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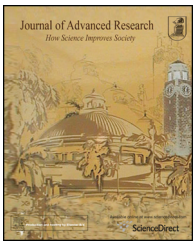

ORIGINAL ARTICLE

# Sulforaphane composition, cytotoxic and antioxidant activity of crucifer vegetables

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**KEYWORDS**

Anticancer;
Antioxidant;
Green cabbage;
*Cruciferae*;
GC-MS;
Sulforaphane

**Abstract** Sulphur compounds in sulphur rich food have been shown to significantly reduce the risk of cancer development. One such compound is sulforaphane (SF), a cancer chemopreventive agent identified in broccoli (*F. cruciferae*). In this study, SF content was assessed in extracts of several crucifer vegetables including broccoli, brussels sprout, green cabbage, red cabbage, Chinese kale and turnip, in parallel with anticancer and antioxidant activity. Among tested crucifers, cabbage demonstrated a pronounced anticancer effect against A-549 lung cancer cells, with an $IC_{50}$ value of 38 μg $mL^{-1}$, and correlated with high SF levels at 540 μg $g^{-1}$. Except for red cabbage and kale, crucifer extracts displayed moderate to weak activity in scavenging 2,2-diphenyl-1-picrylhydrazyl (DDPH) free radicals relative to vitamin E standard.



## Introduction

In recent years, a growing body of epidemiological evidence has emerged pointing to the low incidence of some types of tumours in populations, or sections thereof, whose diet includes large quantities of certain vegetables. Following this, interest in food with chemoprevention properties has been steadily increasing. Cruciferous vegetables in particular have attracted a great deal of attention, since they are rich in aromatic and aliphatic isothiocyanates [1,2]. Of these, sulforaphane [1-isothiocyanato-4-(methylsulfinyl)-butane] (Fig. 1), which has been identified in broccoli as a product of enzymatic or acid hydrolysis of the corresponding ω-(methylsulfinyl)-alkyl-glucosinolate (glucoraphanin), has recently aroused interest as a possible cancer-preventive agent [3,4]. This isothiocyanate can decrease the risk of developing different cancers such as breast cancer [5], gastric cancer [6] and skin cancer [7]. The chemoprotective effect of sulforaphane was thought to be due solely to its ability to behave as an inducer of phase II detoxification enzymes [8]. In subsequent research, however, sulforaphane was also shown to inhibit the CYP2EI isoenzyme of the cytochrome P450, thus emerging as an inhibitor of phase I enzymes [9]. The information in the current literature regarding sulforaphane has focused on its effects in broccoli, with little information on its presence in other cruciferous vegetables [10]. Other common vegetables in this plant family include

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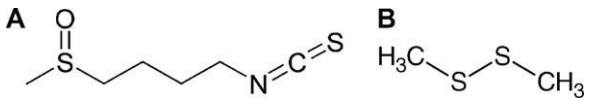


**Figure 1** Structural formula of sulforaphane (A) and dimethyldisulphide (B).

brussels sprout, cabbage, radish, Chinese kale, mustard, turnip, and cauliflower. Given the increasing interest in sulforaphane for cancer chemoprevention, we set out to screen other cruciferous vegetables for their sulforaphane content. Further, as there has been little information in the literature on their extract bioactivities relative to that of broccoli, we decided to assess their anticancer effects against human lung cancer cell line A-549 and antioxidant capacity in scavenging 2,2-diphenyl-1-picrylhydrazyl (DDPH) free radicals. Results from this study reveal further evidence for correlation between SF content and the potent cancer-preventive effects of crucifer vegetables.

## Material and methods

### *Chemicals and plant material*

Samples of crucifer vegetables including broccoli florets (*Brassica oleracea* var. *italica*), green cabbage (*Brassica oleracea L.* var. *capitata*), brussels sprout (*Brassica oleracea L.* var. *gemmifera*), Chinese kale (*Brassica oleracea* var. *acephala*), turnip leaves (*Brassica rapa* var. *rapa*) and red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*) were bought in a retail outlet in Louisville, KY, USA. After purchasing, vegetables were processed as quickly as possible. All chemical reagents were of analytical grade and all solvents were of GC and HPLC grade. R-sulforaphane was purchased from LKT Laboratories (St. Paul, MN, USA). DDPH and α-tocopherol (vitamin E) standards were purchased from Sigma–Aldrich (St. Louis, MO, USA).

