Profiling of organosulphur compounds using HPLC-PDA and GC/MS system and antioxidant activities in hooker chive (*Allium hookeri*)

Sunyoung Kim, Dan-Bi Kim, Sanghee Lee, Jisu Park, Dongbin Shin and Miyoung Yoo

Division of Food Analysis Center, Korea Food Research Institute, Bundang-gu, Sungnam-si, Republic of Korea

**ABSTRACT**

This study investigates the comprehensive organosulphur compounds and evaluation of antioxidant activities in *Allium hookeri*, called hooker chive. The non-volatile and volatile organosulphur compounds were determined by high-performance liquid chromatography-photodiode array detector (HPLC-PDA) and gas chromatography-mass spectrometry (GC-MS), respectively. Among 11 non-volatile organosulphur compounds, methiin and cycloalliin were major compounds. A total of 42 volatile compounds were identified and allyl methyl sulphides and dimethyl sulphides were primarily volatiles. The total phenols ranged from 19.32 to 71.19 mg gallic acid equivalents (GAE)/g. The antioxidant capacities expressed IC$_{50}$ values ranged from 9.59 to 38.54 mg/mL for DPPH radical scavenging activity and from 0.39 to 2.36 for ferric reducing antioxidant power assay. The results were proposed as useful tools for evaluating the nutraceutical of hooker chive.

**ARTICLE HISTORY**

Received 30 November 2015
Accepted 20 February 2016

**KEYWORDS**

*Allium hookeri*; organosulphur compound; total phenol; antioxidant activity

**1. Introduction**

At the present time, the *Allium* genus has various species, distributed all over Europe, America, Africa and Asia, each differing in taste, form and colour, but close in biochemical, phytochemical and nutraceutical content. There are over 120 different documented uses of...
Allium plants, some of which have medicinal properties, such as anti-cancer properties, lipid level, blood pressure, blood cholesterol-level lowering effects, anti-glycative effect and an antioxidant capacity (Fenwick & Hanley 1985; Sendl et al. 1992; Agusti 1996; Lawson 1998; Yeh and Liu 2001; Yanagita et al. 2003; Tan et al. 2015). In recent years, there has been particular interest in the antioxidant activity because of the relationship between oxidative stress and pathologies (e.g. atherosclerosis and tumours) and ageing in which free radicals and reactive oxygen species have been implicated (Halliwell et al. 1992; Maeda et al. 1992; Mihaylova et al. 2014). These beneficial effects have been attributed to a rich content of organosulphur compounds. These compounds include γ-glutamyl peptides, such as γ-L-glutamyl-S-allyl-L-cysteine (GSAC), γ-L-glutamyl-S-(trans-1-propenyl)-L-cysteine (GSPC) and γ-L-glutamyl-S-methyl-L-cysteine (GSMC) and γ-glutamyl-phenylalanine (γGPA). S-alk(en)yl-L-cysteines as S-alk(en)yl-L-cysteine sulphoxides (ACSos) intermediate compounds are produced from γ-glutamyl peptides by γ-glutamyl peptidase and consist of (+)-S-allyl-L-cysteine (SAC), (+)-S-(trans-1-propenyl)-L-cysteine (SPC) and (+)-S-methyl-L-cysteine (SMC). Also, S-alk(en)yl-L-cysteines chemically change in ACSos by oxidase and ACSos are (+)-S-allyl-L-cysteine sulphoxide (alliin), (+)-S-(trans-1-propenyl)-L-cysteine sulphoxide (isoalliin), (+)-S-methyl-L-cysteine sulphoxide (methiin) and (1S,3R,5S)-3-carboxy-5-methyl-1,4-thiazane1-oxide (cycloalliin), respectively (Iciek et al. 2009; Beato et al. 2012).

