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Relationship between Chemical Structure and Antimicrobial Activities of Isothiocyanates from Cruciferous Vegetables against Oral Pathogens

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We evaluated the potentials of 10 isothiocyanates (ITCs) from cruciferous vegetables and radish root hydrolysate for inhibiting the growth of oral pathogens, with an emphasis on assessing any structure-function relationship. Structural differences in ITCs impacted their antimicrobial activities against oral pathogens differently. The indolyl ITC (indol-3-carbinol) was the most potent inhibitor of the growth of oral pathogens, followed by aromatic ITCs (benzyl ITC (BITC) and phenylethyl ITC (PEITC)) and aliphatic ITCs (erucin, iberin, and sulforaphene). Sulforaphene, which is similar in structure, but has one double bond, showed higher antimicrobial activity than sulforaphane. Erucin, which has a thiol group, showed higher antimicrobial activity than sulforaphane, which has a sulfinyl group. BITC and iberin with a short chain exhibited higher antimicrobial potential than PEITC and sulforaphane with a longer chain, respectively. ITCs have strong antimicrobial activities and may be useful in the prevention and management of dental caries.

Keywords: Cruciferous vegetables, isothiocyanates, radish root hydrolysate, antimicrobial activity, oral pathogens

Introduction

Tooth decay is one of the most prevalent oral diseases. Bacteria are normally present in the mouth, and these bacteria convert sugar and starch into acids and cause calcified dental plaque [9]. Dental plaque control is an essential strategy for preventing dental caries. Tooth-brushing is the most accepted method for controlling plaque, but if this is not enough to remove dental plaque, antimicrobial agents are needed to kill oral pathogens [14, 15]. Facultative anaerobic bacteria, such as *Streptococcus mutans*, *S. sobrinus*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Lactobacillus casei*, and a yeast, *Candida albicans*, are the major causative pathogens associated with tooth decay in humans [3, 11, 12, 14, 15].

Various chemicals have been used for killing microorganisms from root canals. Chlorhexidine digluconate (CHX) is used widely with regard to root canals and is effective in killing oral pathogens such as gram-positive and gram-negative bacteria. However, long-term use of CHX may cause discoloration of teeth and pain in the tongue [14]. Thus, in recent years, there has been increasing interest in the use of natural compounds of dietary origin for the management of oral infectious diseases.

Cruciferous vegetables, such as broccoli, cabbage, mustard, horseradish, and radishes, may be an interesting choice because they contain compounds with potent antioxidant, antimicrobial, and anticancer activities [16, 22]. Among these natural compounds are the isothiocyanates (ITCs; R-N=C=S). ITCs are a class of compounds derived from the enzymatic hydrolysis (myrosinase) of glucosinolates (GLs), which are sulfur-containing compounds present in cruciferous vegetables [6]. Major ITCs found in cruciferous vegetables are benzyl ITC (BITC), allyl ITC (AITC), indole-3-carbinol (I3C), phenylethyl ITC (PEITC), sulforaphane, sulforaphene, iberin, and erucin, as shown in Table 1.

The antimicrobial properties of ITCs have been studied previously, mainly in connection with food preservation and plant pathogen control [1, 7, 13, 20]. BITC and AITC showed stronger antibacterial activity against gram-negative

Table 1. Chemical structure of isothiocyanates used in this study.

Common name	Side chain name	Side chain structure	MW	Main dietary source
Aliphatic ITC[a]				
Allyl ITC	2-propenyl	CH_2=CH-CH_2-	99	Cabbage and horseradish
Erucin	4-methylthiobutyl	CH_3-S-$(CH_2)_4$-	161	Turnip and kohlrabi
Hexyl ITC	hexyl	$CH_3(CH_2)_5$-	143	
Iberin	3-methylsulfinylpropyl	CH_3-SO-$(CH_2)_3$-	163	Broccoli and cabbage
Sulforaphane	4-methylsulfinylbutyl	CH_3-SO-$(CH_2)_4$-	177	Broccoli
Sulforaphene	4-methylsulfinyl-3-butenyl	CH_3-SO-CH=CH-$(CH_2)_2$-	175	Radish
Aromatic ITC				
Benzyl ITC	benzyl	C_6H_5-CH_2-	149	Wasabi and mustard
Phenyl ITC	phenyl	C_6H_5-	135	
Phenylethyl ITC	2-phenylethyl	C_6H_5-$(CH_2)_2$-	163	Watercress
Indolyl ITC				
Indole-3-carbinol	1H-indol-3-yl methanol	C_8H_6N-CH_2OH	147	All vegetables

