

Anthocyanins and Other Flavonoids from *Amorphophallus titanum* Having Largest Inflorescence in Plant Kingdom, and Other Two Species

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Abstracts *Amorphophallus titanum* has a largest inflorescence of the plant kingdom. In this survey, five anthocyanins and 14 other flavonoids were isolated from the spathe and spadix appendix of *A. titanum*. Anthocyanins were identified as cyanidin 3-*O*-glucoside, cyanidin 3-*O*-rutinoside, peonidin 3-*O*-glucoside, peonidin 3-*O*-rutinoside and pelargonidin 3-*O*-rhamnosylglucoside. On the other hand, other flavonoids were characterized as vitexin, vitexin 2''-*O*-glucoside, isovitexin, isovitexin 2''-*O*-glucoside, orientin, isoorientin, vicenin-2, lucenin-2, schaftoside, isoschaftoside, isovitexin X''-*O*-rhamnoside, isoscoparin X''-*O*-glucoside (*C*-glycosylflavones), chrysoeriol 7-*O*-glucoside (flavone) and kaempferol 3-*O*-robinobioside (flavonol). Anthocyanins and other flavonoids in the inflorescences of other two *Amorphophallus* species, *A. rivieri* and *A. paeoniifolius* were also isolated and identified.

Key words: *Amorphophallus paeoniifolius*, *Amorphophallus rivieri*, *Amorphophallus titanum*, Anthocyanins, Flavonols, *C*-Glycosylflavones.

Introduction

The genus *Amorphophallus* is mainly distributed in the tropical zone of the Old World and consists of 80–90 species (Ohashi, 1982). Of their species, *A. titanum* is endemic to Sumatra Island and has the largest inflorescence, ca. 3 m high and 1 m wide, of the plant kingdom. Its flowering in the botanical gardens is very rare. In 2012 and 2014, the flowers of the species came out at the Tsukuba Botanical Garden, National Museum of Nature and Science, Japan. The spathe and spadix appendix of the species are dark purple and their anthocyanins have been reported to be cyanidin 3-*O*-glucoside and 3-*O*-rutinoside, and peonidin and pelargonidin 3-*O*-(*p*-coumaloyl)glucosides (Gallori *et al.*,

2004).

Amorphophallus paeoniifolius grows in Sri Lanka, the Philippines, Malaysia, Indonesia, and other Southeast Asia countries. Flavonol, quercetin, has been isolated from the corm of the species (Sharstry *et al.*, 2010). *Amorphophallus rivieri* is native to Indochina and southern China and cultivated in Japan for edible. However, polyphenols including flavonoids are not reported until now.

As the flavonoids of other *Amorphophallus* species, anthocyanin, cyanidin 3-*O*-rutinoside has been found in the spathe of *A. abyssinicus* (A.Rich.) N.E.Brown and *A. stuhlmannii* (Engl.) Engl. & Gehrm. (Williams *et al.*, 1981). A rare flavonol, 3,5-diacetylambulin (7,8,4'-trimethoxy-3,5-diacetoxyflavone), has been isolated from the

tuberous roots of *A. campanulatus* Blume ex Decne (= *A. paeoniifolius*) (Khan *et al.*, 2008). Unknown C-glycosylflavone was detected from the leaves of *A. stuhlmannii* (Williams *et al.*, 1981). In this paper, we describe the isolation and identification of the anthocyanins, flavone, flavonols and C-glycosylflavones from the inflorescences of three *Amorphophallus* species, *A. titanum*, *A. paeoniifolius* and *A. rivieri*.

Materials and Methods

General

UV spectra were recorded on a Shimadzu MPS-2000 Multipurpose recording spectrophotometer (Shimadzu, Japan) according to Mabry *et al.* (1970). LC-MS were measured on Shimadzu LC-MS systems using Inertsil ODS-4 column [I.D. 2.1×100 mm (GL Sciences, Japan)], at a flow-rate of 0.1 ml min^{-1} , eluting with MeCN/H₂O/HCOOH (5:90:5) for anthocyanins and (12:83:5 or 15:80:5) for other flavonoids, ESI⁺ 4.5 kV and ESI⁻ 3.5 kV, 250°C. HPLC survey of the isolated compounds and crude extracts was performed with a Shimadzu HPLC systems using Inertsil ODS-4 column (I.D. 6.0×150 mm) at a flow-rate of 1.0 ml min^{-1} , eluting with MeCN/HOAc/H₂O/H₃PO₄ (10:8:79:3) (Solv. I) for anthocyanins, a Senshu Pak Pegasil ODS column (I.D. 6.0×150 mm, Senshu Scientific Co. Ltd., Japan) at a flow-rate of 1.0 ml min^{-1} , eluting with MeCN/H₂O/H₃PO₄ (15:85:0.2) (Solv. II) and (18:82:0.2) (Solv. III) for other flavonoids. Detection wave length was 530 nm for anthocyanins and 350 nm for other flavonoids. Acid hydrolyses of the anthocyanins and other flavonoids were performed in 12% HCl, 100°C, 30 min. After reaction, isoamyl alcohol (anthocyanins) or diethyl ether (other flavonoids) was added in the solution. Aglycones migrated to organic layer, and glycosidic sugars and C-glycosylflavones remained in aqueous layer. Aglycones and C-glycosylflavones were identified by HPLC comparisons with authentic samples, and sugars were compared with authentic sugars by paper chromatography or TLC using solvent sys-

tems, BBPW (*n*-BuOH/benzene/pyridine/H₂O = 5:1:3:3) and BTPW (*n*-BuOH/toluene/pyridine/H₂O = 5:1:3:3). The solvent systems of qualitative TLC (Merck, Germany) and preparative paper chromatography (PPC) are as follows; BAW (*n*-BuOH/HOAc/H₂O = 4:1:5, upper phase), BEW (*n*-BuOH/EtOH/H₂O = 4:1:2.2) and 15% HOAc. NMR spectra were measured in pyridine-*d*₅ at 600 MHz (¹H NMR) and 150 MHz (¹³C NMR).

Plant materials

Three *Amorphophallus* species, *A. titanum* (Becc.) Becc. ex Arcangeli (TBG157495), *A. paeoniifolius* (Dennst.) Nicolson (TBG84663) and *A. rivieri* Durieu (= *A. konjac* K.Koch) (TBG157597) (Fig. 1) were used as plant materials. They are cultivated in the Tsukuba Botanical Garden, National Museum of Nature and Science, Tsukuba, Japan.