Generally, only a few Allium plants are commonly known as edible vegetables, notably, onion (Allium cepa), garlic (Allium sativum), chive (Allium schoenoprasum), leek (Allium ampe-loprasum var. porrum) and rakkyo (Allium chinense). Recently, Allium hookeri, commonly called hooker chive, was introduced to cultivate in the southern region of South Korea (Rhyu & Park 2013). Hooker chive is a wild herb originating from India and Myanmar. The root of the plant is used in food and medicines in Asia; the leaves are used for teas and seasonings (Sangtam et al. 2012). The health benefits of hooker chive include anti-inflammatory and anti-cancer effects that are related with bioactive compounds, such as organosulphur and phenolic compounds (Bae & Bae 2012). However, hooker chive, despite the known health benefits of its various bioactive compounds, remains under-utilised in the average diet and rarely researched worldwide. Although Rhyu and Park (2013) reported the characterisation of alkyl thiosulphinates in hooker chive using HPLC-ESI-MS, only the amount of diallyl thiosulphinate (allicin) was quantified. Therefore, it is essential that the bioactive compounds in hooker chive should be quantified for the assessment as a functional food. Accordingly, this study reported the identification and quantification of organosulphur compounds in hooker chive by liquid chromatography-photodiode array detector (HPLC-PDA) system. In addition, volatile compounds in hooker chive were identified using gas chromatography-mass spectrometry (GC-MS). Moreover, antioxidant capacities were estimated by ferric reducing antioxidant power and DPPH radical scavenging assays. Thus, the overall nutraceutical values of hooker chive were investigated.

2. Results and discussion

2.1 Determination of non-volatile organosulphur compounds

For simultaneous analysis of all 11 non-volatile organosulphur compounds with an HPLC-PDA system, the analytical method was performed following Yoo et al. (2010). However, identification and quantification of non-volatile organosulphur compounds were difficult due
to complex matrix effect of hookeri chive. For this reason, the analytical methods for determination of non-volatile organosulphur compounds were conducted following Yoo et al. (2010) and Lee et al. (2013).

The non-volatile organosulphur compounds were classified into three groups: γ-glutamyl peptides, S-alk(en)yl-L-cysteines and S-alk(en)yl-L-cysteine sulfoxides (ACSO). The contents of non-volatile organosulphur compounds are shown in Table S1 (supplementary material Table S1). The levels of organosulphur compounds varied according to the domestic or imported sample. The amounts of γ-glutamyl peptides, GSMC, GSAC, GSPC and γ-GPA, were 0.13 to 2.05, 0.34 to 2.63, 0.24 to 1.67 and 0.01 to 0.15 mg/g of dry weight (DW), respectively. The leaves had significantly greater γ-glutamyl peptide levels (except GSPC) than the roots ($p < 0.05$). The imported roots had higher levels of γ-glutamyl peptides than domestic sample roots. Ichikawa et al. (2006) determined the levels of organosulphur compounds in garlic cloves: GSAC, GSPC and GSMC levels were 5.43 to 14.87, 7.89 to 15.12 and 0.78 to 1.27 mg/g of DW, respectively. The contents of GSAC and GSPC were considerably lower compared with their levels in garlic (*A. sativum* L.). However, the GSMC level of the leaves was greater than that of garlic, ranging from approximately 1.6 to 2.6-fold greater. The level of S-alk(en)yl-L-cysteines, S-methyl cysteine (SMC) and trans-1-propenyl cysteine (TPC) was also quantified. The SMC and TPC contents in the roots and leaves were 0.01 to 0.71 and 0.03 to 0.57 mg/g of DW, respectively. The concentrations of S-alk(en)yl-L-cysteines in the leaves of domestic sample were significantly greater than those in the root ($p < 0.05$). The contents of the S-alk(en)yl-L-cysteine sulfoxides (ACSO), alliin, allicin, cycloalliin and methiin were also quantified. The amounts of alliin and allicin in the root sample were 3.66 to 4.02 and 0.30 to 0.35 mg/g of DW, respectively. The alliin and allicin levels ranged from 12.44 to 25.05 and 3.25 to 4.60 mg/g of DW, respectively, in garlic (Ichikawa et al. 2006; Yoo et al. 2010). The level of allicin in the root samples was significantly lower by an approximate factor of 14 when compared with the level in garlic. The level of methiin was 3.99 to 6.39 mg/g of DW. The roots of the imported samples had the highest methiin levels compared with the levels in the roots and leaves. The cycloalliin content ranged from 1.31 to 11.62 mg/g of DW in hooker chive. In the domestic and imported samples, the levels of cycloalliin in the roots were not statistically different, whereas they were considerably higher in the leaves than in the roots ($p < 0.05$). The methiin content ranged from 1.3 to 3.0 mg/g of DW in garlic (*A. sativum* L.), onion (*A. cepa* L) and leek (*Allium porrum* L) (Yamazaki et al. 2011). Yamazaki et al. (2011) determined the cycloalliin content in seven *Allium* vegetables, such as garlic, onion, Chinese chives, rakkyo and leek, to be 0.7 to 4.0 mg/g of DW. Therefore, the amount of methiin and cycloalliin in hooker chive was relatively predominant compared with other *Allium* vegetables. Several studies have indicated various biological activities of methiin, such as anti-oxidative and anti-diabetic properties and having a hypolipidemic effect (Kumari et al. 1995; Kumari & Augusti 2002; Dini et al. 2008). Cycloalliin has been reported to exhibit biological properties, including fibrinolytic activity, reduction of serum triglycerols and induction of quinine reductase in hepatic cells (Agarwal et al. 1977; Xiao & Parkin 2002; Yanagita et al. 2003). Considering the biological activities of methiin and cycloalliin, hooker chive has promising potential as nutraceutical ingredient.
2.2 Identification of volatile organosulphur compounds

