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Glucosinolates and Antioxidant Properties of *Brassica Oleracea* var. *capitata* L.

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Short Communication

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Abstract

The Brassica (Brassicaceae) family includes cruciferous vegetables like cabbage, broccoli, cauliflower and mustards. Climate change is causing evident effects on the life cycle, distribution and phyto-chemical composition of the world's vegetation, including medicinal and aromatic plants. Hence, the current study was aimed to analyze the hydrolysis products of glucosinolates and other volatile compounds by Gas-chromatographic mass spectrometry (GC-MS) method and antioxidant activity by DPPH method. This study is first attempt to analyze phytonutrient content in *Brassica oleracea* var. *capitata* (white cabbage) of Indian variety, Mysuru region. The white cabbage was analyzed for relative percentage, retention time, probability, area occupied and molecular mass of each component. The relative percentage of the total identified glucosinolates including other volatile compounds was 99.99%. Glucosinolates hydrolysis products comprised about 37.39% while the rest of 62.51% were other volatile components identified. The white cabbage extracts was more effective in scavenging the DPPH significantly ($p \leq 0.05$) in a dose-dependent manner. The IC_{50} values were 85.66 mg and 15.57 mg for white cabbage extract and standard ascorbic acid respectively. The present study analyzed cabbage variety had allyl isothiocyanate, iberin and indole-3-carboxyaldehyde in higher amounts and have played a significant role in the higher antioxidant potential. Hence it can be used by the pharmacological industries for bulk production of drugs to cure related diseases.

Keywords: Glucosinolates; Cancer; Cabbage; GC-MS

Introduction

Cruciferous or *Brassica* vegetables plants belong to family cruciferae. They include Broccoli, Brussels sprouts, Cabbage, Cauliflower, Kale, Mustard and Turnips. They are rich source of sulfur-containing compounds called glucosinolates. The most commonly consumed glucosinolates within our diets are derived from methionine, such as sulforaphane, erucin and allyl-glucosinolates and aromatic glucosinolates, such as phenethylglucosinolate and benzylglucosinolate [1]. Scientific studies have been reported that diets rich in

cruciferous vegetables are associated with improved health benefits, which include protection from cancer incidence and progression from cardiovascular disease [2]. Glucosinolates are converted by plant myrosinase and gastrointestinal microflora to isothiocyanates. These isothiocyanates are biochemically active compounds with potent anticancer, anticoagulant, anti-inflammatory, anti-asthma, antibiotic, antifungal and pharmaceutical applications [3]. A number of isothiocyanates and a limited number of glucosinolates were examined effectively, block the chemical carcinogenesis in preclinical studies and also protect cells from redox imbalance that underlies the development of several chronic inflammatory diseases [4].

India has an extraordinary variety of climatic regions, ranging from tropical in the south to temperate and alpine in the Himalayan north, where elevated regions receive sustained winter snowfall. These vast climatic variations may cause a difference in the phytoconstituents of the plant species [5]. Hence, the present study attempts to analyze the hydrolysis products of glucosinolates and other volatile compounds by GC-MS method and antioxidant activity by DPPH method in the Indian variety of *Brassica oleracea* var. *capitata* (white cabbage) cultivated in Mysuru region.

Materials and methods

Sample collection

White cabbages were harvested and collected from Hasiru organic farm, Mysuru district in the month of April 2017. They were cleaned immediately and freeze dried. The dried samples were kept in deep freezer (−20°C) and used for further analysis.

Chemicals

Allyl isothiocynate and Myrosinase were purchased from Sigma-Aldrich Chemicals Co. (India). Dichloromethane (DCM) was purchased from SRL chemicals, India. All other chemicals used were of analytical grade.

Preparation of extract for GC-MS

Preparation of extraction was done as per the method described [6]. Freeze-dried white cabbage (5 g) was mixed with distilled water (250 mL), myrosinase enzyme (5units/13.12mg) and 5 mg of L-ascorbic acid and allowed to hydrolyze for 2h at ambient temperature. DCM (100 mL) was added to the mixture, shaken for 30min and separated by centrifugation for 15min at 3,500rpm.

The separated organic layer was dried over anhydrous sodium sulfate and carefully concentrated with flash evaporator. The concentrated hydrolysate was stored in a freezer (−20°C) until analysis.

One µL aliquots of the concentrated DCM extract were analyzed by GC-MS, using Thermo GC - Trace Ultra Ver: 5.0, Thermo MSDSQII. The column used was DB-35 capillary column (30×0.25 mm×0.25 µm). GC oven initial temperature was 70°C for 2 min and was programmed to 260°C at a rate of 6°C /min, and finally held at 260°C for 2 min. operating condition of GC were as follows: Helium was used as carrier gas (1 ml/min); the temperature of injector and detector was 240°C and 260°C respectively; the volume injected was 1µl in spilt mode (10:1). The mass spectra were performed at 70 eV of the mass range of 60~650. The identified glucosinolates and other volatile compositions were confirmed by comparing the spectra with published spectra from Wiley's database and literature data.

