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Flavonoids and xanthones from the leaves of *Amorphophallus titanum* (Araceae)

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ABSTRACT

The flavonoids and xanthones in the leaves of *Amorphophallus titanum*, which has the largest inflorescence among all Araceous species, were surveyed. Eight *C*-glycosylflavones, five flavonols, one flavone *O*-glycoside and two xanthones were isolated and characterized as vitexin, isovitexin, orientin, isoorientin, schaftoside, isoschaftoside, vicenin-2 and lucenin-2 (*C*-glycosylflavones), kaempferol 3-*O*-robinobioside, 3-*O*-rutinoside and 3-*O*-rhamnosylarabinoside, and quercetin 3-*O*-robinobioside and 3-*O*-rutinoside (flavonols), luteolin 7-*O*-glucoside (flavone), and mangiferin and isomangiferin (xanthones). Although the inflorescence of this species has been surveyed for flavonoids, those of the leaves were reported for the first time.

1. Subject and source

The genus *Amorphophallus* Blume ex Decne. belonging to the family Araceae consists of ca. 290 species and is mainly distributed in the tropical zone of the Old World (Mabberley, 2017). As with other aroids, the *Amorphophallus* inflorescence consists of two main parts, the spathe and spadix (Yuzammi et al., 2017). *A. titanum* (Becc.) Becc. has the largest inflorescence (ca. 3m height) among *Amorphophallus* species.

The leaves of *A. titanum* were collected in Cibodas Botanic Garden, Indonesia, and Tsukuba Botanical Garden, National Museum of Nature and Science, Japan in November 2017. The individuals from Indonesia were cultivated in the open air. On the other hand, individual from Japan was growing in the greenhouse. Voucher specimens were deposited in the herbarium of Cibodas Botanic Garden, LIPI, Indonesia (I.B.28) and National Museum of Nature and Science (TNS), Japan (living specimen, TBG157495).

2. Previous work

Four anthocyanins were isolated from the spathe and spadix of *A. titanum*, together with six minor cyanidin, pelargonidin and peonidin derivatives, and characterized as cyanidin 3-*O*-glucoside, cyanidin 3-*O*-rutinoside, pelargonidin 3-*O*-(*p*-coumaroylglucoside) and peonidin 3-*O*-(*p*-coumaroylglucoside) (Gallori et al., 2004). Two *C*-glycosylflavones, vitexin (**2**) and orientin (**4**) were reported from the spathe of the same species (Iwashina et al., 2015). On the other hand, twelve *C*-glycosylflavones, and each one of flavone and flavonol were isolated from the spadix of this species, and characterized as isovitexin (**1**), **2**, isoorientin (**3**), **4**, isoschaftoside (**5**), schaftoside (**6**), vicenin-2 (**7**), lucenin-2 (**8**), vitexin 2″-*O*-glucoside, isovitexin 2″-*O*-glucoside, isovitexin X″-*O*-rhamnoside and isoscoparin X″-*O*-glucoside (*C*-glycosylflavones), chrysoeriol 7-*O*-glucoside (flavone) and kaempferol 3-*O*-robinobioside (**11**) (flavonol) (Iwashina et al., 2015). In this time, five anthocyanins, cyanidin 3-*O*-glucoside and 3-*O*-rutinoside, peonidin 3-*O*-glucoside and 3-*O*-rutinoside, and pelargonidin 3-*O*-rhamnosylglucoside were also obtained from the spathe and spadix (Iwashina et al., 2015). Six sulphur compounds, dimethyl disulphide, dimethyl trisulphide, dimethyl tetrasulphide, dimethyl pentasulphide, *S*-methyl thioacetate and *S*-methyl thioisobutylate were reported as *Amorphophallus* odour substances from *A. titanium* (Kite and Hetterscheid, 2017). As flavonoids from other *Amorphophallus* species, **1**, **3**, **4**, vitexin 2″-*O*-xyloside and quercetin 3-*O*-glucoside were isolated from the inflorescence of *A. rivieri* Durieu (= *A. konjac* K. Koch) (Iwashina et al., 2015). Four *C*-glycosylflavones, **1**, **2**, **4**, **6** and **7**, and two flavonols, kaempferol and quercetin 3-*O*-glucosides were detected in the inflorescence of *A. paeoniifolium* (Dennst.) Nicolson (Iwashina et al., 2015). A rare flavonol, 3,5-diacetyltambulin (7,8,4′-trimethoxy-3,5-diacetoxyflavone) was isolated from the tuberous roots of *A. campanulatus* Blume ex Decne (= *A. paeoniifolius*)

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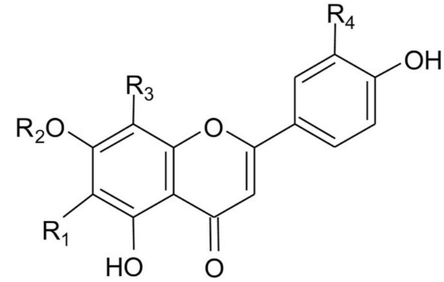
(Khan et al., 2008). However, foliar flavonoids are not surveyed in *Amorphophallus* species including *A. titanum*.

