanate on the viability of HepG2 cells determined at 24 h using the crystal violet viability assay.

Minimum inhibitory concentration (MIC) was applied to β-phenylethyl isothiocyanate that showed an inhibitory effect against the microorganism with micro dilution broth method. The MIC value of β-phenylethyl isothiocyanate against each of the microorganisms is shown in Fig. 5. β-Phenylethyl isothiocyanate at 100 μg/ml inhibited the growth of food-borne pathogens, exhibiting antimicrobial effects at low concentra-

Table 2. Antimicrobial activity of β-phenylethyl isothiocyanate on food-borne pathogens.

Conc. (μg/ml)	Clear zone on plate (mm)			
	Vibrio parahaemolyticus	*Staphylococcus aureus*	*Bacillus cereus*	*Salmonella choleraesuis*
0	-*	-	-	-
100	11.7	-	-	-
500	13.7	14.7	-	-
1000	20.0	22.7	10.7	-
2000	31.0	30.0	16.7	-

*- : Not dectected

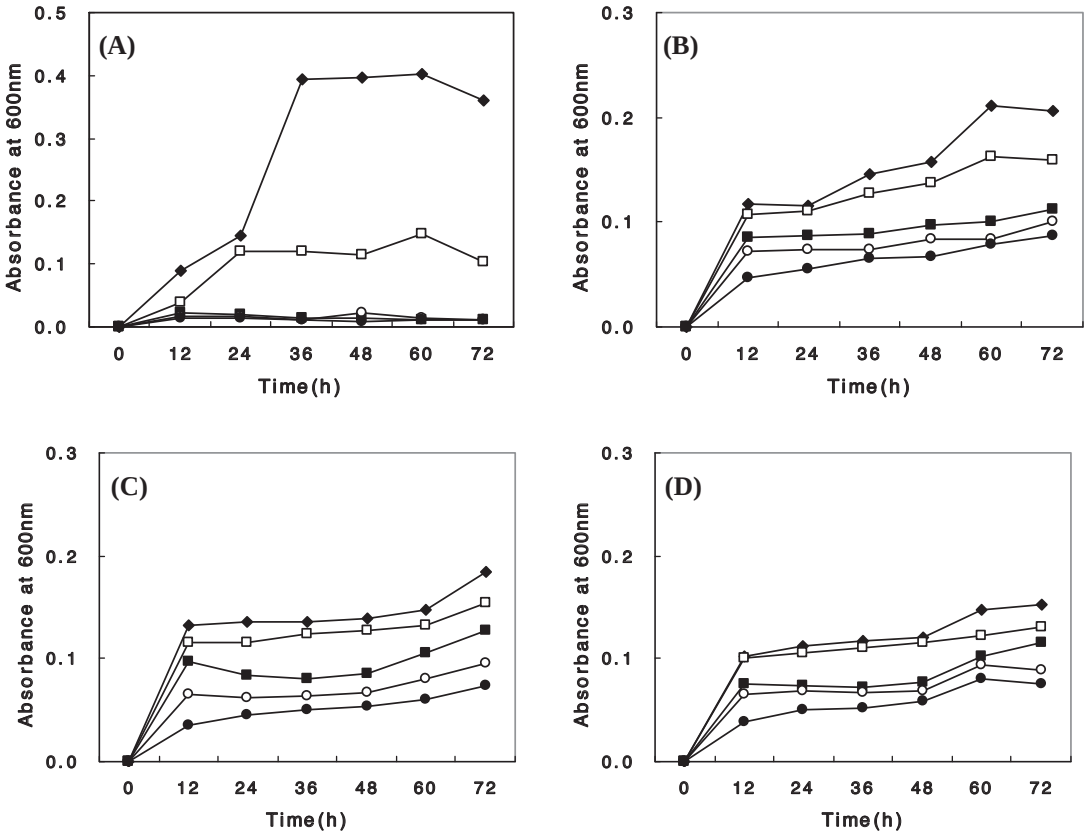

Fig. 5. Growth inhibitory effect by β-phenylethyl isothiocyanate on (A) *Vibrio parahaemolyticus*, (B) *Staphylococcus aureus*, (C) *Bacillus cereus*, (D) *Salmonella choleraesuis*. ◆, 0 mg/l; □, 100 mg/l; ■, 500 mg/l; ○, 1000 mg/l; ●, 2000 mg/l.

tion. β-phenylethyl isothiocyanate inhibited Vibrio parahaemolyticus most effectively.

