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Internal and external flavonoids from the leaves of Japanese *Chrysanthemum* species (Asteraceae)

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ABSTRACT

Flavonoids in the leaves of 17 species and 4 varieties of Japanese *Chrysanthemum* were surveyed. Nine flavonoid glycosides were isolated and identified as quercetin 3-*O*-rutinoside, eriodictyol 7-*O*-glucuronide, luteolin 7-*O*-rutinoside, 7-*O*-glucoside and 7-*O*-glucuronide, apigenin 7-*O*-glucoside and 7-*O*-glucuronide, chrysoeriol 7-*O*-glucuronide and acacetin 7-*O*-rutinoside. The taxa were divided into three chemotypes by their flavonoid characters, i.e. I) occurrence of flavone, flavonol and flavanone, II) occurrence of flavone and flavanone and III) occurrence of flavone alone. The chemotypes almost agreed with the system based on morphological, cytological and geographical characters, i.e. Group I) the taxa growing on seashore and having tubular flowers alone, Group III) the taxa of the Asian Continent origins and having both tubular and ligulate flowers. Others were included in Group II which was subdivided into four groups by flavonoid characters. Twelve external flavonoids were isolated and identified as acacetin 7-*O*-rutinoside, luteolin, nepetin, 5,7,3′,4′-tetrahydroxy-6,5′-dimethoxyflavone, apigenin, hispidulin, 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone, jaceosidin, sudachitin, eupatilin, hymenoxin and pectolinarigenin. The distribution patterns of almost all external flavonoids were apparently irregular among the taxa.

1. Introduction

The genus *Chrysanthemum* (Asteraceae) consists of ca. 50 species and is mainly native to East Asia (Kitamura, 1999). Of their species, eighteen ones are growing in Japan (Ohashi and Yonekura, 2004). *Chrysanthemum* is classified into three sections, *Ajania*, *Chrysanthemum* and *Arctanthemum* (Ohashi and Yonekura, 2004). Morphological characters of each section are as follows. Section *Ajania* has only tubular flowers on the head, and sects. *Chrysanthemum* and *Arctanthemum* have both tubular and ligulate flowers. Cytologically, polyploid series, i.e. diploid, tetraploid, hexaploid, octaploid and decaploid is known in the genus, suggesting that polyploidization has played an important rule in the speciation of *Chrysanthemum* (Tanaka and Shimotomai, 1978).

Some taxonomists consider sects. *Chrysanthemum*, *Ajania* and *Arctanthemum* as to be independent genera, respectively (Bremer and Humphries, 1993; Oberprieler et al., 2009). However, the molecular phylogenic analysis using ITS and/or IGS

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sequences revealed that the boundary among these sections is indefinable (Zhao et al., 2010). Thus, the taxonomy and phylogeny of *Chrysanthemum* are now confused (Koyama, 1995; Nakata, 1999; Ohashi and Yonekura, 2004; Bremer and Humphries, 1993).

Apart from phylogenic classification, Japanese *Chrysanthemum* is practically divided into five groups by morphological and geographical characters, 1) non-ligulate flower group growing on the seashore such as *Chrysanthemum pacificum*, *Chrysanthemum shiwogiku* and *Chrysanthemum kinokuniense*; 2) non-ligulate flower group growing in high mountain range such as *Chrysanthemum rupestre* and *Chrysanthemum pallasianum*; 3) yellow-ligulate flower group such as *Chrysanthemum okiense*, *Chrysanthemum indicum* and *Chrysanthemum seticuspe* f. *boreale*; 4) white-ligulate flower group which is endemic to Japan such as *Chrysanthemum japonense*, *Chrysanthemum wakasaense*, *Chrysanthemum yoshinaganthum*, *Chrysanthemum ornatum*, *Chrysanthemum crassum* and *Chrysanthemum makinoi*; and 5) white-ligulate flower group of the Asian Continent origin such as *Chrysanthemum yezoense*, *Chrysanthemum zawadskii*, *Chrysanthemum weyrichii* and *Chrysanthemum arcticum* L. subsp. *yezoense* (F. Maek.) H. Ohashi & Yonek. (Nakata et al., 1979). However, identification of species is rather difficult because most species closely resemble each other within the groups. Moreover, morphological variations, intraspecific polyploidy and natural hybrids between the species are present (Nakata et al., 1987; Kondo and Abd El-Twab, 2002).