3. Present study

Fresh leaves of three individuals from Cibodas Botanic Garden, Indonesia (two individuals, 244.4 g and 115.8 g, respectively) and Tsukuba Botanical Garden, National Museum of Nature and Science, Japan (one individual, 58.2 g) were extracted with MeOH. It was shown that flavonoid composition is the essentially the same among three individuals from Indonesia and Japan. After concentration, the extracts were gathered and applied to preparative PC using solvent systems, BAW (*n*-BuOH/HOAc/H_2O = 4:1:5, upper phase) and then 15% HOAc. The isolated flavonoids were purified by Sephadex LH-20 column chromatography using solvent system, 70% MeOH. Sixteen compounds were obtained as pale yellow powder [**1** (ca. 30 mg), **3** (ca. 50 mg), **4** (15 mg), **7** (ca. 20 mg) and **8** (ca. 20 mg)] and pure MeOH solutions (**2**, **5**, **6**, **9–16**). Their compounds except for **12** were identified as isovitexin (**1**), vitexin (**2**), isoorientin (**3**), orientin (**4**), isoschaftoside (**5**), schaftoside (**6**), vicenin-2 (**7**), lucenin-2 (**8**), luteolin 7-*O*-glucoside (**9**), kaempferol 3-*O*-rutinoside (**10**), kaempferol 3-*O*-robinobioside (**11**), quercetin 3-*O*-rutinoside (**13**), quercetin 3-*O*-robinobioside (**14**), mangiferin (**15**) and isomangiferin (**16**) by UV spectral analysis (Mabry, et al., 1970) (Shimadzu MPS-2000 multipurpose recording spectrophotometer, Shimadzu, Kyoto), LC-MS (Shimadzu LC-MS-2010 EV systems using Inertsil ODS-4 column, I.D. 2.1 × 100 mm, flow-rate of 0.1 ml min^{-1}, detection wave-length of 350 nm, ionizing voltage 4.5 kV for ESI^+ and 3.5 kV for ESI^-, eluent: MeCN/H_2O/HCOOH = 15:80:5), acid hydrolysis (in 12%HCl, 100 °C, 30 min), and HPLC and TLC (Merck, Germany, solvent systems; BAW, BEW (*n*-BuOH/EtOH/H_2O = 4:1:2.2) and 15% HOAc) comparisons with authentic samples. Origins of the compounds used as authentic samples are as follows: isovitexin (**1**) and vitexin (**2**) from the flowers of *Iris ensata* Thunb. (Iridaceae) (Iwashina et al., 1996), isoorientin (**3**) and orientin (**4**) from the fronds of *Cyrtomium* spp. (Dryopteridaceae) (Iwashina et al., 2006), isoschaftoside (**5**), kaempferol 3-*O*-rutinoside (**10**) and quercetin 3-*O*-rutinoside (**13**) from the aerial parts of *Osyris alba* L. (Santalaceae) (Iwashina et al., 2008), schaftoside (**16**) from Extrasynthese, vicenin-2 (**7**) and lucenin-2 (**8**) from the fronds of *Asplenium normale* D. Don (Aspleniaceae) (Iwashina et al., 2010a), luteolin 7-*O*-glucoside (**9**) from the leaves of *Schmalhausenia nidulans* Petrak (Asteraceae) (Iwashina and Kadota, 1999), kaempferol 3-*O*-robinobioside (**11**) from the aerial parts of *Cassytha filiformis* (Lauraceae) (Murai et al., 2008), quercetin 3-*O*-robinobioside (**14**) from the leaves of *Asarum canadense* L. (Aristolochiaceae) (Iwashina and Kitajima, 2000), and mangiferin (**15**) and isomangiferin (**16**) from the leaves of *Iris setosa* Pallas (Iridaceae) (Hayashi et al., 1989). Flavonoid **12** liberated kaempferol, rhamnose and arabinose by acid hydrolysis. The attachment of each one of rhamnose and arabinose to 3-position of kaempferol was shown by LC-MS and UV spectral survey according to Mabry et al. (1970). Thus, **12** was characterized as kaempferol 3-*O*-rhamnosylarabinoside.

4. Chemotaxonomic significance

Eight *C*-glycosylflavones (**1-8**), one flavone *O*-glycoside (**9**), five flavonol *O*-glycosides (**10–14**) and two *C*-glycosylxanthones (**15**, **16**) were isolated from *Amorphophallus titanum* leaves (Fig. 1). Of their compounds, eight *C*-glycosylflavones (**1–8**) and flavonol (**11**) have already been reported from the inflorescence of this species (Iwashina et al., 2015). Although flavone *O*-glycoside, chrysoeriol 7-*O*-glucoside was found in the inflorescence of this species (Iwashina et al., 2015), it was not isolated from the leaves. Instead, luteolin 7-*O*-glucoside was detected in the leaves of *A. titanum.* Four flavonol *O*-glycosides, kaempferol 3-*O*-rutinoside (**10**) and 3-*O*-rhamnosylarabinoside (**12**), and quercetin 3-*O*-rutinoside (**13**) and 3-*O*-robinobioside (**14**) were also found in this species for the first time. Thus, it was shown that major flavonoids of *A. titanum* are *C*-glycosylflavones and accompanied with minor flavone and flavonol *O*-glycosides. In the family Araceae, 48 genera including *Landoltia*, *Lemna*, *Spirodela*, *Wolffia* and *Wolffillea* which were converted to this family from the Lemnaceae by APG III were surveyed for flavonoids (Iwashina, 2020). Major flavonoids of many genera in the Araceae, e.g. *Lemna*, *Spirodela* (McClure and Alston, 1966), *Cryptocoryne* (Franke et al., 2006), *Pothos* (Iwashina et al.,