[a]ITC, Isothiocyanates.

bacteria (*Escherichia coli*, *Erwinia carotovora*, *Pseudomonas fluorescens*) than gram-positive bacteria (*Listeria monocytogenes*, *Bacillus subtilis*) [1]. AITC and PEITC inhibited various pathogenic microorganisms (*Bacillus*, *Escherichia*, *Klebsiella*, *Listeria*, *Salmonella*, *Serratia*, and *Staphylococcus*) [13, 20]. The diethyl ether extract of horseradish (major ITC: AITC) [8] and the acetone fraction of radish root (major ITC: raphasatin) [4] demonstrated antimicrobial activities against food-poisoning bacteria.

Several studies have attempted to investigate the antibacterial activities of ITCs against oral pathogens. *Salvadora persica* (tooth brush tree) root extracts (major ITC: BITC) exhibited rapid and strong bactericidal effects against oral pathogens as well as against gram-negative bacteria (*Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Salmonella enterica*, etc), whereas gram-positive bacteria (*S. mutans*, *S. aureus*, *E. faecalis*, etc.) showed mainly growth inhibition or were unaffected [17]. The horseradish root (major ITC: PEITC and AITC) showed the strongest antimicrobial activity against *C. albicans* among facultative microorganisms, and against *F. nucleatum* among anaerobic bacteria [14]. Hexane extract of *S. persica* (major ITC: BITC) exhibited maximum antimicrobial activity against *E. faecalis* and *C. albicans* [3].

There have also been a few reports that the antimicrobial potential of ITCs depends on their chemical structure [6, 20]. Aromatic and indolyl groups show a higher antibacterial effect compared with aliphatic groups against plant pathogenic bacteria [2], foodborne pathogens and spoilage bacteria [20], and methicillin-resistant *S. aureus* isolated from diabetic foot-ulcer patients [6].

To date, the antimicrobial activity of ITCs against oral pathogens has only been assessed using a limited number of ITCs, such as BITC, AITC, and PEITC, and the relationship between the structure and relative antimicrobial activity of various types of ITCs against oral pathogens has not yet been determined. Thus, the aim of the present study was to evaluate the potentials of 10 ITCs present in cruciferous plants and radish root hydrolysate to inhibit the growth of six oral pathogens, with an emphasis on a structure-function relationship.

Materials and Methods

Chemicals and Sample

BITC, I3C, AITC, phenyl ITC (PITC), hexyl ITC (HITC), and chlorhexidine digluconate (CHX) were purchased from Sigma-Aldrich (USA). PEITC, L-sulforaphene, *R*(-) iberin, and erucin were purchased from Santa Cruz Biotechnology, Inc. (USA). DL-Sulforaphane was purchased from Calbiochem (Merck Millipore, Germany). Dimethyl sulfoxide (DMSO) was purchased from Daejung Chemistry (Korea).

Radish (*Raphanus sativus* L.) roots were purchased at a local market, and washed, sliced, and freeze-dried. Radish root powders were stored at −20°C until needed.

Preparation of Isothiocyanate Solutions

Ten ITCs were used in this study. A fresh stock solution of each ITC was prepared in 100% DMSO and stored at −20°C. Dilutions of the stock were made in the respective growth medium for each strain. The final concentration of DMSO was adjusted to 1% (v/v),

which was shown to have no discernible effect on the growth of the test strains. Tested concentrations of each ITC were as follows: 0.0312–4.000 mg/ml (BITC, AITC, HITC, and PITC), 0.0078–1.000 mg/ml (sulforaphane, sulforaphene, erucin, iberin, PEITC, and I3C), 0.0078–1.000 mg/ml (CHX), and 0.0039–0.500 mg/ml (radish root hydrolysate).