Acknowledgement This research was supported by Duksung Women's University, Korea, 2007.

References

Barrett, J.E., Klopfenstein, C.F. and Leipold, H.W. (1998). Protective effects of cruciferous seed meals and hulls against colon cancer in mice. *Cancer Lett.*, **127**, 83-88.

Bonnesen, C., Eggleston, I.M. and Hayes, J.D. (2001). Dietary indoles and isothiocyanate that are generated from cruciferous from vegetables can both stimulate apoptosis and confer protection against DNA damage in human colon cell lines. *Cancer Research.*, **61**, 6120-6130.

Brzezinski, W. and Medelewski, P. (1984). Determination of total glucosinolate content in rapeseed meal with Thymol reagent. *Z Pflanzenzuchtg.*, **93**, 177-183.

Chew, F.S. (1988). Biological effects of glucosinolates, in: Cutler HG (Ed.), Biological Active Natural Products for Potential Use

in Agriculture, *The American chemical Society, Washington, DC.*, pp. 155-181.

Chung, F.L., Kelloff, G., Steele, V., Pittman, B., Zang, E., Jiao, D., Rigotty, J., Choi, C.I. and Rivenson, A. (1996). Chemopreventive efficacy of arylalkyl isothiocyanates and N-acetylcysteine for lung tumorigenesis in Fischer rats. *Cancer Res*., **56**, 772-778.

Cummings, J.H. and Bingham, S.A. (1998). Fortnightly review-diet and the prevention of cancer. *British Medical Journal.*, **317**, 1636-1640.

Fahey, J.W., Zalcmenn, A.T. and Talalay, P. (2001). The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry.*, **56**, 5-51.

Fahey, J.W., Zhang, Y. and Talalay, P. (1997). Broccoli sprouts : An exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *Proc Natl Acad Sci USA.*, **94**, 10367-10372.

Gao, X., Dinkova-Kostova, A.T. and Talalay, P. (2001). Powerful and prolonged protection of human retinal pigment epithelial cells, keratinocytes and mouse leukemia cells against oxidative damage: the indirect antioxidant effects of sulforaphane. *Proc. Natl. Acad. Sci. USA.*, **98**, 15221-15226.

Ge, X., Fares, F.A. and Yannai, S. (1999). Induction of apoptosis in MCF-7 cell by indole-3-carbinol is independent of p53 and Bax. *Anticancer Research.*, **19**, 3199-3204.

Gerber, M., Boutron-Ruault, M.C., Hercberg, S., Riboli, E., Scalbert, A. and Sies, M.H. (2002). Food and cancer: state of the art about the protective effect of fruits and vegetables. *Bull Cancer.*, **89**, 293-312.

Gomori. (1974). Buffers for pH and metal ion control. In: Buffers, *NY USA.*, pp.138

Grubbs, C.J., Steele, V.E., Casebolt, T., Juliana, M.M., Eto, I., Whitaker, L.M., Dragnev, K.H., Kelloff, G.J. and Lubet, R.L. (1995). Chemoprevention of chemically-induced mammary carcinogenesis by indole-3-carbinol. *Anticancer Research.*, **15**, 709-716.

Hanen, M.B., Nielsen, S.E. and Berg, K. (1989). Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. *Journal of Immunological Methods.*, **119**, 203-210.

Huang, C., Ma, W.Y., Li, J., Hecht, S.S. and Dong, Z. (1998). Essential role of P53 in phenethyl isothiocyanate-induced apoptosis. *Cancer Reseasch*., 58, 4102-4106.