Extraction and separation

Fresh spathe (454 g) and spadix appendix (945 g) of *A. titanum*, and fresh inflorescences (138 g and 300 g) of *A. paeoniifolius* and *A. rivieri* were extracted with MeOH:HCOOH (92:8). After filtration, the concentrated extracts were applied to PPC using solvent systems, BAW and 15% HOAc. Other flavonoids were further separated with solvent system, BEW. The isolated anthocyanins and other flavonoids were purified by Sephadex LH-20 column chromatography using solvent systems, MeOH/H₂O/HCOOH (70:25:5) and 70% MeOH, respectively.

Identification

The isolated compounds were identified by UV spectroscopy, LC-MS, characterization of acid hydrolysates, ¹H and ¹³C NMR, and TLC and HPLC comparisons with authentic samples. TLC, HPLC, LC-MS and NMR data of the isolated compounds are as follow.

Cyanidin 3-*O*-glucoside (chrysanthemin, **1**). HPLC: *t*R (min) 8.22 (solv. I). UV: λ_{max} (nm) 0.01% MeOH-HCl 282, 529; + AlCl₃ 556; E₄₄₀/E_{max} 28%. LC-MS: *m/z* 449 [M]⁺ (molecular ion



Fig. 1. Three *Amorphophallus* species used as plant materials.

peak, cyanidin + 1 mol glucose), m/z 287 $[M - 162]^+$ (fragment ion peak, cyanidin).

Cyanidin 3-*O*-rutinoside (keracyanin, **2**). HPLC: t_R (min) 9.22 (solv. I). UV: λ_{max} (nm) 0.01%MeOH-HCl 280, 527; + $AlCl_3$ 546; E_{440}/E_{max} 29%. LC-MS: m/z 595 $[M]^+$ (molecular ion peak, cyanidin + each 1 mol rhamnose and glucose), m/z 287 $[M - 308]^+$ (fragment ion peak, cyanidin).

Peonidin 3-*O*-glucoside (oxycoccicyanin, **3**). HPLC: t_R (min) 16.81 (solv. I). LC-MS: m/z 463 $[M]^+$ (molecular ion peak, peonidin + 1 mol glucose), m/z 301 $[M - 162]^+$ (fragment ion peak, peonidin).

Peonidin 3-*O*-rutinoside (**4**). HPLC: t_R (min) 17.82 (solv. I). UV: λ_{max} (nm) 0.01%MeOH-HCl 280, 529; + $AlCl_3$ 528; E_{440}/E_{max} 30%. LC-MS: m/z 609 $[M]^+$ (molecular ion peak, peonidin + each 1 mol rhamnose and glucose), m/z 301 $[M - 308]^+$ (fragment ion peak, peonidin).

Pelargonidin 3-*O*-rhamnosylglucoside (**5**). HPLC: t_R (min) 13.86 (solv. I). UV: λ_{max} (nm) 0.01%MeOH-HCl 277, 512; + $AlCl_3$ 512. LC-MS: m/z 579 $[M]^+$ (molecular ion peak, pelargonidin + each 1 mol rhamnose and glucose), m/z 271 $[M - 308]^+$ (fragment ion peak,

pelargonidin).

Cyanidin 3-*O*-diglucoside (**6**). HPLC: t_R (min) 6.18 (solv. I). UV: λ_{max} (nm) 0.01%MeOH-HCl 280, 527; + $AlCl_3$ 546; E_{440}/E_{max} 25%. LC-MS: m/z 611 $[M]^+$ (molecular ion peak, cyanidin + 2 mol glucose), m/z 287 $[M - 324]^+$ (fragment ion peak, cyanidin).

Pelargonidin 3-*O*-glucoside (callistephin, **7**). HPLC: t_R (min) 11.72 (solv. I). UV: λ_{max} (nm) 0.01%MeOH-HCl 272, 512; + $AlCl_3$ 513; E_{440}/E_{max} 42%. LC-MS: m/z 433 $[M]^+$ (molecular ion peak, pelargonidin + 1 mol glucose).

Vitexin (**8**). TLC: R_f 0.48 (BAW), 0.44 (BEW), 0.16 (15%HOAc); color UV (365 nm) dark purple, UV/NH₃ dark greenish yellow. HPLC: t_R (min) 6.11 (solv. II). UV: λ_{max} (nm) MeOH 270, 329; + NaOMe 278, 326, 394 (inc.); + $AlCl_3$ 275, 304, 346, 377sh; + $AlCl_3$ /HCl 274, 303, 344, 376sh; + NaOAc 279, 310, 390; + NaOAc/H₃BO₃ 271, 344. LC-MS: m/z 431 $[M - H]^-$ (molecular ion peak, apigenin + 1 mol glucose).

Vitexin 2''-*O*-glucoside (**9**). TLC: R_f 0.36 (BAW), 0.40 (BEW), 0.59 (15%HOAc); color UV (365 nm) dark purple, UV/NH₃ dark greenish yellow. HPLC: t_R (min) 6.37 (solv. II). UV: λ_{max} (nm) MeOH 270, 334; + NaOMe 280,

330, 397 (inc.); + AlCl₃ 277, 303, 349, 383; + AlCl₃/HCl 278, 302, 343, 382sh; + NaOAc 280, 312, 392; + NaOAc/H₃BO₃ 271, 345. LC-MS: *m/z* 593 [M – H][–] (molecular ion peak, apigenin + 2 mol glucose).

Isovitexin (**10**). TLC: R_f 0.71 (BAW), 0.68 (BEW), 0.42 (15%HOAc); color UV (365 nm) dark purple, UV/NH₃ dark greenish yellow. HPLC: *t*R (min) 8.02 (solv. II). UV: λ_{max} (nm) MeOH 271, 334; + NaOMe 279, 327, 399 (inc.); + AlCl₃ 278, 303, 350, 377sh; + AlCl₃/HCl 279, 303, 346, 376sh; + NaOAc 278, 313, 394; + NaOAc/H₃BO₃ 272, 347. LC-MS: *m/z* 433 [M + H]⁺, 431 [M – H][–] (molecular ion peaks, apigenin + 1 mol glucose).

Isovitexin 2''-O-glucoside (**11**). TLC: R_f 0.45 (BAW), 0.51 (BEW), 0.69 (15%HOAc); color UV (365 nm) dark purple, UV/NH₃ dark greenish yellow. HPLC: *t*R (min) 6.52 (solv. II). UV: λ_{max} (nm) MeOH 270, 334; + NaOMe 280, 330, 397 (inc.); + AlCl₃ 277, 303, 349, 383; + AlCl₃/HCl 278, 302, 343, 382sh; + NaOAc 280, 312, 392; + NaOAc/H₃BO₃ 271, 345. LC-MS: *m/z* 593 [M – H][–] (molecular ion peak, apigenin + 2 mol glucose).

