Note

Stimulatory Effects of 6-Methylsulfinylhexyl Isothiocyanate on Cultured Human Follicle Dermal Papilla Cells

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Wasabi is a member of the *Brassicaceae* family and produces various isothiocyanates (ITCs). Because 6-methylsulfinylhexyl ITC (6-MSITC) is stable among wasabi ITCs, it has potential as a functional compound. Hair loss can have psychological and social effects on an individual because of its impact on appearance. In this study, we investigated the stimulatory effects of 6-MSITC by culturing on human follicle dermal papilla cells (DPCs). Results showed that 6-MSITC significantly promoted DPC proliferation and upregulated vascular endothelial growth factor (VEGF) mRNA levels compared to control. Furthermore, 6-MSITC significantly upregulated adenosine A2b receptor (ADORA2b) mRNA levels. Previous studies have reported that VEGF was induced through ADORA1 and ADORA2 pathways. We suggest that 6-MSITC stimulates hair growth on DPCs related to the upregulation of ADORA2b mRNA level. Thus, 6-MSITC may be used as a functional compound.

Keywords: hair growth, cell proliferation, vascular endothelial growth factor, human follicle dermal papilla cells, wasabi, 6-methylsulfinylhexyl isothiocyanate

Introduction

Hair loss, such as hair regression and balding, can have psychological and social effects on an individual because of its impact on appearance; thus, the condition is distressing to many people. To address this, functional food extracts and their constituents that stimulate hair growth have been studied (Esfandiari and Kelly, 2005; McElwee *et al.*, 2003; Tsuruki *et al.*, 2005) and developed.

The hair follicle is a skin appendage that is composed of various cells, such as dermal papilla cells (DPCs), hair matrix, melanocytes, and hair follicle stem cells, which stimulate hair growth. DPCs, located at the base of the hair follicle, have an essential function in the control of hair growth and hair cycling by releasing molecular mediators such as growth factors. Therefore, compounds capable of controlling DPCs may be useful as hair growth inducers.

Human follicle DPCs express vascular endothelial growth

factor (VEGF, commonly referred to as VEGF-A) (Lachgar *et al.*, 1996). VEGF is an important angiogenic factor that forms perifollicular capillaries, which disappear during the telogen phase (Lachgar *et al.*, 1996). VEGF also acts on DPCs as an autocrine growth factor to promote cell proliferation of human DPCs through VEGF receptor-2 (VEGFR2) (Li *et al.*, 2012). Therefore, VEGF may stimulate hair growth either by forming perifollicular capillaries or by promoting DPC proliferation.

Wasabi [*Wasabia japonica* (Miq.) Matsum. syn. *Eutrema japonicum* (Sieb.) Maxim.] is a plant of Japanese origin (Yamane *et al.*, 2016) that belongs to the *Brassicaceae* family. Grated wasabi rhizome, which contains various isothiocyanates (ITCs) (Etoh *et al.*, 1990; Kumagai *et al.*, 1994), is a popular condiment in Japan. Most ITCs are pungent and flavorful compounds and are the source of the wasabi flavor. 6-Methylsulfinylhexyl ITC (6-MSITC) is particularly stable among ITCs in wasabi because it has a weak wasabi flavor

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(Etoh *et al.*, 1990). In addition, 6-MSITC is the most abundant ITC in wasabi after allyl ITC (AITC; Etoh *et al.*, 1990). Morimitsu *et al.* (2002) reported that when orally administered, 6-MSITC was absorbed and entered the circulatory system in Wistar rats. Although 6-MSITC absorption has not been reported in humans, 4-MSITC (commonly referred to as sulforaphane), a 6-MSITC analog, was reported to be absorbed in humans following oral intake of broccoli sprout extract containing 4-MSITC (Atwell *et al.*, 2015). Thus, 6-MSITC may also be absorbed in humans and be useful as a functional compound.

6-MSITC has been studied for its physiological functions, including antioxidative (Hou *et al.*, 2011, Morimitsu *et al.*, 2002, Yamada-Kato *et al.*, 2017), neuroprotective (Trio *et al.*, 2016), antiplatelet (Morimitsu *et al.*, 2000), anticancer (Morimitsu *et al.*, 2000; Watanabe *et al.*, 2003), detoxifying (Morimitsu *et al.*, 2000; Toyama *et al.*, 2011), anti-inflammatory (Uto *et al.*, 2005), antidiabetic (Fukuchi *et al.*, 2004; Yoshida *et al.*, 2011), and antiallergenic properties (Nagai and Okunishi, 2009; Yamada-Kato *et al.*, 2012). However, the stimulatory effects of 6-MSITC and other ITCs on hair growth have not been reported. Broccoli is a member of the *Brassicaceae* family and contains 4-MSITC. Although broccoli sprout extract has been reported to stimulate hair growth on DPCs, the key compound remains unknown (Tsuzuki *et al.*, 2016).