Preparation and GC/MS Analysis of Radish Root Hydrolysate

Radish root hydrolysate was prepared as follows [10]. Distilled water (8 ml) was added to freeze-dried radish root powders (0.5 g) in a 50 ml centrifuge tube. Endogenous enzymatic hydrolysis was performed at room temperature for 10 min. Dichloromethane was added and the mixture was further hydrolyzed in a shaking water bath at room temperature for 15 min. The hydrolysate was extracted three times with dichloromethane and the combined extracts were filtered through a No. 5A filter paper (Advantec, Japan) with 2 g of anhydrous sodium sulfate. The filtrate was evaporated using a rotary vacuum evaporator (Rotavapor R-124; Büchi Labortechnik AG, Switzerland) at room temperature. The dried residue was dissolved in DMSO for antimicrobial tests and in dichloromethane for GC/MS analysis. ITCs in the hydrolysis product of radish root were analyzed to clarify the main active components involved in the antimicrobial activity with an Agilent 6890N GC/5973 MSD (Agilent Technologies, USA) according to Kim *et al*. [10].

Microorganisms and Culture Media

The gram-positive bacteria *S. mutans* KCOM 1054, *S. sobrinus* KCOM 1157, *S. aureus* KCOM 1492, *E. faecalis* KCOM 1083, and *L. casei* KCTC 3109, and the yeast *C. albicans* KCTC 7965 were obtained from the Korean Collection for Oral Microbiology (KCOM, Korea) and the Korean Collection for Type Cultures (KCTC, Korea).

Brain heart infusion (BHI) broth (Becton, Dickinson and Company, USA) was used for the culture of *S. mutans*, *S. sobrinus*, and *S. aureus*. Tryptic soy broth (TSB; Becton, Dickinson and Company) was used for the culture of *E. faecalis*. Lactobacilli MRS broth (Becton, Dickinson and Company) was used for the culture of *L. casei*. Yeast malt broth (YMB; Becton, Dickinson and Company) was used for the culture of *C. albicans*. *S. mutans*, *S. sobrinus*, *S. aureus*, *L. casei*, and *E. faecalis* were incubated at 37°C, and *C. albicans* was incubated at 25°C.

Antimicrobial Activity Test

The inhibitory potential of 10 ITCs and radish root hydrolysate against growth of six test strains was assessed in terms of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using broth microdilution techniques following Clinical and Laboratory Standard Institute methods [5]. Stock culture suspensions were prepared by a direct colony suspension method and were adjusted to 0.5 McFarland standard solution turbidity (1×10^8 colony forming unit (CFU)/ml). A stock culture was diluted 1:20 to obtain a working culture containing ~5×10^6 CFU/ml.

Next, 5 µl of each ITC solution was mixed with 495 µl of sterile culture medium and 100 µl of working solution was added to each well of a 96-well round-bottom microplate (Nunc Ltd., Denmark). Then, 5 µl of working culture was inoculated into each well. The 1% DMSO in the sterile culture medium was used as a negative control, and CHX was used as a positive control. The plates were incubated under the conditions described above. The MIC was defined as the lowest concentration that completely inhibited growth of the organism in the wells, as detected by the unaided eye after incubation for 24 h.

The MBC was also measured. A loopful of each microbial culture in each 96-well round-bottom microplate at the MIC value determined above was inoculated onto a culture medium agar plate, and incubated under the same conditions described above. The MBC was defined as the lowest concentration at which no microorganism growth was detected on the agar plate.

Statistical Analysis

All experiments were performed in quadruplicates. Data are presented as the mean ± SD. Statistical analyses were performed using the SPSS ver. 18.0 software (SPSS Inc., USA) and significant differences ($p < 0.05$) among the means were determined by Duncan's multiple range test.