Orientin (**12**). TLC: R_f 0.31 (BAW), 0.27 (BEW), 0.11 (15%HOAc); color UV (365 nm) dark purple, UV/NH₃ dark yellow. HPLC: *t*R (min) 5.27 (solv. II). UV: λ_{max} (nm) MeOH 257, 269, 349; + NaOMe 270, 327sh, 407 (inc.); + AlCl₃ 274, 422; + AlCl₃/HCl 263sh, 276, 296, 358, 385sh; + NaOAc 272, 276, 327sh, 400; + NaOAc/H₃BO₃ 266, 376, 427sh. LC-MS: *m/z* 449 [M + H]⁺, 447 [M – H][–] (molecular ion peaks, luteolin + 1 mol glucose).

Isoorientin (**13**). TLC: R_f 0.47 (BAW), 0.45 (BEW), 0.28 (15%HOAc); color UV (365 nm) dark purple, UV/NH₃ dark yellow. HPLC: *t*R (min) 6.01 (solv. II). UV: λ_{max} (nm) MeOH 258, 270, 350; + NaOMe 270, 337sh, 411 (inc.); + AlCl₃ 276, 422; + AlCl₃/HCl 262sh, 278, 296sh, 357, 384sh; + NaOAc 270, 402; + NaOAc/H₃BO₃ 267, 378, 426sh. LC-MS: *m/z* 449 [M + H]⁺, 447 [M – H][–] (molecular ion peaks, luteolin + 1 mol glucose).

Vicenin-2 (**14**). TLC: R_f 0.17 (BAW), 0.19

(BEW), 0.40 (15%HOAc); color UV (365 nm) dark purple, UV/NH₃ dark greenish yellow. HPLC: *t*R (min) 4.73 (solv. II). UV: λ_{max} (nm) MeOH 272, 334; + NaOMe 282, 335, 399 (inc.); + AlCl₃ 279, 303, 353, 384sh; + AlCl₃/HCl 279, 303, 347, 383sh; + NaOAc 282, 398; + NaOAc/H₃BO₃ 278, 283sh, 323, 349, 410sh. LC-MS: *m/z* 595 [M + H]⁺, 593 [M – H][–] (molecular ion peaks, apigenin + 2 mol glucose).

Lucenin-2 (**15**). TLC: R_f 0.09 (BAW), 0.09 (BEW), 0.31 (15%HOAc); color UV (365 nm) dark purple, UV/NH₃ dark yellow. HPLC: *t*R (min) 4.14 (solv. II). UV: λ_{max} (nm) MeOH 255sh, 272, 344; + NaOMe 268, 280, 340sh, 411 (inc.); + AlCl₃ 279, 402; + AlCl₃/HCl 260sh, 279, 296sh, 356, 385sh; + NaOAc 268sh, 281, 327sh, 404; + NaOAc/H₃BO₃ 275, 285sh, 361, 422. LC-MS: *m/z* 611 [M + H]⁺, 609 [M – H][–] (molecular ion peaks, luteolin + 2 mol glucose).

Schaftoside (**16**). TLC: R_f 0.21 (BAW), 0.20 (BEW), 0.40 (15%HOAc); color UV (365 nm) dark purple, UV/NH₃ dark greenish yellow. HPLC: *t*R (min) 5.63 (solv. II). UV: λ_{max} (nm) MeOH 272, 333; + NaOMe 283, 334, 399 (inc.); + AlCl₃ 279, 305, 350, 384sh; + AlCl₃/HCl 279, 303, 345, 384sh; + NaOAc 282, 314sh, 397; + NaOAc/H₃BO₃ 276sh, 284, 322, 350sh, 410. LC-MS: *m/z* 565 [M + H]⁺, 563 [M – H][–] (molecular ion peaks, apigenin + each 1 mol arabinose and glucose).

Isoschaftoside (**17**). TLC: R_f 0.32 (BAW), 0.35 (BEW), 0.40 (15%HOAc); color UV (365 nm) dark purple, UV/NH₃ dark greenish yellow. HPLC: *t*R (min) 5.63 (solv. II). UV: λ_{max} (nm) MeOH 272, 333; + NaOMe 283, 334, 399 (inc.); + AlCl₃ 279, 305, 350, 384sh; + AlCl₃/HCl 279, 303, 345, 384sh; + NaOAc 282, 314sh, 397; + NaOAc/H₃BO₃ 276sh, 284, 322, 350sh, 410. LC-MS: *m/z* 565 [M + H]⁺, 563 [M – H][–] (molecular ion peaks, apigenin + each 1 mol arabinose and glucose).

Isovitexin X''-O-rhamnoside (**18**). TLC: R_f 0.53 (BAW), 0.53 (BEW), 0.75 (15%HOAc); color UV (365 nm) dark purple, UV/NH₃ dark greenish yellow. HPLC: *t*R (min) 6.75 (solv. II). UV: λ_{max} (nm) MeOH 270, 329; + NaOMe 280,

331, 393 (inc.); + AlCl₃ 273, 304, 349, 377sh; + AlCl₃/HCl 278, 303, 339, 380sh; + NaOAc 280, 313, 389; + NaOAc/H₃BO₃ 270, 347. LC-MS: *m/z* 579 [M + H]⁺, 577 [M – H][–] (molecular ion peaks, apigenin + each 1 mol rhamnose and glucose).

Isoscoparin X''-O-glucoside (**19**). TLC: Rf 0.14 (BAW), 0.12 (BEW), 0.36 (15%HOAc); color UV (365 nm) dark purple, UV/NH₃ dark greenish yellow. HPLC: *t*R (min) 5.25 (solv. II). UV: λ_{max} (nm) MeOH 273, 345; + NaOMe 282, 341sh, 411 (inc.); + AlCl₃ 262sh, 277, 300sh, 360, 387sh; + AlCl₃/HCl 257sh, 277, 298sh, 358, 387sh; + NaOAc 282, 337sh, 409; + NaOAc/H₃BO₃ 276, 285sh, 344, 412. LC-MS: *m/z* 625 [M + H]⁺ (molecular ion peak, chrysoeriol + 2 mol glucose), *m/z* 461 [M – 162 – H][–] (fragment ion peak, chrysoeriol + 1 mol glucose).

Chrysoeriol 7-O-glucoside (**20**). TLC: Rf 0.48 (BAW), 0.41 (BEW), 0.09 (15%HOAc); color UV (365 nm) dark purple, UV/NH₃ yellow. HPLC: *t*R (min) 16.16 (solv. II). UV: λ_{max} (nm) MeOH 252, 269, 342; + NaOMe 264, 276sh, 391 (inc.); + AlCl₃ 262sh, 274, 296, 358, 385sh; + AlCl₃/HCl 259sh, 276, 297, 354, 385sh; + NaOAc 258, 265sh, 404; + NaOAc/H₃BO₃ 269, 348. LC-MS: *m/z* 463 [M + H]⁺, 461 [M – H][–] (molecular ion peaks, chrysoeriol + 1 mol glucose).