In this study, to utilize 6-MSITC as a functional compound, we investigated its stimulatory effects on cultured human follicle DPCs.

Materials and Methods

Reagents 6-MSITC was synthesized by the oxidation of 6-methylthiohexyl ITC (Ogawa & Co., Ltd., Tokyo, Japan) according to the method described by Murata *et al.* (2004). The purity of 6-MSITC was determined to be 96% by quantitative nuclear magnetic resonance spectroscopy (data not shown). The chemical structure of 6-MSITC is shown in Fig. 1. Minoxidil was purchased from Sigma (St. Louis, USA). Hair follicle DPC growth medium (TMTPGM-250) was purchased from Toyobo Co., Ltd. (Osaka, Japan). Cell Count Reagent SF was purchased from Nacalai Tesque, Inc. (Kyoto, Japan).

Cell culture Human hair follicle DPCs (CA60205a) were purchased from Cell Applications, Inc. (San Diego, USA). For DPCs, subculture number 3 or less is guaranteed. In this study, DPCs with subculture number 2 or less were used. Cells were cultured on type I collagen-coated flasks in hair follicle DPC growth medium at 37°C in a 5% CO₂ incubator.

Cell viability Cell viability was determined by the WST assay using 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfonyl)-2H-tetrazolium (WST-8). DPCs were suspended at 5.0×10^4 cells/mL in hair follicle DPC growth medium. The cell suspension was transferred to 96-well type I collagen-coated plates (100 µL/well) and incubated at 37°C for

Fig. 1. Chemical structure of 6-MSITC.

1 d in a 5% CO₂ incubator. The medium was removed from approximately 50% of confluent cell cultures, and then 100 µL of hair follicle DPC growth medium with 6-MSITC was added and incubated at 37°C for 3 days in a 5% CO₂ incubator. After removing the medium, the cells were then washed with phosphate-buffered saline (PBS), and then 100 µL of the hair follicle DPC growth medium with Cell Count Reagent SF (final concentration 10%) was added and incubated at 37°C in a 5% CO₂ incubator. After 30 and 90 min, absorption at 450 nm was measured with the reference wavelength at 595 nm using a plate reader (Precision microplate reader; Molecular Devices Corporation, San Jose, USA). The absorption change per time unit was calculated to determinate cell viability.

Cell proliferation assay Cell proliferation was determined using the WST assay. DPCs were suspended at 4.0×10^4 cells/mL in hair follicle DPC growth medium. The cell suspension was transferred to 48-well type I collagen-coated plates (300 µL/well) and incubated at 37°C for 1 d in a 5% CO₂ incubator. The medium was removed from cell cultures, and then 300 µL of human follicle DPC growth medium with test samples was added and incubated at 37°C for 1 or 3 d in a 5% CO₂ incubator. After 3 d, DPCs were observed with a phase-contrast microscope. After removing the medium, the cells were washed with PBS, 300 µL of hair follicle DPC growth medium with Cell Count Reagent SF (final concentration 10%) was added and the cells were incubated at 37°C in a 5% CO₂ incubator. After 30 and 90 min, absorption at 450 nm was measured with the reference wavelength at 595 nm using a plate reader. The absorption change per time unit was calculated to determinate cell proliferation.

Quantitative real-time polymerase chain reaction (PCR) DPCs were suspended at 3.33×10^5 cells/mL in hair follicle DPC growth medium. The cell suspension was transferred to 48-well type I collagen-coated plates (300 µL/well) and incubated at 37°C for 1 d in a 5% CO₂ incubator. The medium was removed from 100% confluent cell cultures, 300 µL of hair follicle DPC growth medium with test samples was added, and the cells were incubated at 37°C for 2 h in a 5% CO₂ incubator. After washing the cells with PBS, total RNA was extracted from the cells and reverse transcribed into cDNA using the FastLane Cell cDNA kit (Qiagen, Tokyo, Japan) according to the manufacturer's instructions. Real-time PCR reactions were performed using the LightCycler 96 system (Roche Diagnostics K. K., Tokyo, Japan) with SYBR *Premix Ex Taq* (Tli RNaseH Plus; Takara Bio Inc., Shiga, Japan). After DNA polymerase was activated by heating at 95°C for 30 s, PCR cycling began with 40 cycles at 95°C for 10 s and 60°C for 30 s. PCR primer