Antioxidant Assay

The ability of white cabbage extracts to scavenge 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals was determined by the Radical scavenging activity (RSA) method [7].

Statistical Analysis

The data was analyzed by ANOVA followed by Tukey's multiple comparisons test for significant differences, using SPSS 16.0 computer software. The values were considered significant when $p \leq 0.05$. IC_{50} values were calculated by Boltzmann's dose response analysis using Origin 8.1 software.

Results

The white cabbage was analyzed for relative percentage, retention time, probability, area occupied and molecular mass of each component. The relative percentage of the total identified glucosinolates including other volatile compounds was 99.99%. Glucosinolates hydrolysis products comprised about 37.39% while the rest of 62.51% were other volatile components identified as shown in the (Tables 1 & 2) respectively. The white cabbage contained the major compounds such allyl isothiocyanate (5.04%), iberin (3.85%) and indole-3-carboxyaldehyde (9.04%) in higher amounts.

Sl. No	Identified compounds	Molecular formula	Probability	Area %	RT[1] (min)	[M]+
1	Allyl Isothiocyanate	C_4H_5NS	46.87	5.09	4.34	99
2	1-Propene, 3-isothiocyanato	C_4H_5NS	46.87		4.34	99
3	Cyclopropane, isothiocyanato	C_4H_5NS	10.47		4.34	99
4	1-Butene, 4-isothiocyanato	C_5H_7NS	84.48	4.35	5.79	113
5	2-Isopropyl-4-Methyl-2,5-dihydrooxazole	$C_7H_{13}NO$	11.45	0.36	6.58	127
6	Methyl methanethiosulfoxide	$C_2H_6O_2S_2$	1.24	3.60	8.23	126
7	S-Methyl methanethiosulphonate	$C_2H_6O_2S_2$	30.11		8.23	126
8	Indole	C_8H_7N	27.41	0.88	11.92	117
9	2-tert-Butyl-4-isopropyl-5-methylphenol	$C_{14}H_{22}O$	24.89	4.19	13.97	206
10	Iberin	$C_5H_9NOS_2$	97.68	3.85	21.29	163
11	1H-Indole-3-carboxaldehyde	C_9H_7NO	32.94	9.04	25.53	145
12	1H-Indole-3-carboxaldehyde, 5-methoxy	$C_{10}H_9NO_2$	4.67	1.07	29.98	175
13	Methyl 3,7-diaza-4-indol-3'-ylmethyl-7(N)-methyl-6,8-dioxo-2-thia-cis-bicyclo[3.3.0]octyl-exo-4-carboxylate-2(S)-oxide	$C_{17}H_{17}N_3O_5S$	2.95	4.38	33.67	375
14	Di-(2ethylhexyl)phthalate	$C_{24}H_{38}O_4$	48.10	0.58	33.20	390

Table 1: GC-MS analysis of glucosinolates hydrolysis products of white cabbage.
*RT- Retention time, M – Molecular weight

Sl. No	Identified compounds	Molecular formula	Probability	Area %	RT[1] (min)	[M]+
1	Nonacosane	$C_{29}H_{60}$	21.05	33.69	36.38	408
2	Triacontane	$C_{30}H_{62}$	11.94	3.17	38.88	422
3	13-Docosenamide, (Z)	$C_{22}H_{43}NO$	65.15	3.05	39.19	337
4	E-15-Heptadecenal	$C_{17}H_{32}O$	7.91	2.70	17.70	252
5	1-Heptacosanol	$C_{27}H_{56}O$	4.97	2.70	31.41	396
6	1-Octadecene	$C_{18}H_{36}$	5.82	2.69	21.78	252
7	n-Tetracosanol-1	$C_{24}H_{50}O$	15.55	2.56	28.96	354
8	Phthalic acid, hept-4-yl isobutyl ester	$C_{19}H_{28}O_4$	5.54	1.74	22.84	320
9	Octacosyl heptafluorobutyrate	$C_{32}H_{57}F_7O_2$	3.80	1.62	34.48	606
10	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	92.79	1.53	23.91	276
11	3',5'-Dinitrobenzoic acid (1S,2R,5R,6R,9R)-2,6,10,10Tetramethyltri cyclo [7.2.0.0(2,5)] undecane-6-yl ester	$C_{22}H_{28}N_2O_6$	10.75	1.45	15.72	416
12	Hexadecane	$C_{16}H_{34}$	33.363	1.34	13.32	226
13	5à,17à-Dihydroxy-1-oxo-6à, 7 alpha.-Epoxy(22R)-witha-2,24-dienolide	$C_{28}H_{38}O_6$	30.69	0.57	24.48	470
14	1-(methylsulfonyl) methyl-2-methyldisulfide	$C_3H_8O_2S_3$	41.54	0.54	16.91	172
15	Dotriacontane	$C_{32}H_{66}$	8.29	0.50	19.97	450
16	Tetradecane	$C_{14}H_{30}$	46.80	0.49	9.65	198
17	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	$C_{16}H_{22}O_4$	7.61	0.49	24.97	278
18	8-Quinolinol	C_9H_7NO	9.44	0.46	19.43	145
19	4-Allenyl-1,4-dihydro-1-methyl-2,4,6-triphenylphosphorin-1-Oxide	$C_{27}H_{23}OP$	39.24	0.41	35.52	394