Kaempferol 3-O-robinobioside (**21**). TLC: Rf 0.69 (BAW), 0.63 (BEW), 0.75 (15%HOAc); color UV (365 nm) dark purple, UV/NH₃ dark greenish yellow. HPLC: *t*R (min) 9.33 (solv. II). UV: λ_{max} (nm) MeOH 267, 343; + NaOMe 276, 327, 400 (inc.); + AlCl₃ 275, 303, 352, 384; + AlCl₃/HCl 276, 302, 347, 387sh; + NaOAc 275, 316, 394; + NaOAc/H₃BO₃ 267, 353. LC-MS: *m/z* 593 [M – H][–] (molecular ion peak, kaempferol + each 1 mol rhamnose and galactose), *m/z* 287 [M – 308 + H]⁺ (fragment ion peak, kaempferol).

Quercetin 3-O-glucoside (isoquercitrin, **22**). TLC: Rf 0.65 (BAW), 0.65 (BEW), 0.31 (15%HOAc); color UV (365 nm) dark purple, UV/NH₃ yellow. HPLC: *t*R (min) 7.33 (solv. II). UV: λ_{max} (nm) MeOH 257, 265sh, 358;

+ NaOMe 274, 331, 407 (inc.); + AlCl₃ 274, 431; + AlCl₃/HCl 268, 300, 359, 394sh; + NaOAc 273, 327, 395; + NaOAc/H₃BO₃ 261, 380. LC-MS: *m/z* 463 [M – H][–] (molecular ion peak, quercetin + 1 mol glucose), *m/z* 302 [M – 162 + H]⁺ (fragment ion peak, quercetin).

Vitexin 2''-β-O-xylopyranoside (**23**). TLC: Rf 0.47 (BAW), 0.55 (BEW), 0.73 (15%HOAc); color UV (365 nm) dark purple, UV/NH₃ dark greenish yellow. HPLC: *t*R (min) 6.22 (solv. II). UV: λ_{max} (nm) MeOH 270, 330; + NaOMe 279, 331, 394 (inc.); + AlCl₃ 276, 304, 348, 384sh; + AlCl₃/HCl 277, 302, 343, 383sh; + NaOAc 279, 311, 390; + NaOAc/H₃BO₃ 271, 345. LC-MS: *m/z* 565 [M + H]⁺, 563 [M – H][–] (molecular ion peaks, apigenin + each 1 mol xylose and glucose). ¹H NMR (600 MHz, pyridine-*d*₅): δ 13.85 (1H, s, 5-OH), 8.25 (2H, d, *J* = 6.7 Hz, H-2',6'), 7.24 (2H, d, *J* = 6.7 Hz, H-3',5'), 6.82 (1H, s, H-3), 6.74 (1H, s, H-6), 5.84 (1H, d, *J* = 9.2 Hz, glucosyl H-1), 5.01 (1H, brs, xylosyl H-1), 4.95 (1H, m, glucosyl H-2), 4.53 (1H, m, glucosyl H-4), 4.52 (1H, m, glucosyl H-6a), 4.42 (1H, m, glucosyl H-6b), 4.41 (1H, m, glucosyl H-3), 4.08 (1H, m, glucosyl H-5), 3.83 (1H, m, xylosyl H-3), 3.82 (1H, m, xylosyl H-5a), 3.82 (1H, m, xylosyl H-2), 3.76 (1H, m, xylosyl H-4), 3.18 (1H, m, xylosyl H-5b). ¹³C NMR (150 MHz, pyridine-*d*₅): (apigenin) δ 165.1 (C-2), 103.5 (C-3), 183.2 (C-4), 162.5 (C-5), 99.4 (C-6), 164.7 (C-7), 105.4 (C-8), 158.3 (C-9), 105.4 (C-10), 123.2 (C-1'), 129.5 (C-2',6'), 117.0 (C-3',5'), 162.6 (C-4'); (C-glucose) δ 73.5 (C-1), 82.0 (C-2), 80.6 (C-3), 72.2 (C-4), 83.5 (C-5), 62.9 (C-6); (xylose) δ 106.7 (C-1), 75.0 (C-2), 77.4 (C-3), 70.8 (C-4), 66.5 (C-5).

Kaempferol 3-O-glucoside (astragalin, **24**). TLC: Rf 0.81 (BAW), 0.77 (BEW), 0.39 (15%HOAc); color UV (365 nm) dark purple, UV/NH₃ dark greenish yellow. HPLC: *t*R (min) 8.34 (solv. III). UV: λ_{max} (nm) MeOH 266, 349; + NaOMe 275, 325, 398 (inc.); + AlCl₃ 274, 303, 351, 391; + AlCl₃/HCl 274, 302, 348, 389; + NaOAc 275, 313, 392; + NaOAc/H₃BO₃ 267, 353. LC-MS: *m/z* 447 [M – H][–] (molecular ion

peak, kaempferol + 1 mol glucose), m/z 287 $[M - 162 + H]^+$ (fragment ion peak, kaempferol).

Authentic samples

Authentic samples, which were used in this survey, and their origin were as follows: cyanidin 3-*O*-glucoside, cyanidin 3-*O*-rutinoside, peonidin 3-*O*-glucoside, peonidin 3-*O*-rutinoside and pelargonidin 3-*O*-glucoside from Extra Synthese, France, vitexin from the fronds of *Adiantum venustum* Don (Parkeriaceae) (Iwashina *et al.*, 1995), isovitexin from the flowers of *Iris ensata* Thunb. (Iridaceae) (Iwashina *et al.*, 1996), isovitexin 2''-*O*-glucoside and vitexin 2''-*O*-glucoside from the fronds of *Cyrtomium falcatum* (L.f.) C. Presl (Dryopteridaceae) (Iwashina *et al.*, 2006), orientin from the leaves of *Begonia formosana* (Hayata) Masam. (Begoniaceae) (Iwashina *et al.*, 2008a), isoorientin from the leaves of *Vitex rotundifolia* L.fil. (Verbenaceae) (Iwashina *et al.*, 2011), vicianin-2 and lucenin-2 from the fronds of *Asplenium normale* D.Don (Aspleniaceae) (Iwashina *et al.*, 2010), schaftoside and isoschaftoside from the aerial parts of *Osyris alba* L. (Santalaceae) (Iwashina *et al.*, 2008b), kaempferol 3-*O*-robinobioside from the aerial parts of *Cassytha filiformis* L. (Lauraceae) (Murai *et al.*, 2008), kaempferol 3-*O*-glucoside from the flowers of *Glycine max* (L.) Merr. (Leguminosae) (Iwashina *et al.*, 2007), and quercetin 3-*O*-glucoside from the leaves of *Calystegia sordanella* (L.) Roem. et Schult. (Convolvulaceae) (Murai *et al.*, 2014).