Solid-phase micro extraction (SPME) was combined with GC-MS to efficiently investigate the volatile compounds of hooker chive. SPME minimizes preparation time, solvent use and equipment requirements. It has been widely used in combination with GC and GC/MS, and has been successfully applied to the extraction of volatile compounds from diverse food sample (Kataoka et al. 2000). Therefore, we identified volatile compounds extracted by SPME. The identification of each volatile compound was confirmed by comparing its mass spectral data and retention time with the Wiley 275&7N mass spectral database.

Table S2 lists the volatile compounds, their peak area and RIs on a DB-WAX column identified in the roots and leaves of hooker chive. A total of 42 volatile compounds were identified by SPME, including 21 sulphides, 9 aldehydes, 2 vinyldithiins, 3 ketones and 7 miscellaneous compounds. There were significant differences in the compositions of the volatile compounds between the roots and leaves and between the domestic and imported samples. Aldehydes can be produced by the decarboxylation of amino acids and deamination by mono amine oxidase (Gatfield 1999). The levels of aldehyde, 2-butenal and hexanal in the domestic and imported roots were higher than those in leaves. The area values of 2-methyl-2-butenal and 2-methyl-2-pentanal in the roots were significantly greater than those in the leaves. These compounds can be formed by an aldol condensation and a subsequent dehydration of two molecules of propanol (Boelens et al. 1971). However, 2-methyl butanal, 3-methyl butanal and nonanal could be found in the leaves. The predominant volatile group in the roots and leaves was sulfides, containing 83.1 to 91.2 area%. The main sulphides in the roots were allyl methyl sulphides, such as allyl methylmonosulphide, allyl methyl disulphide, allyl methyl trisulphide and allyl sulphides, including allyl monosulphide, allyl disulphide and allyl trisulphide, whereas these compounds were observed rarely in the leaves. These compounds are related to allicin, which decomposed in the oven of a gas chromatograph, at moderate temperatures. Brodnitz et al. (1971) reported that the allicin is primarily transformed to allyl sulphides, including allyl monosulphide, allyl disulphide and allyl trisulphide. As an intermediate, allyl mercaptan and allyl alcohol were formed during the transformation of allicin (Block 1992). In addition, vinyldithiins are known to be major degradation products of allicin and were generated as artefacts during the gas chromatography analysis of allicin. Therefore, the identification of these compounds in the roots indicated the presence of allicin. Furthermore, these compounds produce a characteristic garlic-like odour. Otherwise, dimethyl sulphides, such as dimethyl disulphide and dimethyltrisulphide, and methyl propenyl disulphide isomers were the major volatiles present in the leaves. In particular, the dimethyl sulphides have a characteristic odour commonly described as cabbage and beet like when cooked (Parliment et al. 1997). Additionally, the hydrolysis of the S-methyl-\(\text{L}\)-cysteinesulphoxides(ACSos), isoalliin, cycloalliin and methiin, and most of the ACSos resulted in the production of pyruvic acid, ammonia and dimethyl sulphides. In addition, the propenyl-containing sulphides, such as methyl propenyl sulphide isomers and propenyl propysulphideisomers possess the flavour of cooked onions (Brodnitz et al. 1971). Overall, the composition of the volatiles of the imported and domestic samples varied, i.e. the volatiles were influenced by the amount of organosulphur compounds, the cultivation and storage conditions. Therefore, we suggest that our data on volatile compounds may be useful as chemical markers for the quality control of hooker chive.
2.3. Antioxidant activities