(**1**) R_1 = glucosyl, R_2 = R_3 = R_4 = H

(**2**) R_1 = R_2 = R_4 = H, R_3 = glucosyl

(**3**) R_1 = glucosyl, R_2 = R_3 =H, R_4 = OH

(**4**) R_1 = R_2 = H, R_3 = glucosyl, R_4 = OH

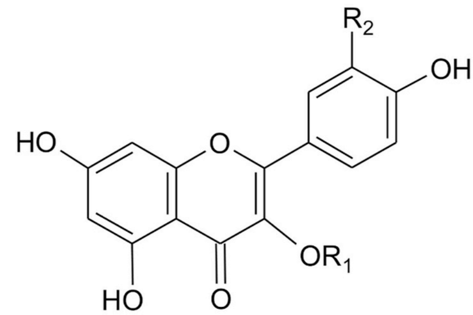
(**5**) R_1 = arabinosyl, R_2 = R_4 = H, R_3 = glucosyl

(**6**) R_1 = glucosyl, R_2 = R_4 =H, R_3 = arabinosyl

(**7**) R_1 = R_3 = glucosyl, R_2 = R_4 = H

(**8**) R_1 = R_3 = glucosyl, R_2 = H, R_4 = OH

(**9**) R_1 = R_3 = H, R_2 = glucosyl, R_4 = OH



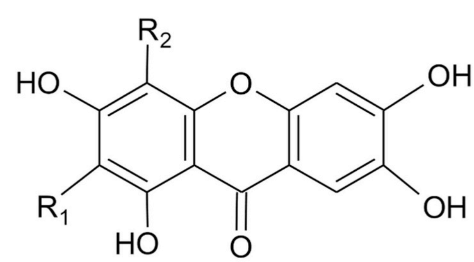
(**10**) R_1 = rutinosyl, R_2 = H

(**11**) R_1 = robinobiosyl, R_2 = H

(**12**) R_1 = rhamnoarabinosyl, R_2 = H

(**13**) R_1 = rutinosyl, R_2 = OH

(**14**) R_1 = robinobiosyl, R_2 = OH



(**15**) R_1 = glucosyl, R_2 = H

(**16**) R_1 = H, R_2 = glucosyl

Fig. 1. Chemical structures of flavonoids and xanthones isolated from *Amorphophallus titanium* leaves.

2010b), *Colocasia* (Iwashina et al., 1999), *Xanthosoma* (Picerno et al., 2003) and *Anthurium* (Williams et al., 1981; Aquno et al., 2001; Clark et al., 2012), were *C*-glycosylflavones. On the other hand, some genera, e.g. *Gymnostachys* (Williams et al., 1971), *Lisichiton*, *Orontium* and *Symplocarpus* (Williams et al., 1981), synthesized flavonol *O*-glycosides as major flavonoids. Although foliar flavonoids of *Amorphophallus* species were surveyed in only one species, *A. titanium* in this experiment, major flavonoids of the genus may be *C*-glycosylflavones. *C*-Glycosylxanthone, mangiferin (**15**) is most popular xanthone in vascular plants (Richardson, 1983). Two *C*-glycosylxanthones, **15** and isomangiferin (**16**), were found in *A. titanum* in this survey. This is first report of xanthones from the Araceae (Richardson, 1983; Al-Hiaimi and Miana, 1990; Peres and Nagem, 1997). Compounds **9**, **10**, **13**–**16** were isolated from the leaves only. On the other hand, *C*-glycosylflavone *O*-glycosides such as isovitexin 2″-*O*-glucoside and X″-*O*-rhamnoside, vitexin 2″-*O*-glucoside, and isoscoparin X″-*O*-glucoside have been found in the spadix only, and were not isolated from the spathe (Iwashina et al., 2015) and also leaves (present paper). It has been shown that flavonoids have various functions and activities such as antioxidant, antibacterial, UV shield, phytoalexin, allelopathy etc. (Iwashina, 2003). The difference of flavonoid composition among their organs in *A. titanium* may be show the difference of function and activities of their flavonoids. In this survey, flavonoids and xanthones of *A. titanum* leaves were characterized. Although their compounds were known flavonoids and xanthones, chemical characters of the genus *Amorphophallus* leaves were reported for the first time. However, the genus consists of ca. 290 species in the World, and their foliar flavonoids are not surveyed except for *A. titanum*. We now survey the polyphenols of other *Amorphophallus* leaves, especially in Indonesia in which many species are abundantly growing.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bse.2020.104036.

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