Results and Discussion

Anthocyanins from *Amorphophallus titanum*, *A. rivieri* and *A. paeoniifolius*

Five anthocyanins (**1**–**5**) were isolated from the spathe and spadix appendix of *Amorphophallus titanum* and the inflorescence of *A. rivieri*. Their anthocyanin composition was the same with each other. On the other hand, three anthocyanins (**1**, **6** and **7**) were found in the inflorescence of *A. paeoniifolius* (Fig. 2).

Acid hydrolysis of **1** and **2** produced cyanidin as anthocyanidin. And also, glucose, and glucose and rhamnose were detected as glycosidic sugars, respectively. As the results, they were identified as cyanidin 3-*O*-glucoside (chrysanthemin, **1**, Fig. 3) and cyanidin 3-*O*-rutinoside (keracyanin, **2**, Fig. 3) by HPLC comparisons with authentic samples. Similarly, **3** and **4** were identified as peonidin 3-*O*-glucoside (oxycoccicyanin, Fig. 3) and peonidin 3-*O*-rutinoside (Fig. 3). Anthocyanin **5** was obtained as a minor compo-

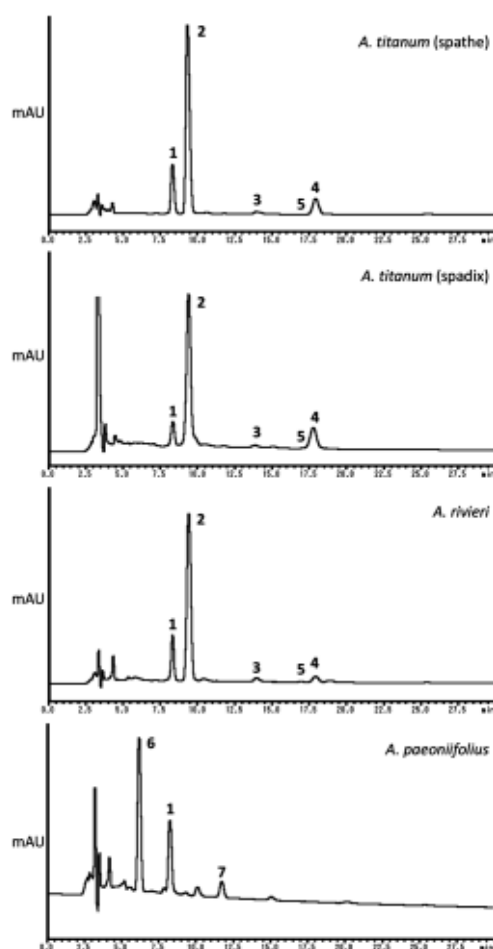


Fig. 2. HPLC patterns of the anthocyanins in the inflorescences of three *Amorphophallus* species. **1**: cyanidin 3-*O*-glucoside, **2**: cyanidin 3-*O*-rutinoside, **3**: peonidin 3-*O*-glucoside, **4**: peonidin 3-*O*-rutinoside, **5**: pelargonidin 3-*O*-rhamnosylglucoside, **6**: cyanidin 3-*O*-diglucoside and **7**: pelargonidin 3-*O*-glucoside.

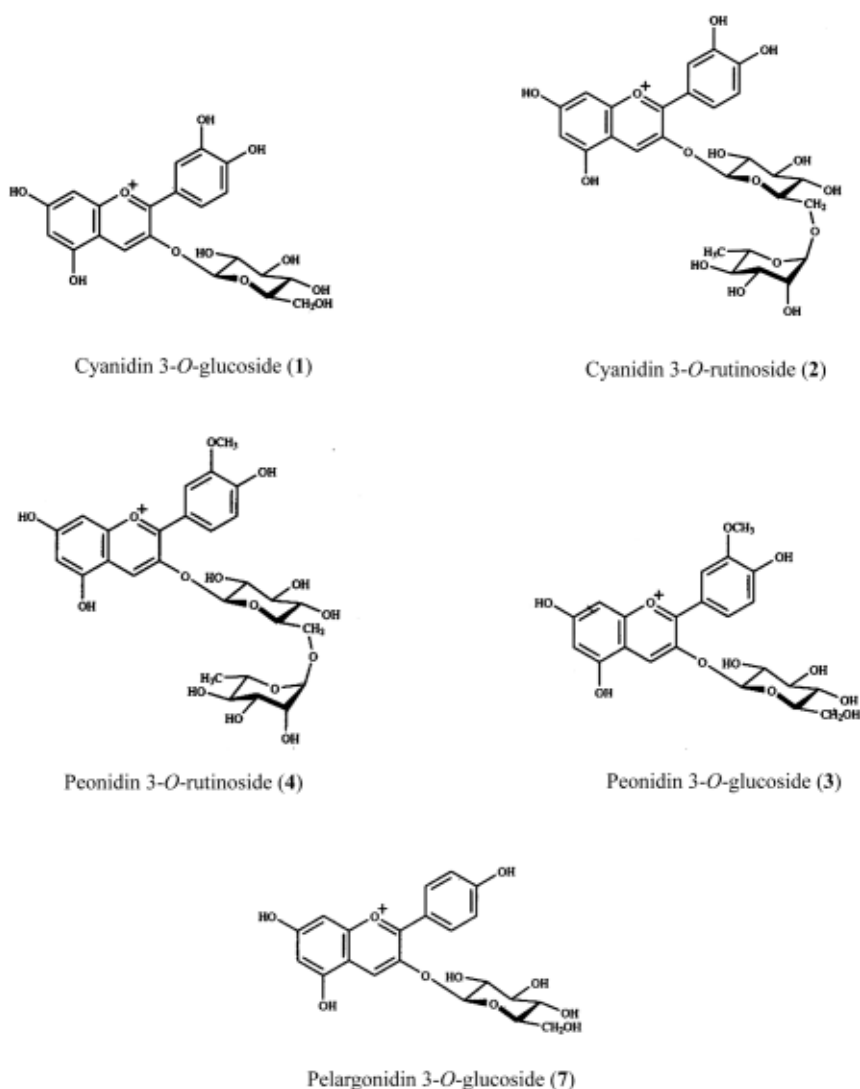


Fig. 3. Anthocyanins isolated from the inflorescences of three *Amorphophallus* species.

ment from *A. titanum* and *A. rivieri*. Pelargonidin, glucose and rhamnose were liberated by acid hydrolysis. Attachment of each 1 mol glucose and rhamnose to pelargonidin was shown by appearance of molecular ion peak, m/z 579 $[M]^+$ of LC-MS. Thus, **5** was characterized as pelargonidin 3-*O*-rhamnosylglucoside. Anthocyanins **6** and **7** were isolated from *A. paeoniifolius*. They produced cyanidin and pelargonidin by acid hydrolysis, respectively. Attachment of 2 mol glucose to 3-position of cyanidin was shown by LC-MS survey, i.e. appearance of molecular ion

peak, m/z 611 $[M]^+$, and UV spectral survey. From the results described above, **6** was characterized as cyanidin 3-*O*-diglucoside. On the other hand, **7** was identified as pelargonidin 3-*O*-glucoside (callistephin) (Fig. 3) by HPLC comparison with authentic sample.