Fig. 2. Effects on DPC viability. DPCs were preincubated with 6-MSITC at 37°C for 3 days. Cells were then supplemented with Cell Count Reagent and incubated for 30 and 90 min. The absorption change per time unit was calculated to determinate cell viability. Data are expressed as mean $\pm$ SD (n = 3).

nucleotide sequences were as follows: VEGF forward: 5'-aaagc attgtttgtacaagatccg-3', reverse: 3'-ctgtcacatctgcaagtacgttcg-5'; adenosine A2b receptor (ADORA2b) forward: 5'-caateccattgtct atgcttaccgg-3', reverse: 3'-gctgtaccccagcctgaccattccc-5'; glyceraldehyde 3-phosphate dehydrogenase (GAPDH) forward: 5'-catccctgcctctactggcgtgcc-3', reverse: 3'-ccaggatgcccttgaggg ggcctc-5'. GAPDH was used for the internal control.

Statistical analysis Results are expressed as mean $\pm$ standard deviation. The results were tested by an ANOVA, followed by Turkey's test to identify significant differences. A level of $p < 0.05$ was considered statistically significant.

Results and Discussion

Cell viability was remarkably decreased by $> 8 \mu\text{M}$ 6-MSITC in the WST assay, indicating cytotoxic effects (Fig. 2). Based on the cell viability results, the stimulation of hair growth was assessed at a maximum concentration of $2 \mu\text{M}$ 6-MSITC. The incubation time of the cell viability assay was 3 d, which is the longest incubation time of a hair growth stimulatory assay. No cytotoxic effect was detected at $30 \mu\text{M}$ minoxidil as the positive control (data not shown).

Cell proliferation was also determined using the WST assay. Concentrations of 0.08 – $2 \mu\text{M}$ 6-MSITC and $30 \mu\text{M}$ minoxidil significantly promoted cell proliferation compared to the control (Fig. 3A and 3B). 6-MSITC at $0.4 \mu\text{M}$ significantly promoted cell proliferation compared to 0.08 and $2 \mu\text{M}$ 6-MSITC and $30 \mu\text{M}$ minoxidil after 1 day (Fig. 3A), whereas 0.08 – $0.4 \mu\text{M}$ 6-MSITC significantly promoted cell proliferation compared to $2 \mu\text{M}$ 6-MSITC and $30 \mu\text{M}$ minoxidil after 3 d (Fig. 3B).

In observation with a phase-contrast microscope, the number of viable cells in the control and $0.08 \mu\text{M}$ 6-MSITC

Fig. 3. DPC proliferation effect. DPCs were preincubated with test samples at 37°C for 1 (A) or 3 d (B). Cells were then supplemented with Cell Count Reagent and incubated for 30 and 90 min. The absorption change per time unit was calculated to determine cell proliferation. Different symbols express $p < 0.05$ as compared with the other groups. Data are expressed as mean $\pm$ SD (n = 5).

appeared to be very similar (Fig. 4), although $0.08 \mu\text{M}$ 6-MSITC potently promoted DPC proliferation. Similarly, the number of viable cells in the control and 0.4 – $2 \mu\text{M}$ 6-MSITC also appeared to be highly similar (data not shown). It is suggested that 6-MSITC might enhance the activity of cells rather than increasing the number of viable cells. Future research on cell proliferation is anticipated.

Real-time PCR was used to determine the reiterative expression level. Although there was no significant difference between $30 \mu\text{M}$ minoxidil and the control, $30 \mu\text{M}$ minoxidil showed a tendency to upregulate VEGF mRNA levels (Fig. 5A). Li *et al.* (2012) reported that human follicle DPCs release VEGF, which promotes DPC proliferation through VEGFR2.

Fig. 4. DPCs under phase-contrast microscopy. After DPCs were incubated with test samples at 37°C for 3 days, the cells were observed with a phase-contrast microscope.