The genus *Chrysanthemum* includes many important medicinal and ornamental species such as *Chrysanthemum morifolium* Ramat. and *C. indicum*. The sesquiterpene, chrysanthemol, which acts as anti-inflammatory substance, has been reported from *C. indicum* (Yu and Xie, 1987). Some flavonoids have been isolated from a few species. Eriodictyol 7-*O*-glucuronide, which inhibit aldose reductase activities in rat lens, was isolated from the flowers of *C. indicum* together with luteolin and its 7-*O*-glucoside (Matsuda et al., 2002). Apigenin 7-*O*-(4″-caffeoylglucuronide), which has been reported HIV-1 integrase inhibitory activity, was obtained from the flowers of *C. morifolium* (Lee et al., 2003). An anthocyanin, cyanidin 3-*O*-glucoside, i.e. chrysanthemin, was isolated from the flowers of *C. morifolium* as a flower pigment (Willstätter and Bolton, 1916; Hayashi, 1937; Kawase et al., 1970). The same species was reinvestigated, and two acylated cyanidin glycosides, cyanidin 3-*O*-(6″-malonylglucoside) and 3-*O*-(3″,6″-dimalonylglucoside), were isolated as major flower pigments (Saito et al., 1988; Nakayama et al., 1997). The flavonoids of *Chrysanthemum* species growing in Japan have also been reported from a few species. Acacetin and its 7-*O*-galactoside and 7-*O*-rutinoside, apigenin and its 7-*O*-glucoside and 7-*O*-glucuronide, vitexin, eupatilin, luteolin and its 7-*O*-glucoside and 7-*O*-glucuronide, eriodictyol 7-*O*-glucuronide, diosmetin 7-*O*-glucoside, kaempferol, quercetin and its 3-*O*-glucoside, 3-*O*-galactoside, 3-*O*-rutinoside and 3,7-di-*O*-glucoside, and myricetin have been reported from the flowers of *C. indicum* (Yu and Xie, 1987; Matsuda et al., 2002; Chatterjee et al., 1981; Wu et al., 2010). Apigenin 7-*O*-glucuronide, patuletin 7-*O*-glucoside, quercetin and its 7-*O*-glucoside were isolated from the flowers of *C. arcticum* (Harborne et al., 1970). From the whole plants of *Chrysanthemum boreale* (=*C. seticuspe* f. *boreale*), acacetin and its 7-*O*-galactoside, apigenin and luteolin were reported (Shin et al., 1995). However, flavonoids were hardly reported from the leaves, especially wild Japanese *Chrysanthemum* species.

In this paper, we describe and chemotaxonomically discuss internal and external flavonoids in the leaves of 21 Japanese *Chrysanthemum* taxa.

2. Materials and methods

2.1. Plant materials

Seventeen species and four varieties of *Chrysanthemum* were used as plant materials in this experiment. The taxa and collection sites are shown in Table 1. In this paper we use scientific names according to Ohashi and Yonekura (2004). Voucher specimens were deposited in the herbarium of National Museum of Nature and Science, Japan (TNS).

2.2. Extraction and isolation

Fresh leaves (each ca. 200 g) of *C. japonense* var. *ashizuriense*, *C. shiwogiku*, *C. pacificum* and *C. seticuspe* f. *boreale* were rinsed with acetone for external flavonoids and then extracted with MeOH for internal flavonoids. After concentration, both extracts were applied to preparative paper chromatography using solvent systems: BAW (*n*-BuOH/HOAc/H_2O = 4:1:5, upper phase), 15% HOAc and BEW (*n*-BuOH/EtOH/H_2O = 4:1:2.2), and then to prep. HPLC (see below). The isolated flavonoids were purified by Sephadex LH-20 column chromatography using solvent system: 70% MeOH.

Fresh leaves (each 5 g) were extracted with MeOH (50 ml) for qualitative and quantitative HPLC survey of internal and external flavonoid composition.

2.3. HPLC

Prep. HPLC was performed with Tosoh HPLC systems using Senshu Pak, PEGASIL ODS column (I.D. 10 × 250 mm, Senshu Scientific Co. Ltd., Japan), at a flow-rate: 1.5 ml/min, injection: 350-400 μl, detection wavelength: 350 nm, and eluent: MeCN/H_2O/HCOOH (24:75:1) for internal glycosides, and MeCN/H_2O/HCOOH (40:59:1) for external flavonoids. Qualitative and quantitative HPLC was performed with Shimadzu HPLC systems using L-column 2 ODS column (I.D. 6 × 150 mm, Chemicals Evaluation and Research Institute, Japan), at a flow-rate: 1.0 ml/min, injection: 10 μl, detection wavelength: 190–400 nm, and eluent: MeCN/H_2O/H_3PO_4 (22:78:0.2) for internal glycosides, and MeCN/H_2O/H_3PO_4 (35:65:0.2) for external flavonoids.