An antioxidant, which can quench reactive free radicals, can prevent the oxidation of other molecules and may, therefore, have health-promoting effects in the prevention of diseases (Kim et al. 2003). The DPPH radical is one of the most commonly used substrates for the rapid evaluation of antioxidant capacity because of its stability in radical form and the simplicity of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical assay. In the DPPH radical assay, the investigated extracts act as hydrogen atoms or electron donors in the transformation of the DPPH radical into its reduced, stable, purple-coloured radical and subsequently, a yellow colour indicating 50% reduction (Bozin et al. 2008). Therefore, we determined the total antioxidant activity as evaluated by DPPH radical scavenging activity.

The ability of the examined hooker chive to scavenge radicals is shown in Table S3. The following IC$_{50}$ values were found: 9.59 mg/mL for leaf extracts, 35.11 mg/mL for root extracts from imported sample and 38.54 mg/mL for root extracts from domestic sample. The majority of antioxidant capacities from plants are derived from phenolic compounds. Similar to the results of total phenolic contents, the leaves showed the highest antioxidant capacity.

Ferric reducing antioxidant power assay (FRAP), which provides reliable and reproducible results, measures the ability of an antioxidant to reduce the ferric tripyridyltriazine (Fe$_3$+-TPTZ) complex to a blue-coloured ferrous tripyridyltriazine (Fe$_2$+-TPTZ) complex by antioxidants and chemical reductants (Benzie & Strain 1996). In this study, FRAP activity of hooker chive obtained is shown in Table S3. Importantly, the activity of leaf extracts (2.36) was significantly higher ($p < 0.05$) than the activity of root extracts (Cheongju: 0.39 and Myanmar: 0.44).

In addition, a highly significant correlation was observed between the total phenolic contents and antioxidant activities involving DPPH radical scavenging activity ($R = 0.998$) and FRAP activity ($R = 0.999$). Although antioxidant capacity significantly correlated with phenolic compounds, this effect did not always relate with the presence of large quantities of phenolics. The antioxidant activities in Allium tissue extracts have been of particular interest, because the thiosulfinate or related organosulfur compounds are primarily responsible for the observed antioxidant effects (Kourounakis & Rekka 1991; Rekka & Kourounakis 1994; Prasad et al. 1995). In this study, good correlation between antioxidant activity and cycloalliin concentration in hooker chive was observed with a high significance level ($R = 0.992$). Therefore, the further study regarding the antioxidant functions of organosulfur compounds using various antioxidant activity assays would be performed.