Results and Discussion

Relationship between Chemical Structure and Antimicrobial Activities of Isothiocyanates

The antimicrobial effects of 10 ITCs on oral pathogenic microorganisms were investigated and are reported as MICs (Table 2) and MBCs (Table 3). A selection of 10 ITCs from the three major groups, aliphatic ITCs ($n = 6$), aromatic ITCs ($n = 3$), and indolyl ITC ($n = 1$), was used. CHX was used as a positive control; it is used widely for root canal treatment and is effective in killing oral pathogens [14].

Wide variations in the susceptibility to ITCs were observed among the test strains. *S. mutans* showed highest susceptibility to the ITCs tested, with a mean MIC of 0.597 ± 0.749 mg/ml, versus *C. albicans* (0.724 ± 1.204 mg/ml), *L. casei* (0.975 ± 1.139 mg/ml), *S. aureus* (1.489 ± 1.447 mg/ml), *S. sobrinus* (1.682 ± 1.517 mg/ml), and *E. faecalis* (1.900 ± 1.449 mg/ml).

Of the 10 ITCs tested, eight ITCs showed favorable inhibition of growth against five strains, but not *E. faecalis*. *E. faecalis* strains are known to survive under harsh environments, and resist the antimicrobial actions of a number of commonly used antimicrobial agents, including calcium hydroxide [3]. I3C was the most potent (MIC range = 0.125–0.5 mg/ml), followed by BITC (0.047–1 mg/ml), PEITC (0.156–1 mg/ml), erucin (0.055–1 mg/ml), iberin (0.188–1 mg/ml), sulforaphene (0.25–1 mg/ml), sulforaphane

Table 2. Minimum inhibitory concentration (mg/ml) of pure isothiocyanates and radish root hydrolysate against oral microorganisms.

Isothiocyanates	Oral pathogens					
	S. mutans	*S. sobrinus*	*S. aureus*	*E. faecalis*	*L. casei*	*C. albicans*
Aliphatic ITC						
Allyl ITC	1.375 ± 0.750^{b}	4.000 ± 0.000^{a}	3.000 ± 1.155^{a}	>4.000	1.000 ± 0.000^{b}	0.219 ± 0.063^{de}
Erucin	0.055 ± 0.016^{c}	1.000 ± 0.000^{b}	0.625 ± 0.250^{bcd}	>1.000	1.000 ± 0.000^{b}	0.063 ± 0.000^{fg}
Hexyl ITC	2.250 ± 1.258^{a}	>4.000	>4.000	>4.000	>4.000	>4.000
Iberin	0.375 ± 0.144^{c}	1.000 ± 0.000^{b}	1.000 ± 0.000^{b}	1.000 ± 0.000^{a}	0.250 ± 0.000^{cd}	0.188 ± 0.072^{de}
Sulforaphane	0.250 ± 0.177^{c}	1.000 ± 0.000^{b}	1.000 ± 0.000^{b}	1.000 ± 0.000^{a}	0.500 ± 0.000^{c}	1.000 ± 0.000^{a}
Sulforaphene	0.250 ± 0.000^{c}	1.000 ± 0.000^{b}	1.000 ± 0.000^{b}	1.000 ± 0.000^{a}	0.250 ± 0.000^{cd}	0.344 ± 0.188^{c}
Aromatic ITC						
Benzyl ITC	0.109 ± 0.031^{c}	1.000 ± 0.000^{b}	0.500 ± 0.000^{bcd}	1.000 ± 0.000^{a}	0.250 ± 0.000^{cd}	0.047 ± 0.018^{fg}
Phenyl ITC	1.500 ± 0.577^{b}	4.000 ± 0.000^{a}	>4.000	>4.000	1.500 ± 0.577^{a}	1.000 ± 0.000^{a}
Phenylethyl ITC	0.156 ± 0.063^{c}	1.000 ± 0.000^{b}	0.750 ± 0.289^{bc}	>1.000	0.500 ± 0.000^{c}	0.250 ± 0.000^{d}
Indolyl ITC						
Indole-3-carbinol	0.125 ± 0.000^{c}	0.250 ± 0.000^{c}	0.250 ± 0.000^{cd}	>1.000	0.500 ± 0.000^{c}	0.125 ± 0.000^{ef}
Hydrolysate of radish root	0.188 ± 0.072^{c}	>0.500	>0.500	>0.500	0.500 ± 0.000^{c}	0.500 ± 0.000^{b}
Chlorhexidine digluconate (Positive control)	0.003 ± 0.000^{c}	0.005 ± 0.002^{d}	0.006 ± 0.000^{d}	0.009 ± 0.003^{b}	0.013 ± 0.000^{d}	0.022 ± 0.006^{g}