6-MSITC at 0.4–2 μM significantly upregulated VEGF mRNA levels compared to the control and 0.08 μM 6-MSITC (Fig. 5A). On the other hand, 0.08–2 μM 6-MSITC significantly promoted cell proliferation. While DPC proliferation induced by 0.08 μM 6-MSITC might be attributed to mechanisms other than upregulation of VEGF mRNA level, effects produced by 0.4–2 μM 6-MSITC might be attributed to the upregulation of VEGF mRNA level as one possibility. The effective concentration of 6-MSITC in DPC proliferation depended on the incubation time of 6-MSITC. Similarly, the expression level of VEGF mRNA may also depend on the incubation time of 6-MSITC. In future, it is necessary to clarify the detailed mechanism by investigating the relationship between incubation time and effective concentration of 6-MSITC.

Minoxidil, a drug that stimulates hair growth, has been reported to increase hair count in humans (De Villez, 1987). Han *et al.* (2004) suggested that the promotion of DPC proliferation by minoxidil stimulates hair growth in humans. Furthermore, Lachgar *et al.* (1998) suggested that the upregulation of VEGF mRNA level by minoxidil in DPCs

Fig. 5. Upregulation effects of mRNA relative expression level. DPCs were incubated with test samples at 37°C for 2 h. After total RNA was extracted and reverse transcribed, real-time PCR reactions were performed. The mRNA expression of (A) VEGF and (B) ADORA2b are shown. Different symbols express $p < 0.05$ as compared with the other groups. Data are expressed as mean $\pm$ SD ($n = 3$).

maintains good vascularization of hair follicles in androgenetic alopecia.

Li *et al.* (2001) reported that minoxidil probably activates and opens the sulfonylurea receptor 2B of DPCs, and the converted adenosine from ATP induces VEGF production through ADORA1 and ADORA2 pathways. As a preliminary test, we performed DNA microarray analyses (3D-Gene human oligo chip 25 k; Toray Industries, Inc., Tokyo, Japan) of DPCs. Although ADORA1 mRNA levels did not change with 6-MSITC and minoxidil in DNA microarray analyses, 6-MSITC and minoxidil upregulated ADORA2b mRNA levels (data not shown). Therefore, as a final test, a quantitative analysis of ADORA2b mRNA was performed with real-time PCR. 6-MSITC at 2 μM and 30 μM minoxidil were found to

significantly upregulate ADORA2b mRNA levels compared to the control and 0.08–0.4 μ M MSITC (Fig. 5B). On the other hand, 0.4–2 μ M 6-MSITC significantly upregulated VEGF mRNA levels. The upregulation of VEGF mRNA level by 2 μ M 6-MSITC might be attributed to the upregulation in ADORA2b mRNA level, whereas the upregulation of VEGF mRNA level by 0.4 μ M 6-MSITC might be attributed to a mechanism that is not related to alterations in ADORA2b mRNA expression. These data suggest that the upregulation of ADORA2b mRNA level by 6-MSITC may be related to the upregulation of VEGF mRNA level.

Lewis *et al.* (2015) reported that AITC inactivates protein tyrosine phosphatase via modification of the active site Cys-215. 6-MSITC was reported to conjugate with a thiol group of *N*-acetyl-L-cysteine (Yamaguchi *et al.*, 2008) and a cysteine residue of heat shock protein 90 β (Shibata *et al.*, 2011). In this study, 6-MSITC stimulated human DPCs. Fetal bovine serum (FBS) in hair follicle DPC growth medium contains proteins such as growth factors. 6-MSITC might stimulate human DPCs directly, or indirectly via modifying the cysteine of proteins in FBS.

We reported for the first time that 6-MSITC promoted cell proliferation and upregulated VEGF mRNA levels in human DPCs. The stimulatory effects of 6-MSITC on DPCs may be related to the upregulation of ADORA2b mRNA level. These results indicate that 6-MSITC is a potential functional compound that has a stimulatory effect on hair growth. Future research and development of 6-MSITC is needed.