In this survey, five anthocyanins, cyanidin 3-*O*-glucoside (**1**), cyanidin 3-*O*-rutinoside (**2**), peonidin 3-*O*-glucoside (**3**), peonidin 3-*O*-rutinoside (**4**) and pelargonidin 3-*O*-rhamnosylglucoside (perhaps 3-*O*-rutinoside, **5**), were isolated and characterized from *A. titanum* and *A. rivieri*.

Though the spathe and spadix appendix of *A. titanum* were separately extracted, their anthocyanin composition was qualitatively the same. On the other hand, three anthocyanins, cyanidin 3-*O*-glucoside (**1**), cyanidin 3-*O*-diglucoside (**6**) and pelargonidin 3-*O*-glucoside (**7**), were isolated from the inflorescence of *A. paeoniifolius*. *A. titanum* and *A. paeoniifolius*, and *A. rivieri* are native to tropical Asia, and temperate zones of Asia, respectively. However, anthocyanin pattern was the same between *A. titanum* and *A. rivieri*, but not *A. paeoniifolius*. Anthocyanins in the inflorescence of *A. titanum* have been surveyed, and 3-*O*-glucoside and 3-*O*-rutinoside of cyanidin, and peonidin and pelargonidin 3-*O*-(*p*-coumaroylglucosides) were reported (Gallori *et al.*, 2004). Of their anthocyanins, though the former two were found in this survey, the latter two acylated anthocyanins were not detected. Instead, three anthocyanins, peonidin 3-*O*-glucoside (**3**), peonidin 3-*O*-rutinoside (**4**) and pelargonidin 3-*O*-rhamnosylglucoside (**5**) were newly isolated. The anthocyanins in the inflorescences of *A. rivieri* and *A. paeoniifolius* were reported for the first time.

Flavonoids in the spathe and spadix appendix of *Amorphophallus titanum*

Fourteen flavonoids were isolated from the spathe and spadix appendix of *A. titanum*. UV spectral properties of flavonoids **8** and **10** showed that they are flavones having 5-, 7- and 4'-hydroxyl groups (Mabry *et al.*, 1970). Since they were unhydrolyzable by hot acid treatment, it was shown that they are *C*-glycosylflavones. Attachment of 1 mol hexose to apigenin was cleared by LC-MS, i.e. appearance of molecular

ion peaks, m/z 433 $[M+H]^+$ and 431 $[M-H]^-$. Finally, they were identified as vitexin (5,7,4'-trihydroxyflavone 8-*C*-glucoside, **8**) and isovitexin (5,7,4'-trihydroxyflavone 6-*C*-glucoside, **10**) (Fig. 4) by TLC and HPLC comparisons with authentic samples. UV spectral properties of **9** and **11** were also the same with those of **8** and **10**, showing that they are 5,7,4'-trihydroxyflavone derivatives. Vitexin and isovitexin were liberated by acid hydrolysis of **9** and **11**, respectively. Attachment of additional 1 mol glucose to vitexin and isovitexin was proved by LC-MS, i.e. appearance of molecular ion peak, m/z 593 $[M-H]^-$. Finally, **9** and **11** were identified as vitexin 2''-*O*-glucoside and isovitexin 2''-*O*-glucoside (Fig. 5) by TLC and HPLC comparisons with authentic standards. It was shown by UV, hot acid treatment and LC-MS that **12** and **13** are 5,7,3',4'-tetrahydroxylated flavone *C*-monohexosides. Thus, they were identified as orientin (5,7,3',4'-tetrahydroxyflavone 8-*C*-glucoside, **12**) and isoorientin (5,7,3',4'-tetrahydroxyflavone 6-*C*-glucoside, **13**) (Fig. 6) by TLC and HPLC comparisons with authentic samples. Flavonoid **14** was unhydrolyzable by hot acid treatment, showing that it is *C*-glycosylflavone. Attachment of 2 mol hexose to 5,7,4'-trihydroxyflavone was shown by LC-MS, i.e. appearance of molecular ion peaks, m/z 595 $[M+H]^+$ and 593 $[M-H]^-$. Finally, **14** was identified as vicenin-2 (5,7,4'-trihydroxyflavone 6,8-di-*C*-glucoside) (Fig. 7) by TLC and HPLC comparison with authentic sample. Similarly, **15** was estimated as lucenin-2 (5,7,3',4'-tetrahydroxyflavone 6,8-di-*C*-glucoside) (Fig. 7). UV spectral survey and hot acid treatment of **16** and **17** showed that they are *C*-glycosylflavones having free 5-, 7- and 4'-hydroxyl

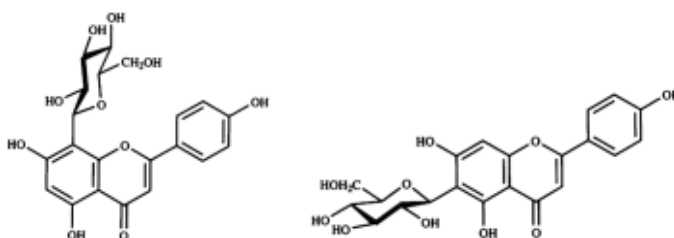


Fig. 4. Chemical structures of vitexin (left, **8**) and isovitexin (right, **10**).

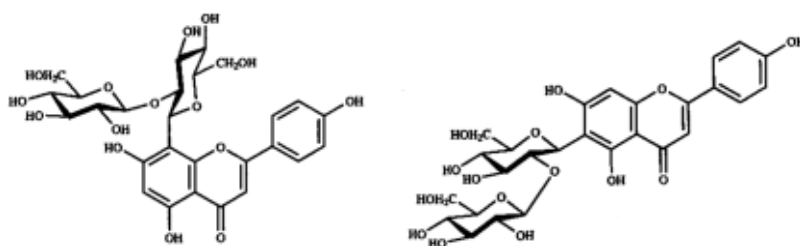


Fig. 5. Chemical structures of vitexin 2''-O-glucoside (left, **9**) and isovitexin 2''-O-glucoside (right, **11**).