Kim, J., Marshall, M.R. and Wei, C. (1995). Antibacterial activity of some essential oil components against five food borne pathogens. *J. of Agric. Food Chem.*, **43**, 2839-2845

La Vecchia, C., Altieri, A. and Tafani, A. (2001). Vegetables, fruits, antioxidants and cancer: a review of Italian studies. *Eur J Nutr.*, **40**, 261-267.

Loft, S., Otte, J., Poulsen, H.E. and Sørecsen, H. (1992). Influence of intact and myrosinase-treated indolyl glucosinolates on the metabolism in vivo of metronidazole and antipyrine in the rat. *Food Chem. Toxicol.*, **30**, 927-935.

McDanell, R., McLean, A.E., Hanley, A.B., Heaney, R.K. and Fenwick, G.R. (1989). The effect of feeding Brassica vegetables and intact glucosinolates on mixed-function oxidase activity in the livers and intestines of rats. *Food Chem. Toxicol.*, **27**, 289-294.

Nachschon-Kedmi, M., Yannai, S., Haj, A. and Fares, F.A. (2003). Indole-3-carbinol and 3,3'-diindolylmethane induce apoptosis in human prostate cancer cells. *Food Chem. Toxicol.*, **41**, 745-752.

Rosa, E.A.S., Heaney, R.K., Fenwick, G.R. and Portas, C.A.M. (1997). Glucosinolates in crop plants. *Horticultural Reviews.*, **19**, 9-215.

Shen, H.M., Yong, C.F. and Ong, C.N. (1999). Sodium selenite-induced oxidative stress and apotosis in human hepatoma HepG2 cells. *International Journal of Cancer.*, **81**, 820-828.

Stoewsand, G.S. (1995). Bioactive organosulfur phytochemicals in Brassica oleracea vegetables - a review. *Food and Chemical Toxicology.*, **33**, 537-543.

Stoner, G.D. and Morse, M.A. (1997). Isothiocyanates and plants polyphenols as inhibitors of lung and esophageal cancer. *Cancer Lett.*, **114**, 113-119.

Suganuma, M., Okabe, S., Kai, Y., Sueoka, N., Sueoka, E. and Fujiki, H. (1999). Synergistic effects of (-)-epigallocatechin gallate with (-)- epicatechin, sulindac, or tamoxifen on cancer-preventive activity in the human lung cancer cell line PC-9. *Cancer Research.*, **59**, 44-47.

Vekerk, R., Dekker, M. and onger, W.M.F. (1998). Glucosinolates. In: 'Natural Toxicant in Food' *Sheffield Academic Press London.*, pp. 29-53.

Verhoeven, D.T.H., Verhagen, H., Goldbohm, R.A., van den Brandt, P.A. and van Poppel, G. (1997). A review of mechanisms underlying anticarcinogenicity by brassica vegetables. *Chemico-Biological Interactions.*, **103**, 79-129.

Yang, Y.M., Conaway, C.C., Chiao, J.W., Wang, C.W., Amin, S., Whysner, J., Dai, W., Reinhardt, J. and Chung, F.L. (2002). Inhibition of benzo(a)pyrene-induced lung tumorigenesis in A/J mice by dietary N-acetylcysteine conjugates of benzyl and isothiocyanates during the postinitiation phase is associated with activation of mitogen-activated protein kinases and p53 activity and induction of apoptosis. *Cancer Research.*, **62**, 2-7.

Zhang, Y., Kensler, T.W., Cho, D.G., Posner, G.H. and Talalay, P. (1994). Anticarcinogenic activities of sulforaphane and structurally related synthetic norbornyl isothicyanates. *Proceedings of the National Academy of Sciences of the USA.*, **91**, 3147-3150.

Zhang, Y. and Talalay, P. (1994). Anticarcinoenic activities of organic isothiocyanates: Chemistry and mechanisms. *Cancer Research.*, **54**, 1976-1981.