Table 2: GC-MS analysis of other volatile compounds of white cabbage.
*RT- Retention time, M – Molecular weight

The RSA of white cabbage extracts analyzed at different concentration (25–150 mg) is given in Figure 1. The white cabbage extracts was more effective in scavenging the DPPH significantly ($p \leq 0.05$) in a dose-dependent manner. The IC_{50} values were 85.66 mg and 15.57 mg for white cabbage extracts and standard ascorbic acid respectively.

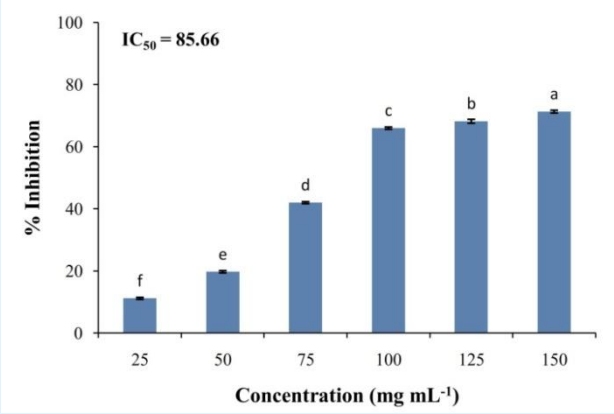

Figure 1: Antioxidant activity of white cabbage extract.

Discussion

The Gas-chromatographic results of the white cabbage showed presence of glucosinolates hydrolysis products which may have played a significant role in the higher antioxidant potential of the extract. Also, studies have shown that different glucosinolates content might attribute to the variation in genetics as compared to the cabbage cultivated in other regions [5]. Apart from genetic variation in the plant, environmental conditions, a period of harvesting, storage, processing and meal preparation may have contributed to their variation in bioactive compounds [8]. The present studies emphasis on the different range of bioactive component can help the public to make informed choices about diet and this leads to an understanding of genetic regulation of these variations, resulting in the generation of a consistent supply of nutritionally enhanced plant foods in the market.

The antioxidant activity in white cabbage extracts might be due to the presence of the bioactive constituents such as allyl isothiocyanate, iberin and indole-3-carboxyaldehyde was in higher amounts and it's confirmed by GC-MS method. Vitamin C, E and carotenoids, neutralize the free radicals before they can harm cells. Glucosinolates and their hydrolysis products are considered as indirect antioxidants, as they do not neutralize free radicals directly, but rather by modulating the activity of xenobiotic metabolizing enzymes (phase I and phase II) enzymes, which trigger the long lasting antioxidant activity. Phase I enzymes (cytochrome P450 enzymes) generally increase the reactivity of fat soluble compounds and as a consequence of this process, some reactive molecules are produced which may be more toxic than parent molecule. While phase II enzymes (glutathione-S-transferase, aldehyde reductase, S-methyl transferase, N-acetyltransferase) increase water solubility and promote the excretion of these metabolites from the body. Hence, inhibition of phase I and induction of phase II enzymes are necessary for the protection of cells against DNA damage by carcinogens and reactive oxygen species. Several mechanisms have been proposed for antioxidant activity due to presence of glucosinolates hydrolysis product (allyl isothiocyanates) as potent inducers of phase II enzymes which are important in the detoxification of electrophiles and protection against oxidative stress [9].

Conclusion

Phytochemical analysis of plants is commercially important in the pharmaceutical industries for the production of new drugs to cure various diseases. It could be concluded that different agro-climatic conditions influence the phytochemical content, composition and antioxidant potential of the white cabbage. In our present study, white cabbage showed the presence of medicinally important phyto-constituents such as allyl isothiocyanate, iberin and indole-3-carboxyaldehyde which are proven antioxidants and linked with the prevention of cancers of the colon, rectum, and breast cancer and hence this plant species can be used by the pharmacological industries for bulk production of drugs to treat related diseases.

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