ITC, Isothiocyanates.

Values are expressed as the mean ± SD (n = 4). Means with different superscripts in the same column are significantly different by Duncan's multiple range test ($p < 0.05$).

Table 3. Minimum bactericidal concentration (mg/ml) of pure isothiocyanates and radish root hydrolysate against oral microorganisms.

Isothiocyanates	Oral pathogens					
	S. mutans	*S. sobrinus*	*S. aureus*	*E. faecalis*	*L. casei*	*C. albicans*
Aliphatic ITC						
Allyl ITC	4.000 ± 0.000^{a}	>4.000	>4.000	>4.000	>4.000	>0.500
Erucin	>0.125	>1.000	>1.000	>1.000	>1.000	0.094 ± 0.036^{ef}
Hexyl ITC	>4.000	>4.000	>4.000	>4.000	>4.000	>4.000
Iberin	0.375 ± 0.144^{b}	1.000 ± 0.000^{a}	>1.000	>1.000	>1.000	0.250 ± 0.000^{cdef}
Sulforaphane	0.250 ± 0.000^{b}	>1.000	>1.000	>1.000	>1.000	1.000 ± 0.000^{b}
Sulforaphene	0.250 ± 0.000^{b}	1.000 ± 0.000^{a}	>1.000	>1.000	>1.000	0.375 ± 0.144^{cd}
Aromatic ITC						
Benzyl ITC	0.250 ± 0.000^{b}	1.000 ± 0.000^{a}	>1.000	2.000 ± 0.000	>0.500	0.125 ± 0.000^{def}
Phenyl ITC	>4.000	>4.000	>4.000	>4.000	>4.000	1.750 ± 0.500^{a}
Phenylethyl ITC	0.313 ± 0.125^{b}	1.000 ± 0.000^{a}	>1.000	>1.000	>1.000	0.313 ± 0.125^{cde}
Indolyl ITC						
Indole-3-carbinol	0.250 ± 0.000^{b}	0.313 ± 0.125^{b}	0.500 ± 0.000	>1.000	>1.000	0.125 ± 0.000^{def}
Hydrolysate of radish root	0.313 ± 0.125^{b}	>0.500	>0.500	>0.500	>0.500	0.500 ± 0.000^{c}
Chlorhexidine digluconate (Positive control)	0.003 ± 0.000^{c}	0.005 ± 0.002^{c}	0.006 ± 0.000	>0.012	>0.025	0.025 ± 0.000^{f}

ITC, Isothiocyanates.

Values are expressed as the mean ± SD (n = 4). Means with different superscripts in the same column are significantly different by Duncan's multiple range test ($p < 0.05$).

(0.25–1 mg/ml), and AITC (0.219–4 mg/ml).

The MBCs for I3C, BITC, PEITC, erucin, and iberin were either the same or 2 × MIC and those ITCs were bactericidal. However, the MBCs for sulforaphene, sulforaphane, and AITC were less than 4 × MIC and those ITCs were bacteriostatic.

