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Phenolic Compounds from *Sanguisorba obtusa* Endemic to Japan

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Abstract Phenolic compounds in the leaves of *Sanguisorba obtusa* endemic to Mt. Hayachine, Japan were surveyed. Quercetin 3-*O*-glucuronide was isolated as major flavonoid, and kaempferol 3-*O*-glucuronide was found as minor flavonoid in addition to ellagic acid and its derivatives. Flavonoid composition of the plant was similar to those of the other alpine rosaceous species, *Geum calthifolium* var. *nipponicum* and *Sieversia pentapetala*. Their ecological significance was also discussed.

Key words: Chemical ecology, chemotaxonomy, phenolic compounds, Rosaceae, *Sanguisorba obtusa*.

Introduction

The genus *Sanguisorba* (Rosaceae) consists of ca. 15 species that are distributed in temperate to boreal zone of the North Hemisphere (Mabberley, 2008), and seven species occurs in alpine and highland area of Japan (Shimizu, 2004). Of their species, *Sanguisorba obtusa* Maxim. is endemic to Mt. Hayachine, Iwate Prefecture, Japan and listed as endangered species.

Quercitrin (quercetin 3-*O*-rhamnoside) has been reported from the leaves of *S. hakusanensis* Makino (Nakaoki and Morita, 1960). El-Mousallamy (2002) has reported two new flavonol glycosides, corniculatusin 3-*O*-sophoroside and kaempferol 3-*O*-[(2″-galloylglucosyl)-(1→2)-glucoside], and five known flavonoids from the aerial parts of *Sanguisorba minor* Scop. In addition, several phenolic compounds, e.g., phenolic carboxylic acids, gallic acid, ellagic acid, and three flavonols including quercetin 3-*O*-(6″-galloylglucoside), quercetin and kaempferol, were isolated from the whole plants of *S. minor* (Ayoub, 2003). Earlier studies have shown that *S. officinalis* contains several catechins and ellagitannins (e.g., Nonaka *et al.*, 1982a, 1982b; Tanaka *et al.*, 1983). Furthermore, two ellagic acids, i.e., 3,4,3′-tri-*O*-methylellagic acid 4′-*O*-*β*-D-xyloside and 3,4,3′-tri-*O*-methylellagic acid, have been isolated from the roots of *S. officinalis* L. They promotes proliferation of megakaryocyte progenitor cells and megakaryocyte differentiation (Gao *et al.*, 2014). However, the number of studies on foliar phenolic compounds of *Sanguisorba* species is limited, and no information is available for *Sanguisorba obtusa*.

Flavonoids are one of the noble UV-absorbing compounds that are synthesized in plants (Caldwell *et al.*, 1983). Plants that occur in highland areas are thought to be adaptive to several environmental stresses (e.g., intense UV-B and cold) by accumulation of UV-absorbing compounds such as flavonoids and phenolic acids (Spitaler *et al.*, 2006; Murai *et al.*, 2009, 2014, 2015; Murai and Iwashina, 2010, 2015). These compounds are also known as efficient antioxidants.

In this study, we analyzed *Sanguisorba obtusa* for their phenolic compounds, and describe the chemotaxonomical and ecological significance of their compounds.

Materials and Methods

Plant materials

Sanguisorba obtusa (Fig. 1) was collected from Mt. Hayachine, Iwate Prefecture, Japan in August 2014. The plant collection was carried out under the permissions of Iwate Prefecture, the Forestry Agency, and the Agency for Cultural Affairs, Japan. Voucher specimen was deposited in the Herbarium of National Museum of Nature and Science, Japan (TNS).

Extraction and separation

Fresh leaves of *S. obtusa* (4.2 g) were extracted with MeOH. The concentrated extracts were applied to preparative paper chromatography using solvent systems: BAW (*n*-BuOH/HOAc/H_2O = 4:1:5, upper phase) and 15%HOAc. The compounds were purified by Sephadex LH-20 column chromatography (solvent system: 70% MeOH).

Fig. 1. *Sanguisorba obtusa*.

Quantitative HPLC analysis of flavonoids

Fresh leaves (0.2 g) of each population were extracted with 4 ml MeOH. After filtration with GL Chromatodisk 13N (0.45 μm pore size, GL Sciences Inc., Japan), the extracts were analyzed using a Shimadzu HPLC system with SunShell C18 column (2.6 μm particle material, I.D. 4.6 × 100 mm, ChromaNik Technologies Inc., Japan), at flow-rate: 0.8 ml min^{-1}, detection: 190–400 nm and eluents: MeCN/H_2O/H_3PO_4 (15:85:0.2), injection: 2 μl. HPLC chromatogram is shown in Fig. 2.