### *Preparation of crucifer aqueous and organic extracts for biological activity*

Crucifer aqueous extracts were prepared according to the method of Bertelli et al. [11] with few modifications. Briefly, plant material was ground to a fine homogenous powder and kept at −80 °C with liquid nitrogen. Ten grams of powdered material was left to autolyse in 10 mL de-ionised water at room temperature for 12 h to allow complete hydrolysis of the sulphur glycosides. Aqueous extracts were then centrifuged at 5000*g* for 15 min to discard the leaves' mark. Respective plant extracts were obtained after lyophilisation. Dried aqueous extracts were then dissolved in PBS buffer pH 7.0, filter sterilised through 0.2 μm Milix filter (Millipore, MA, USA) to give stock aqueous solutions of the aqueous extract at a concentration of 50 mg $mL^{-1}$.

For preparation of organic extracts, 1 mL of plant aqueous extract prepared as above was fractionated with chloroform (2 × 1 mL) at room temperature to extract sulphur aglycone compounds. The chloroform layer was evaporated in a speed-Vac (Vacufuge TM, Eppendorf, Westbury, NY, USA) and residue was resuspended in 1 mL PBS buffer, stored at –20 °C until further testing.

### *GC-MS quantification of sulforaphane in crucifer extracts*

Concentration of sulforaphane in crucifer extracts was determined using the method reported by Matusheski et al. [12] with few modifications. About 1 mL plant aqueous extract prepared as above was extracted with chloroform (2 × 1 mL) twice at room temperature. The chloroform layers were dried over anhydrous sodium sulfate and stored at −20 °C until processed. About 1 μL of the chloroform extract was injected on a GC (Thermoquest) connected to a PolarisQ ion trap MS (ThermoFinnigan, Austin, TX, USA). The column was 0.15 mm i.d. × 50 m fused silica open-tubular, coated with 0.2 μm BPX-5 (5% phenylmethylphenylsiloxane). Injections were made in the splitless mode for 30 s, and the gas chromatograph was operated under the following conditions: injector 220 °C, column oven 40 °C for 3 min, then programmed at a rate of 12 °C/min to 180 °C, kept at 180 °C for 5 min and finally ramped at a rate of 40 °C/min to 250° C, which was then maintained for 2 min. He carrier gas linear flow velocity was maintained at 30 cm $s^{-1}$. The transfer line and ion-source temperature were adjusted at 230 °C and 190 °C, respectively. The ion trap mass spectrometer was operated in the electron ionisation mode at 70 eV and a source temperature of 180 °C. Volatile components were identified by mass spectrum matching to the EPA/NIH database and with authentic standards. Peaks were quantified by selected abundant fragments ($m/z$), calculated using MET-IDEA software to extract peak areas of individual ions characteristic of each component. For SF absolute quantification, an external standard calibration curve of sulforaphane SF (0.1–10 mM) was prepared and extracted under exact conditions.

### *Anticancer activity*

The human lung cancer A-549 cell line (ATCC#CCL-185) was obtained from the American type culture collection. Cells were maintained at 37 °C in a 5% $CO_2$ atmosphere. A-549 cells line were grown in RPMI 1640 medium (MP Biomedicals Inc., Irvine, CA, USA) containing 10% foetal bovine serum (FBS, Hyclone, Logan, UT, USA) as well as 0.2% glucose, 2 mM glutamine, 500 μg $mL^{-1}$ streptomycin, and 500 IU $mL^{-1}$ penicillin.

Exponentially growing cells were plated in 96-well microplates (Costar, Corning Inc., USA) at a density of $3 \times 10^3$ cells per well in 100 μL of culture medium, and were allowed to adhere for 16 h before treatment. Increasing concentrations of plant extract in ethanol were then added (100 μL per well). Final concentration of ethanol in the culture medium was maintained at 0.5% (v/v) to avoid solvent toxicity. Sulforaphane was used as a positive control at a concentration range from 0.5 to 100 μM. The cells were incubated for 48 h in the presence and absence of the extract. Cytotoxicity was assessed using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) according to the vendor's protocol (Promega, Madison, WI, USA). Absorbance was measured on automated 96-well microplate SpectraMax M5 (Molecular devices, CA, USA) at wavelength 570 nm. Cytotoxicity here is expressed as the concentration of plant extract inhibiting cell growth by 50% relative to cells incubated in the presence of 0.5% ethanol ($IC_{50}$ value). Each measurement was performed in triplicate.