Structural differences in ITCs, such as the presence of particular functions (*e.g.*, thiol group and double bond), molecular size, or the length of a hydrocarbon chain, impacted their antimicrobial activities. ITCs are grouped into three main classes based on their chemical structures: aliphatic-, aromatic-, and indolyl-ITCs. ITC structures vary by side chains because they are derived from different amino acids, and these differences confer different biological properties [7]. Indolyl ITC (containing an indole group) was the most potent inhibitor of the growth of oral pathogens, followed by aromatic ITCs (containing a benzene ring) and aliphatic ITCs. For example, I3C (an indolyl ITC) showed high activity against five of the six oral pathogens tested (*S. mutans*, *C. albicans*, *S. aureus*, *S. sobrinus*, and *L. casei*). Sung and Lee [18] reported that I3C exerted its antimicrobial activity against clinically isolated antibiotic-resistant bacterial strains by disrupting the structure of the cell membrane.

BITC and PEITC (aromatic ITCs containing a benzene ring) exhibited potent activities against five of the six oral pathogens tested (*S. mutans*, *C. albicans*, *S. aureus*, *L. casei*, and *S. sobrinus*). Dias *et al.* [6] evaluated the MICs of purified BITC and PEITC from cruciferous plants against 15 isolates of methicillin-resistant *S. aureus* isolated from diabetic foot-ulcer patients, and BITC and PEITC were effective because they may be more capable of moving throughout bacterial structures and interfering with the bacterial redox system [6]. However, sulforaphane, sulforaphene, iberin, and erucin, all aliphatic compounds, were less effective and showed high antimicrobial activities against only three of the six oral pathogens (*S. mutans*, *C. albicans*, and *L. casei*).

The presence of a double bond in the chemical structure of the ITCs seemed to increase the antimicrobial activity. For example, sulforaphene (CH_3-SO-CH=CH-CH_2-CH_2-), which is similar in structure but has one double bond, showed higher antimicrobial activity (0.344 mg/ml) against *C. albicans* than sulforaphane (CH_3-SO-CH_2-CH_2-CH_2-CH_2-) (1.000 mg/ml).

Thiol (-S-) or sulfinyl (-SO-) groups in the chemical structure also made a difference to the antimicrobial activity. Erucin (CH_3- S-CH_2-CH_2-CH_2-CH_2-), which is similar in structure but has a thiol group, showed higher antimicrobial activities (0.055, 0.625, and 0.063 mg/ml) against *S. mutans*, *S. aureus*, and *C. albicans* than sulforaphane (CH_3-SO-CH_2-CH_2-CH_2-CH_2-), which has a sulfinyl group (0.25, 1, and 1 mg/ml, respectively).

The antimicrobial activity was also dependent on the length of the hydrocarbon chain. BITC (C_6H_5-CH_2-), with a short chain, exhibited higher antimicrobial potential (0.047, 0.109, 0.25, and 0.5 mg/ml) against *C. albicans*, *S. mutans*, *L. casei*, and *S. aureus* than PEITC (C_6H_5-CH_2-CH_2-) with a longer chain (0.25, 0.156, 0.5, and 0.75 mg/ml). A similar trend was observed for iberin and sulforaphane. Iberin (CH_3-SO-CH_2-CH_2-CH_2-) showed higher activities (0.188 and 0.25 mg/ml) against *C. albicans* and *L. casei* than sulforaphane (CH_3-SO-CH_2-CH_2-CH_2-CH_2-) (1 and 0.5 mg/ml). The chemical structure of ITCs certainly affect the capacity to interact with microbial cells, to penetrate the cell envelope, to cross the plasma membrane, and to persist and accumulate in cells [7, 20, 21, 23].

Antimicrobial Activity of Radish Root Hydrolysate

Radishes are readily available and their primary GLs are glucoraphasatin and glucoraphenin. Glucoraphasatin is the predominant GL accounting for about 80% of the total GLs, whereas glucoraphenin accounts for less than 10% in mature radishes [10]. These compounds do not have any direct biological activities in their original forms, but their ITCs, raphasatin and sulforaphene, may have antioxidant, antimicrobial, antiviral, antimutagenic, and anticancer activities [10].