3. Conclusion

This study is the first to report the comprehensive organosulfur compound profiles of A. hookeri, as obtained by an HPLC-PDA system and GC-MS. Our data show that the non-volatile organosulfur compounds of hooker chive were mainly composed of methiin and cycloalliin. The volatile compounds were mainly allyl sulfides and propenyl containing sulphones compound. Additionally, antioxidant activities were evaluated via DPPH radical scavenging assay and FARP activity. As a result, the antioxidant activities of hooker chive were significantly powerful. These results correlated with total phenolic and cycloalliin. However, the antioxidant function of cycloalliin will be researched in this regard.
Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research was supported by the Korea Food Research Institute [grant number E0143033672].

References

Agarwal RK, Dewar HA, Newell DJ, Das B. 1977. Controlled trial of the effect of cycloalliin on the fibrinolytic activity of venous blood. Atherosclerosis. 27:347–351.

Agusti K. Therapeutic values of onion (Allium cepa L.) and garlic (Allium sativum L.). 1996. Indian J Exp Biol. 34:634–640.

Bae GC, Bae DY. 2012. The anti-inflammatory effects of ethanol extract of Allium Hookeri cultivated in South Korea. Korea J Herbol. 27:55–61.

Beato VM, Sánchez AH, de Castro A, Montaño A. 2012. Effect of processing and storage time on the contents of organosulfur compounds in pickled blanched garlic. J Agric Food Chem. 60:3485–3491.

Benzie IF, Strain JJ. 1996. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. Anal Biochem. 239:70–76.

Block E. 1992. The organosulfur chemistry of the genus Allium – implications for the organic chemistry of sulfur. Angew Chem Int Ed Engl. 31:1135–1178.

Boelens M, De Valois PJ, Wobben HJ, Van der Gen A. 1971. Volatile flavor compounds from onion. J Agric Food Chem. 19:984–991.

Bozin B, Mimica-Dukic N, Samojlik I, Goran A, Icgi R. 2008. Phenolics as antioxidants in garlic (Allium sativum L., Alliaceae). Food Chem. 111:925–929.

Brodnitz MH, Pascale JV, Van Derslice LV. 1971. Flavor components of garlic extract. J Agric Food Chem. 19:273–275.

Dini I, Tenore GC, Dini A. 2008. S-alkenyl cysteine sulfoxide and its antioxidant properties from Allium cepa var. tropeana (red onion) seeds. J Nat Prod. 71:2036–2037.

Fenwick GR, Hanley AB. 1985. The genus Allium – part 1. Crit Rev Food Sci Nutr. 22:199–271.

Gatfield IL. 1999. Flavor chemistry, thirty years of progress: biotechnological production of natural flavor materials. New York, NY: Kluwer academic Plenum publisher.

Halliwell B, Gutteridge JM, Cross CE. 1992. Free radicals, antioxidants, and human disease: where are we now? J Lab Clin Med. 119:598–620.

Ichikawa M, Ide N, Yoshida J, Yamaguchi H, Ono K. 2006. Determination of seven organosulfur compounds in garlic by high-performance liquid chromatography. J Agric Food Chem. 54:1535–1540.

Iciek M, Kwiecień I, Włodek L. 2009. Biological properties of garlic and garlic-derived organosulfur compounds. Environ Mol Mutagen. 50:247–265.

Kataoka H, Lord HL, Pawliszyn J. 2000. Applications of solid-phase microextraction in food analysis. J Chromatogr A. 880:35–62.

Kim DO, Jeong SW, Lee CY. 2003. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chem. 81:321–326.

Kourounakis PN, Recka EA. 1991. Effect on active oxygen species of allylii and Allium sativum (garlic) powder. Res Commun Chem Pathol Pharmacol. 74:249–252.

Kumari K, Augusti KT. 2002. Antidiabetic and antioxidant effects of S-methyl cysteine sulfoxide isolated from onions (Allium cepa Linn) as compared to standard drugs in alloxan diabetic rats. Indian J Exp Biol. 40:1005–1009.