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References

- Atwell, L.L., Hsu, A., Wong, C.P., Stevens, J.F., Bella, D., Yu, T.W., Pereira, C.B., Lohr, C.V., Christensen, J.M., Dashwood, R.H., Williams, D.E., Shannon, J., and Ho, E. (2015). Absorption and chemopreventive targets of sulforaphane in humans following consumption of broccoli sprouts or a myrosinase-treated broccoli sprout extract. *Mol. Nutr. Food Res.*, **59**, 424–433.
- De Villez, R.L. (1987). Androgenetic alopecia treated with topical minoxidil. *J. Am. Acad. Dermatol.*, **16**, 669–672.
- Esfandiari, A. and Kelly, A.P. (2005). The effects of tea polyphenolic compounds on hair loss among rodents. *J. Natl. Med. Assoc.*, **97**, 1165–1169.
- Etoh, H., Nishimura, A., Takasawa, R., Yagi, A., Saito, K., Sakuta, K., Kishima, I., and Ina, K. (1990). ω -Methylsulfinylalkyl isothiocyanates in wasabi, *Wasabia japonica* Matsum. *Agric. Biol. Chem.*, **54**, 1587–1589.
- Fukuchi, Y., Kato, Y., Okunishi, I., Matsutani, Y., and Osawa, T. (2004). 6-Methylsulfinylhexyl isothiocyanate, an antioxidant derived from *Wasabia japonica* MATUM, ameliorates diabetic nephropathy in type 2 diabetic mice. *Food Sci. Technol. Res.*, **10**, 290–295.
- Han, J.H., Kwon, O.S., Chung, J.H., Cho, K.H., Eun, H.C., and Kim, K.H. (2004). Effect of minoxidil on proliferation and apoptosis in dermal papilla cells of human hair follicle. *J. Dermatol. Sci.*, **34**, 91–98.
- Hou, D.X., Korenori, Y., Tanigawa, S., Yamada-Kato, T., Nagai, M., He, X., and He, J. (2011). Dynamics of Nrf2 and Keap1 in ARE-mediated NQO1 expression by wasabi 6-(methylsulfinyl)hexyl isothiocyanate. *J. Agric. Food Chem.*, **59**, 11975–11982.
- Kumagai, H., Kashima, N., Seki, T., Sakurai, H., Ishii, K., and Ariga, T. (1994). Analysis of volatile components in essential oil of upland *Wasabi* and their inhibitory effects on platelet aggregation. *Biosci. Biotech. Biochem.*, **58**, 2131–2135.
- Lachgar, S., Moukadi, H., Jonca, F., Charveron, M., Bouhaddiou, N., Gall, Y., Bonafe, J.L., and Plouet, J. (1996). Vascular endothelial growth factor is an autocrine growth factor for hair dermal papilla cells. *J. Invest. Dermatol.*, **106**, 17–23.
- Lachgar, S., Charveron, M., Gall, Y., and Bonafe, J.L. (1998). Minoxidil upregulates the expression of vascular endothelial growth factor in human hair dermal papilla cells. *Br. J. Dermatol.*, **138**, 407–411.
- Lewis, S.M., Li, Y., Catalano, M.J., Laciak, A.R., Singh, H., Seiner, D.R., Reilly, T.J., Tanner, J.J., and Gates, K.S. (2015). Inactivation of protein tyrosine phosphatases by dietary isothiocyanates. *Bioorg. Med. Chem. Lett.*, **25**, 4549–4552.
- Li, M., Marubayashi, A., Nakaya, Y., Fukui, K., and Arase, S. (2001). Minoxidil-induced hair growth is mediated by adenosine in cultured dermal papilla cells: Possible involvement of sulfonyleurea receptor 2B as a target of minoxidil. *J. Invest. Dermatol.*, **117**, 1594–1600.
- Li, W., Man, X.Y., Li, C.M., Chen, J.Q., Zhou, J., Cai, S.Q., Lu, Z.F., and Zheng, M. (2012). VEGF induces proliferation of human hair follicle dermal papilla cells through VEGFR-2-mediated activation of ERK. *Exp. Cell Res.*, **318**, 1633–1640.
- McElwee, K.J., Niiyama, S., Freyschmidt-Paul, P., Wenzel, E., Kissling, S., Sundberg, J.P., and Hoffmann, R. (2003). Dietary soy oil content and soy-derived phytoestrogen genistein increase resistance to alopecia areata onset in C3H/HeJ mice. *Exp. Dermatol.*, **12**, 30–36.
- Morimitsu Y., Hayashi K., Nakagawa Y., Fujii H., Horio F., Uchida K., and Osawa T. (2000). Antiplatelet and anticancer isothiocyanates in Japanese domestic horseradish, *Wasabi*. *Mech. Ageing Dev.*, **116**, 125–134.
- Morimitsu, Y., Nakagawa, Y., Hayashi, K., Fujii, H., Kumagai, T., Nakamura, Y., Osawa, T., Horio, F., Itoh, K., Iida, K., Yamamoto, M., and Uchida, K. (2002). A sulforaphane analogue that potently activates the Nrf2-dependent detoxification pathway. *J. Biol. Chem.*, **277**, 3456–3463.
- Murata, M., Uno, M., Nagai, Y., Nakagawa, K., and Okunishi, I. (2004). Content of 6-methylsulfinylhexyl isothiocyanate in wasabi and processed wasabi products. *J. Jpn. Soc. Food Sci. Technol. (Nippon Shokuhin Kagaku Kogaku Kaishi)* **51**, 477–482 (in Japanese).
- Nagai, M. and Okunishi, I. (2009). The effect of wasabi rhizome extract on atopic dermatitis-like symptoms in HR-1 hairless mice. *J. Nutr. Sci. Vitaminol.*, **55**, 195–200.