The antimicrobial effects of radish root hydrolysate on oral pathogenic microorganisms were also investigated and reported in terms of MIC (Table 2) and MBC (Table 3). The MIC values of radish root hydrolysate were 0.188 mg/ml against *S. mutans*, 0.500 mg/ml against *C. albicans* and *L. casei*, and >0.500 mg/ml against *S. sobrinus*, *S. aureus*, and *E. faecalis* in terms of the concentration of raphasatin. The antimicrobial activity of radish root hydrolysate was lower than that of erucin, especially for *S. mutans* and *C. albicans*, even though raphasatin (CH_3-S-CH=CH-$(CH_2)_2$-) is similar to erucin (CH_3-S-CH_2-CH_2-CH_2-CH_2-) in chemical structure, but is very unstable [10].

The MBC for radish root hydrolysate was either the same or less than 2 × MIC, and radish root hydrolysate was bactericidal in its activity against the test strains.

Radish root hydrolysate was analyzed by GC/MS to clarify the main components involved in its antimicrobial activity (Fig. 1). Radish root hydrolysate contained 82.2% raphasatin, 15.6% sulforaphene, and 2.2% unknowns (as % peak area). The concentration (mg/ml) ratio of raphasatin

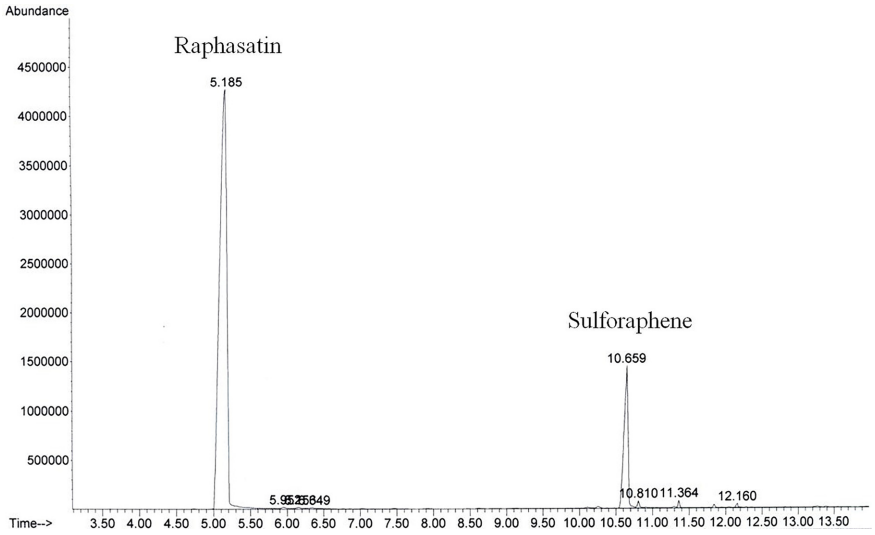

Fig. 1. GC/MS chromatogram of the radish root hydrolysate.

to sulforaphene was 1:0.16.

Beevi *et al*. [4] reported that the ethyl acetate extract of India radish root had potent antibacterial activity, with significant inhibition of pathogenic bacteria such as *B. subtilis*, *S. aureus*, *S. epidermidis*, *E. faecalis*, *S. typhimurium*, *E. coli*, *E. aerogenes*, *E. cloacae*, and *P. aeruginosa*. Shukla *et al*. [19] also reported that India radish root juice exhibited high antimicrobial activity against *Klebsiella pneumoniae, S. aureus, Pseudomonas aeruginosa, E. faecalis, and E. coli* at a MIC ranging from 0.078 to 0.625 mg/ml.

The results of this study suggest that ITCs from cruciferous vegetables have strong antimicrobial activities and may be useful as intra oral medicaments (*e.g*., as a mouthwash or root canal irrigant). Comparing the activities of ITCs showed that, within a structural group, the activity can vary dramatically, and that structural features of ITCs may be of importance, such as the presence of particular functions (*e.g*., thiol group and double bond), molecular size, or the length of a hydrocarbon chain. However, the antimicrobial susceptibilities and relationships between ITC structures and antimicrobial activities need to be evaluated further using more clinical strains.

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