Kumari K, Mathew BC, Augusti KT. 1995. Antidiabetic and hypolipidemic effects of S-methyl cysteine sulfoxide isolated from Allium cepa Linn. Indian J Biochem Biophy. 32:49–54.

Lawson LD. 1998. Phytomedicines of Europe chemistry and biological activity: garlic: a review of its medicinal effects and indicated active compounds. Washington, DC: American chemical society.
Lee HJ, Suh HJ, Park YH. 2013. Utilization of hydrolytic enzymes for the extraction of cycloalliin from garlic (*Allium sativum* L.). Process Biochem. 48:1111–1117.

Maeda H, Katsuki T, Akaike T, Yasutake R. 1992. High correlation between lipid peroxide radical and tumor-promoter effect: suppression of tumor promotion in the Epstein-Barr virus/B-Lymphocyte system and scavenging of alkyl peroxide radicals by various vegetable extracts. Jpn J Cancer Res. 83:923–928.

Mihaylova DS, Lante A, Tinello F, Krastanov Al. 2014. Study on the antioxidant and antimicrobial activities of *Allium ursinum* L. pressurised-liquid extract. Nat Prod Res. 28:2000–2005.

Parliment TH, Kolor MG, Maing IY. 1997. Identification of the major volatile components of cooked beets. J Food Sci. 42:1592–1593.

Prasad K, Laxdal VA, Yu M, Raney BL. 1995. Antioxidant activity of allicin, an active principle in garlic. Mole Cell Biochem. 148:183–189.

Rekka EA, Kourounakis PN. 1994. Investigation of the molecular mechanism of the antioxidant activity of some *Allium sativum* ingredients. Pharmazie. 49:539–540.

Rhyu DY, Park SH. 2013. Characterization of alkyl thiosulfinate in *Allium hookeri* root using HPLC-ESI-MS. J Korean Soc Appl Biol Chem. 56:457–459.

Sangtam TL, Jamir NS, Deb CR, Jamir S. 2012. A study on the medicinal plants used by the Sangtam Naga Tribe in Kiphire district, Nagaland, India. Int J Ayurveda & Altern Med. 2:267–275.

Sendl A, Elbl G, Steinke B, Redl K, Breu W, Wagner H. 1992. Comparative pharmacological investigations of *Allium ursinum* and *Allium sativum*. Planta Med. 58:1–7.

Tan D, Zhang Y, Chen L, Liu L, Zhang X, Wu Z, Bai B, Ji S. 2015. Decreased glycation and structural protection properties of γ-glutamyl-S-allyl-cysteine peptide isolated from fresh garlic scales (*Allium sativum* L.). Nat Prod Res. 29:2219–2222.

Xiao H, Parkin KL. 2002. Antioxidant functions of selected *Allium* thiosulfinates and S-alk(en)yl-L-cysteine sulfoxides. J Agric Food Chem. 50:2488–2493.

Yamazaki Y, Iwasaki K, Mikami M, Yagihashi A. 2011. Distribution of eleven flavor precursors, S-alk(en)yl-L-cysteine derivatives, in seven *Allium* vegetables. Food Sci Technol Res. 17:55–62.

Yanagita T, Han SY, Wang YM, Tsuruta Y, Anno T. 2003. Cycloalliin, a cyclic sulfur imino acid, reduces serum triacylglycerol in rats. Nutrition. 19:140–143.

Yeh YY, Liu L. 2001. Cholesterol-lowering effect of garlic extracts and organosulfur compounds: human and animal studies. J Nutr. 131:989S–993S.

Yoo MY, Lee SH, Lee SI, Seog HM, Shin DB. 2010. Validation of high performance liquid chromatography methods for determination of bioactive sulfur compounds in garlic bulbs. Food Sci Biotechnol. 19:1619–1626.