Liquid chromatograph-mass spectra (LC-MS)

LC-MS were measured with Shimadzu LC-MS system using Inertsil ODS-4 (3 μm particle material, I.D. 2.1 × 100 mm, GL Sciences Inc.), at a flow-rate of 0.2 ml min^{-1}, eluting with HCOOH/MeCN/H_2O (1:15:84), injection: 3 μl, ESI^+ 4.5 kV, ESI^- 3.5 kV, 250°C.

Identification of compounds

In this survey, two flavonoids, ellagic acid and its two derivatives were isolated from the leaves of *S. obtusa*. Flavonoids (**4** and **5**) were identified by UV spectroscopy according to Mabry *et al.* (1970), LC-MS, HPLC comparisons with authentic standards (Murai and Iwashina, 2010) and characterization of acid hydrolysates (in 12% HCl, 100°C, 30 min), and ellagic acid and its two derivatives were characterized by UV spectroscopy, LC-MS and HPLC comparisons with authentic sample (ellagic acid) gifted from Dr. Y. Yazaki. Chemical data of the isolated compounds are as follows.

Ellagic acid derivative A (**1**). Pale yellow solution. UV: λmax (nm) MeOH 200, 255, 345sh, 355. HPLC: *Rt* 3.7 min. LC-MS: *m/z* 453 $[M+H]^+$, 433 $[M-H]^-$ (ellagic acid + 1 mol pentose) and 303 $[M-132+H]^+$, 301 $[M-132-H]^-$ (ellagic acid).

Ellagic acid derivative B (**2**). Pale yellow solution. UV: λmax (nm) MeOH 200, 255, 345sh, 355. HPLC: *Rt* 4.1 min. LC-MS: *m/z* 453 $[M+H]^+$, 433 $[M-H]^-$ (ellagic acid + 1 mol pentose) and 303 $[M-132+H]^+$, 301 $[M-132-$

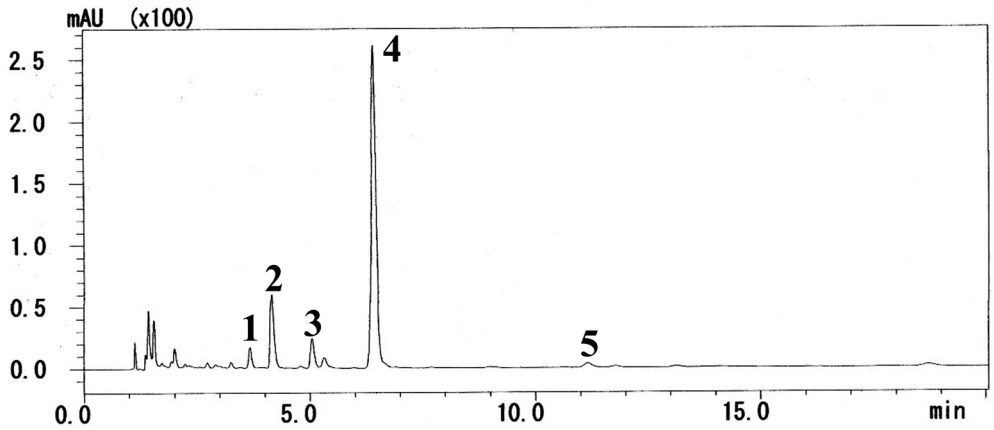

Fig. 2. HPLC chromatogram of MeOH extracts from *Sanguisorba obtusa*. 1. Ellagic acid derivative A. 2. Ellagic acid derivative B. 3. Ellagic acid. 4. Quercetin 3-*O*-glucuronide. 5. Kaempferol 3-*O*-glucuronide. Detection: 350 nm.

$H]^-$ (ellagic acid).

Ellagic acid (**3**). White powder. UV: λmax (nm) MeOH 201, 255, 350sh, 363. HPLC: *Rt* 5.0 min. LC-MS: *m/z* 303 $[M+H]^+$, 301 $[M-H]^-$ (ellagic acid).

Quercetin 3-*O*-glucuronide (**4**). Pale yellow powder. UV: λmax (nm) MeOH 255, 268sh, 355; +NaOMe 271, 330, 398 (inc.); +$AlCl_3$ 272, 410; +$AlCl_3$/HCl 270, 298, 359sh, 399sh; +NaOAc 273, 320, 386; +NaOAc/H_3BO_3 262, 294sh, 373. HPLC: *Rt* 6.4 min. Acid hydrolysis: quercetin and glucuronic acid. LC-MS: *m/z* 479 $[M+H]^+$, 477 $[M-H]^-$ (quercetin + 1 mol glucuronic acid) and 303 $[M-176+H]^+$, 301 $[M-176-H]^-$ (quercetin).