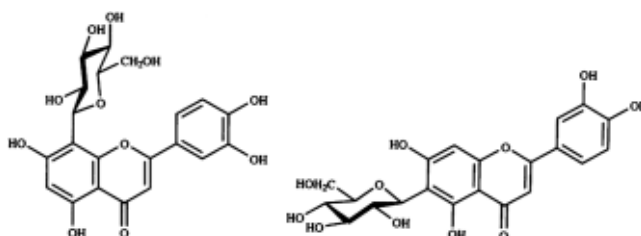


Fig. 6. Chemical structures of orientin (left, **12**) and isoorientin (right, **13**).

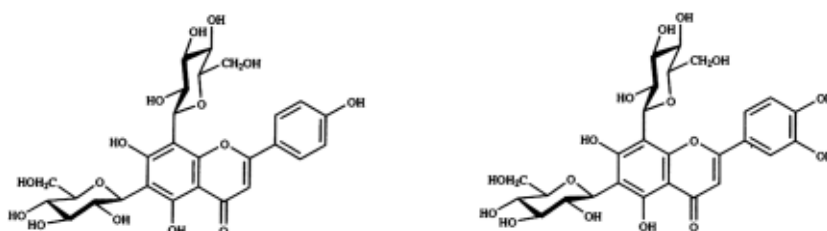


Fig. 7. Chemical structures of vicenin-2 (left, **14**) and lucenin-2 (right, **15**).

groups. By LC-MS survey of their compounds, molecular ion peaks, m/z 565 $[M+H]^+$ and 563 $[M-H]^-$, appeared on the chromatograms, showing that they are apigenin monopentosylhexoside. It was cleared that they are schaftoside (apigenin 6-C-glucoside-8-C-arabioside, **16**) and isoschaftoside (apigenin 6-C-arabioside-8-C-glucoside, **17**) by TLC and HPLC comparisons with authentic samples (Fig. 8). Flavonoid **18** produced isovitexin and rhamnose by acid hydrolysis. Since UV spectral properties of original compound were essentially the same with those of isovitexin, rhamnosyl moiety was considered to be attached to 6-C-glucosyl group of isovitexin. From the results described above, **18** was characterized as isovitexin X''-O-rhamnoside. However, interglycosidic linkage could not be determined.

To flavonoid **19** is trihydroxy-monomethoxyflavone dihexoside proved by LC-MS, i.e. appearance of molecular ion peak, m/z 625 $[M+H]^+$. Isoscaparin (5,7,4'-trihydroxy-3'-methoxyflavone 6-C-glucoside) and glucose were liberated by acid hydrolysis of **19**. Since UV spectral properties of **19** were the same with those of isoscaparin, it was shown that O-glucose is attached to 6-C-glucosyl group. Thus, **19** was characterized as isoscaparin X''-O-glucoside. UV spectral survey in addition of various shift reagents showed that **20** is 7,3'-substituted luteolin (Mabry *et al.*, 1970). Chrysoeriol (5,7,4'-trihydroxy-3'-methoxyflavone) and glucose were produced by acid hydrolysis. Since molecular ion peaks, m/z 463 $[M+H]^+$ and 461 $[M-H]^-$, appeared on the chromatogram of LC-MS, it was shown that

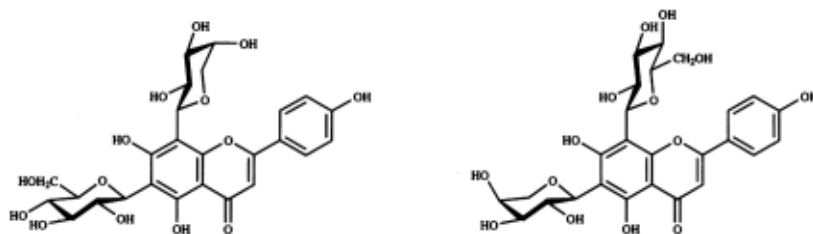


Fig. 8. Chemical structures of schaftoside (left, **16**) and isoschaftoside (right, **17**).

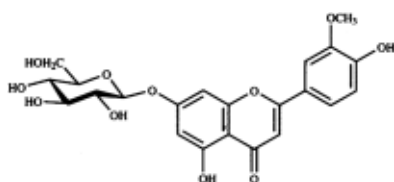


Fig. 9. Chemical structure of chrysoeriol 7-O-glucoside (**20**).

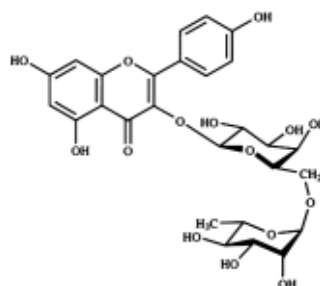


Fig. 10. Chemical structure of kaempferol 3-O-robinobioside (**21**).

1 mol glucose is attached to chrysoeriol. Thus, **20** was identified as chrysoeriol 7-O-glucoside (Fig. 9). Flavonoid **21** was only one flavonol from the inflorescence of *A. titanum*. Kaempferol, rhamnose and galactose were liberated by acid hydrolysis of **21**. UV spectral properties of the compound showed that this is 3-substituted kaempferol. Molecular ion peak, m/z 593 $[M - H]^-$, appeared on the chromatogram of LC-MS, showing the attachment of each 1 mol rhamnose and galactose to kaempferol. Finally, **21** was identified as kaempferol 3-O-robinobioside [= 3-O-rhamnosyl-(1→6)-galactoside] (Fig. 10) by TLC and HPLC comparison with authentic sample. Though the presence of other flavonoids was noticed by HPLC analysis of the crude extract of *A. titanum*, they could not be isolated for small amounts.

Flavonoids in the inflorescences of *A. rivieri* and *A. paeoniifolius*

Five flavonoids were isolated from the inflorescence of *A. rivieri*. On the other hand, seven flavonoids were identified from *A. paeoniifolius*. Of the flavonoids in *A. rivieri*, three were identified as isovitexin (**10**), orientin (**12**) and isoorientin (**13**). Flavonoid **22** liberated quercetin and

glucose by acid hydrolysis. Since UV spectral properties of original compound showed that **22** is 3-substituted quercetin, it was proved that glucose is attached to 3-position of quercetin. Finally, **22** was identified as quercetin 3-O-glucoside (isoquercitrin) (Fig. 11) by TLC and HPLC comparison with authentic sample. Flavonoid **23** was obtained as pale yellow powder (yield, ca. 30 mg). Vitexin and xylose were found as acid hydrolysates of **23**. Attachment of 1 mol xylose to 8-C-glucosyl group of vitexin was shown by m/z 565 $[M + H]^+$ and 563 $[M - H]^-$ of LC-MS and UV spectral properties. In 1H NMR, six aromatic proton signals, δ 6.82, 6.74, 8.25 and 7.24 derived from H-3, H-6, H-2',6' and H-3',5', and two anomeric proton signals, δ 5.84 (d, $J=9.2$ Hz) and 5.01 (brs) derived from glucose and xylose, appeared. The interglycosidic linkage was determined as xylosyl-(1→2)-glucose from HMBC correlation between xylosyl anomeric proton at δ 5.01 and C-2 signal of 8-C-glucose at δ 82.0. Thus, **23** was identified as apigenin 8-C- β -xylopyranosyl-(1→2)-glucopyranoside (vitexin 2''-O-xyloside) (Fig. 12).