- Shibata, T., Kimura, Y., Mukai, A., Mori, H., Ito, S., Asaka, Y., Oe, S., Tanaka, H., Takahashi, T., and Uchida, K. (2011). Transthiocarbamylation of proteins by thiolated isothiocyanates. *J. Biol. Chem.*, **286**, 42150–42161.
- Toyama, T., Shinkai, Y., Yasutake, A., Uchida, K., Yamamoto, M., and Kumagai, Y. (2011). Isothiocyanates reduce mercury accumulation via an Nrf2-dependent mechanism during exposure of mice to methylmercury. *Environ. Health Perspec.*, **119**, 1117–1122.
- Trio, P.Z., Fujisaki, S., Hisanaga, A., Sakao, K., and Hou, D.X. (2016). DNA microarray highlights nfr2-mediated neuron protection targeted by wasabi-derived isothiocyanates in IMR-32 cells. *GENE Regul. Syst. Bio.*, **10**, 73–83.
- Tsuruki, T., Takahata, K., and Yoshikawa, M. (2005). Anti-alopecia mechanisms of soymetide-4, an immunostimulating peptide derived from soy β -conglycinin. *Peptides*, **26**, 707–711.
- Tsuzuki, H., Watanabe, N., Okamura, K., Yamada, H., Ogata, F., Kawasaki, N. (2016). Elucidation of hair loss factors and hair growth promoting effects of broccoli sprouts extract. *Fragrance Journal*, **44**, 14–20 (in Japanese).
- Uto, T., Fujii M, and Hou, D.X. (2005). Inhibition of lipopolysaccharide-induced cyclooxygenase-2 transcription by 6-(methylsulfinyl) hexyl isothiocyanate, a chemopreventive compound from *Wasabia japonica* (Miq.) Matsumura, in mouse macrophages. *Biochem. Pharmacol.*, **70**, 1772–1784.
- Watanabe, M., Ohata, M., Hayakawa, S., Isemura, M., Kumazawa, S., Nakayama, T., Furugori, M., and Kinae, N. (2003). Identification of 6-methylsulfinylhexyl isothiocyanate as an apoptosis-inducing component in wasabi. *Phytochemistry*, **62**, 733–739.
- Yamada-Kato, T., Nagai, M., Ohnishi, M., and Yoshida, K. (2012). Inhibitory effects of wasabi isothiocyanates on chemical mediator release in RBL-2H3 rat basophilic leukemia cells. *J. Nutr. Sci. Vitaminol.*, **58**, 303–307.
- Yamada-Kato, T., Okunishi, I., Fukamatsu, Y., and Yoshida, Y. (2017). Inhibitory effects of 6-methylsulfinylhexyl isothiocyanate on superoxide anion generation from differentiated HL-60 human promyelocytic leukemia cells. *Food Sci. Technol. Res.*, **23**, 343–348.
- Yamaguchi, H., Noshita, T., Kidachi, Y., Umetsu, H., Fuke, Y., and Ryoyama, K. (2008). Detection of 6-(methylsulfinyl)hexyl isothiocyanate (6-MITC) and its conjugate with *N*-acetyl-L-cysteine (NAC) by high performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry (HPLC-MS/APCI). *Chem. Pharm. Bull.*, **56**, 715–719.
- Yamane, K., Sugiyama, Y., Lu, Y.X., Lü Na., Tanno, K., Kimura, E., and Yamaguchi, H. (2016). Genetic differentiation, molecular phylogenetic analysis, and ethnobotanical study of *Eutrema japonicum* and *E. tenue* in Japan and *E. yunnanense* in China. *Hort J.*, **85**, 46–54.
- Yoshida, J., Nomura, S., Nishizawa, N., Ito, Y., and Kimura, K. (2011). Glycogen synthase kinase-3 β inhibition of 6-(methylsulfinyl)hexyl isothiocyanate derived from wasabi (*Wasabia japonica* Matsum.). *Biosci. Biotechnol. Biochem.*, **75**, 136–139.