Kaempferol 3-*O*-glucuronide (**5**). Pale yellow solution. UV: λmax (nm) MeOH 266, 349; +NaOMe 274, 325, 396 (inc.); +$AlCl_3$ 276, 305, 352sh, 399sh; +$AlCl_3$/HCl 274, 302, 348sh, 395sh; +NaOAc 274, 306, 384; +NaOAc/H_3BO_3 266, 300sh, 352. HPLC: *Rt* 11.2 min. Acid hydrolysis: kaempferol and glucuronic acid. LC-MS: *m/z* 463 $[M+H]^+$, 461 $[M-H]^-$ (kaempferol + 1 mol glucuronic acid) and 287 $[M-176+H]^+$, 285 $[M-176-H]^-$ (kaempferol).

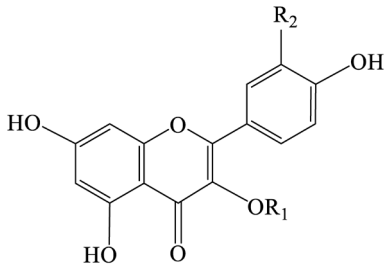

Fig. 3. Chemical structures of flavonols from *Sanguisorba obtusa*. quercetin 3-*O*-glucuronide (R_1 = glucuronyl, R_2 = OH, **4**). kaempferol 3-*O*-glucuronide (R_1 = glucuronyl, R_2 = H, **5**).

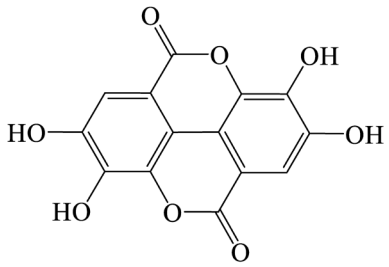

Fig. 4. Chemical structure of ellagic acid (**3**).

Results and Discussion

Flavonoid and ellagic acid composition of Sanguisorba obtusa

The present study revealed the presence of phenolic compounds of *S. obtusa* for the first time. Two flavonol glycosides, quercetin

3-*O*-glucuronide (**4**) and kaempferol 3-*O*-glucuronide (**5**) (Fig. 3), and ellagic acid (**3**) (Fig. 4) and its derivatives (**1** and **2**) were found from the leaves of *S. obtusa*. We formerly surveyed the phenolic compounds of alpine rosaceous species, *Geum calthifolium* Menzies ex Sm. var. *nipponicum* (F.Bolle) Ohwi and *Sieversia pentapetala* (L.) Greene, and **4** was isolated as a major phenolic compound, and **5** and isorhamnetin 3-*O*-glucuronide were found as minor compounds from their leaves (Murai and Iwashina, 2010). These rosaceous species showed the similar flavonoid composition. On the other hand, we preliminary surveyed the HPLC elusion pattern of *S. albiflora* (Makino) Makino, which is also distributed in Mt. Hayachine and other mountains in Tohoku district, Japan, was different from that of *S. obtusa*. *S. albiflora* contained some phenolic acid, caffeic acid derivatives extrapolated from their spectra by HPLC photodiode array detector, in addition to flavonoids, ellagic acid and its derivatives, and did not contain quercetin 3-*O*-glucuronide (data not shown). The survey of phenolic compounds of other alpine *Sanguisorba* species including *S. albiflora* is now in progress.

Ecological significance

S. obtusa contained quercetin 3-*O*-glucuronide as major phenolic compound. The compound is B-ring-*ortho*-dihydroxy flavonoid, which has been reported as an efficient antioxidant in flavonoid compounds (Rice-Evans *et al.*, 1996; Pietta, 2000). Recent numerous studies have shown that several plants growing in higher altitudes synthesize such B-ring-*ortho*-dihydroxy flavonoids (Spitaler *et al.*, 2006; Murai and Iwashina, 2010; Murai *et al.*, 2009, 2014, 2015). Furthermore, ellagic acid is also known as a noble antioxidant. UV radiation cases not only direct physiological damages but also indirect oxidation damages to plant cells and tissues. Oxidation stresses are also caused by cold and other environmental factors. These compounds may play an important role in protection against several environmental stresses in high altitudes. Further studies (e.g., physiological experiment) using highland and alpine species are required for the understanding of their functions.

Acknowledgments

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