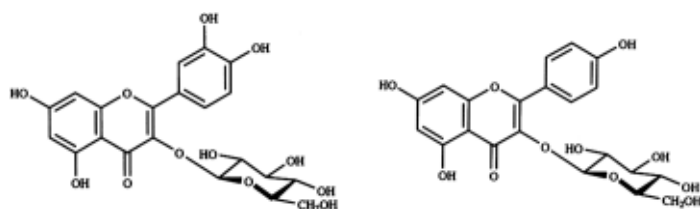


Fig. 11. Chemical structures of quercetin 3-*O*-glucoside (left, **22**) and kaempferol 3-*O*-glucoside (right, **24**).

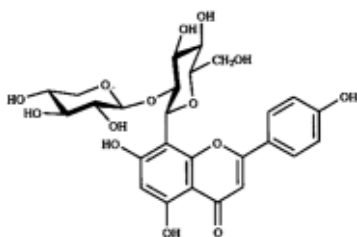


Fig. 12. Chemical structure of vitexin 2''-*O*-xyloside (**23**).

Of seven flavonoids isolated from the inflorescence of *A. paeoniifolius*, six were identified as vitexin (**8**), isovitexin (**10**), orientin (**12**), vicenin-2 (**14**), schaftoside (**16**) and quercetin 3-*O*-glucoside (**22**). Acid hydrolysis of another flavonoid **24** liberated kaempferol and glucose. Attachment of 1 mol glucose to 3-position of kaempferol was determined by LC-MS and UV spectra. Finally, **24** was identified as kaempferol 3-*O*-glucoside (astragalin) (Fig. 11) by TLC and HPLC comparison with authentic sample. In *A. paeoniifolius* (= *A. campanulatus*), quercetin and a rare flavonol, diacetyltambulin, have been isolated from the corm and tuberous roots (Sharstry *et al.*, 2010; Khan *et al.*, 2008). However, they were not found in this survey.

Distribution of anthocyanins and other flavonoids in the inflorescences of three Amorphophallus species

The anthocyanins and other flavonoids isolated from three *Amorphophallus* species are shown in Table 1. In *A. titanum*, flavonoids were separately extracted from the spathe and spadix appendix. Though anthocyanin composition of their organs was qualitatively the same, other fla-

vonoid composition was different. In the spadix appendix, all flavonoids which were found in *A. titanum* in this survey were present. However, two flavonoids only, vitexin (**8**) and orientin (**12**), were isolated from the spathe. This may be means the difference of the function of the flavonoids between the spathe and spadix appendix of *A. titanum*. Among the three *Amorphophallus* species used as plant materials, their flavonoid pattern was different to each other. However, their major flavonoids are *C*-glycosylflavones, together with minor flavonols. Anthocyanins and other flavonoids have been surveyed in many species of the family Araceae, and it has been reported that major anthocyanins are cyanidin 3-*O*-rutinoside and 3-*O*-glucoside (Williams *et al.*, 1981). Furthermore, *C*-glycosylflavones were found in many Araceous species with minor flavonols, kaempferol and quercetin glycosides (Williams *et al.*, 1981). In *A. titanum*, though chrysoeriol 7-*O*-glucoside (**20**) was found as only one flavone *O*-glycoside, another flavone *O*-glycoside, luteolin 7-*O*-glucoside, has been reported from the leaves of *Colocasia esculenta* (Iwashina *et al.*, 1999). Thus, the anthocyanin and other flavonoid characters of three *Amorphophallus* species, *A. titanum*, *A. rivieri* and *A. paeoniifolius* show the same patterns with those of many Araceous species.

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Table 1. Anthocyanins and other flavonoids isolated from the inflorescences of three *Amorphophallus* species

<i>A. titanum</i> (spathe)	
Anthocyanins:	cyanidin 3- <i>O</i> -glucoside, cyanidin 3- <i>O</i> -rutinoside, peonidin 3- <i>O</i> -glucoside, peonidin 3- <i>O</i> -rutinoside, pelargonidin 3- <i>O</i> -rhamnosylglucoside
C-Glycosylflavones:	vitexin, orientin
<i>A. titanum</i> (spadix appendix)	
Anthocyanins:	cyanidin 3- <i>O</i> -glucoside, cyanidin 3- <i>O</i> -rutinoside, peonidin 3- <i>O</i> -glucoside, peonidin 3- <i>O</i> -rutinoside, pelargonidin 3- <i>O</i> -rhamnosylglucoside
C-Glycosylflavones:	vitexin, vitexin 2''- <i>O</i> -glucoside, isovitexin, isovitexin 2''- <i>O</i> -glucoside, orientin, isoorientin, vicenin-2, lucenin-2, schaftoside, isoschaftoside, isovitexin X''- <i>O</i> -rhamnoside, isoscoparin X''- <i>O</i> -glucoside
Flavone:	chrysoeriol 7- <i>O</i> -glucoside
Flavonol:	kaempferol 3- <i>O</i> -robinobioside
<i>A. rivieri</i> (inflorescence)	
Anthocyanins:	cyanidin 3- <i>O</i> -glucoside, cyanidin 3- <i>O</i> -rutinoside, peonidin 3- <i>O</i> -glucoside, peonidin 3- <i>O</i> -rutinoside, pelargonidin 3- <i>O</i> -rhamnosylglucoside
C-Glycosylflavones:	isovitexin, orientin, isoorientin, vitexin 2''- <i>O</i> -xyloside
Flavonol:	quercetin 3- <i>O</i> -glucoside
<i>A. paeoniifolius</i> (inflorescence)	
Anthocyanins:	cyanidin 3- <i>O</i> -glucoside, cyanidin 3- <i>O</i> -diglucoside, pelargonidin 3- <i>O</i> -glucoside
C-Glycosylflavones:	vitexin, isovitexin, orientin, vicenin-2, schaftoside
Flavonols:	kaempferol 3- <i>O</i> -glucoside, quercetin 3- <i>O</i> -glucoside

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