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Flavone *O*- and *C*-Glycosides from *Pothos chinensis* (Araceae)

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Abastract Nine flavonoid glycosides were isolated from the aerial parts of *Pothos chinensis*. Of their flavonoids, eight ones were identified as vitexin, vitexin 7-*O*-glucoside, isovitexin 7-*O*-glucoside, scoparin 7-*O*-glucoside, isoscoparin 7-*O*-glucoside, schaftoside, isoschaftoside and chrysoeriol 7-*O*-rhamnosylglucoside. Another flavonoid was characterized as vitexin 7-*O*-diglucoside or vitexin 7,X″-di-*O*-glucoside. The flavonoids of *P. chinensis* were reported in this paper for the first time.

Key words : Araceae, flavone glycosides, flavonoids, *Pothos chinensis*.

Introduction

The genus *Pothos* belongs to the family Araceae and consists of ca. 50 species (Mabberley, 1997). In Taiwan and Japan, *P. chinensis* (Raf.) Merr. is only growing. Though the species is abundant at medium elevations through Taiwan (Huang, 2000), it is endemic to Daito Islands in Japan (Hatusima and Amano, 1994) and nominated as endangered plant by the Ministry of Environment, Japan (Environment Agency of Japan, 2000).

Flavonoids of the family Araceae have roughly been investigated by Williams *et al*. (1981). They were surveyed the leaf flavonoids of 144 species from 58 genera and showed that flavone *C*-glycosides are the most characteristic flavonoid constituents of the family. In inflorescence, fruits, leaf or petiole of 59 representative species, they were detected cyanidin 3-*O*-rutinoside as a common anthocyanin, together with pelargonidin 3-*O*-rutinoside and cyanidin 3-*O*-glucoside (Williams *et al*., 1981). Eight *C*-glycosylflavones and a flavone *O*-glycoside were isolated from the leaves of cultivated taro (*Colocasia esculenta* (L.) Schott) and identified as orientin, isoorientin, isovitexin, vicenin-2, orientin 7-*O*-glucoside, isovitexin 4′-*O*-glucoside, vitexin X″-*O*-glucoside, and luteolin 7-*O*-glucoside (Iwashina *et al.*, 1999). The anthocyanins, cyanidin and pelargonidin glycosides were found in two *Anthurium* species, and cyanidin and delphinidin glycosides in three *Philodendron* species (Forsyth and Simmonds, 1954), cyanidin 3,5-di-*O*-glucoside in the spathe of *Arisaema serratum* Schott. f. *thunbergii* Makino (Ueno *et al*., 1969), pelargonidin 3-*O*-glucoside, cyanidin 3-*O*-rhamnoside and cyanidin 3-*O*-glucoside in the peeled corms of taro (Chan Jr. *et al*., 1977), and cyanidin and peonidin glycosides in the spathe of *Symplocarpus foetidus* Nutt. var. *latissimus* (Makino) Hara (Yoshitama *et al*., 1980). Common flavones and *C*-glycosylflavones, apigenin, luteolin, vitexin, isovitexin, orientin, isoorientin and isovitexin 7-*O*-glucoside were isolated from the leaves of *Synandrospadix vermitoxicus* (Grisebach) Engler which is a monotypic species and grows in Argentina and Bolivia (Sosa *et al*., 1978). Vitexin 2″-*O*-glucoside and its sinapoyl derivative were reported from *Cryptocoryne* spp. as their chemo-

Fig. 1. *Pothos chinensis* (Raf.) Merr.

taxonomic marker (Franke *et al*., 2006). The former compound was also found in *Lasia spinosa* (L.) Thw. native to Vietnam, together with vitexin and a flavonol glycoside, isorhamnetin 3-*O*-rutinoside (Hong Van *et al*., 2006). Another flavonol glycoside, kaempferol 3-*O*-sophoroside-7-*O*-rhamnoside was isolated from the leaves of *Gymnostachys anceps* R. Br. (Williams *et al*., 1971). However, flavonoid compounds in *Pothos* species have not been reported until now. In this paper, flavonoid glycosides in the aerial parts of *Pothos chinensis* are described.

Materials and Methods

Plant materials

Pothos chinensis (Raf.) Merr. (Fig. 1) was collected in hiking way to Shihfen Waterfall, Pingchi Hsiang, Taipei, Taiwan, during 5 November, 2006.

Extraction and isolation of flavonoids

Fresh aerial parts (35.4 g) of *P. chinensis* 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), 15% HOAc and then BEW (*n*-BuOH/EtOH/H_2O=4 : 1 : 2.2). The isolated flavonoids were purified by Sephadex LH-20 column chromatography using solvent system: 70% MeOH.

High performance liquid chromatography (HPLC)

HPLC was performed with Shimadzu HPLC systems using a PEGASIL ODS column (I.D. 6.0×150 mm: Senshu Scientific Co. Ltd.), at a flow-rate of 1.0 ml min^{-1}. Detection was 190–700 nm and eluent was MeCN/H_2O/H_3PO_4 (18 : 82 : 0.2).

Liquid chromatograph-mass spectra (LC-MS)

LC-MS was measured using a PEGASIL ODS column (I.D. 2.0×150 mm, Senshu Scientific Co.

Ltd.), at a flow-rate of 0.2 ml min^{-1}, eluent was $MeCN/H_2O/HCOOH$ (15 : 80 : 5), by ESI^+ 4.5 kV and ESI^- 3.5 kV, 250°C.

Identification of flavonoids

The isolated flavonoids were identified by UV spectra according to Mabry *et al*. (1970), LC-MS, acid hydrolysis and characterization of their products, and direct TLC and HPLC comparisons with authentic standards. TLC, HPLC, UV and LC-MS data of the isolated flavonoids were as follows.

Vitexin (**1**). TLC: Rf 0.45 (BAW), 0.48 (BEW), 0.16 (15% HOAc); UV—dark purple, UV/NH_3—dark greenish yellow. HPLC: Rt 10.90 min. UV: λ_{max} (nm) MeOH 270, 331; +NaOMe 279, 328, 393 (inc.); $+AlCl_3$ 276, 305, 354, 384sh; $+AlCl_3$/HCl 276, 303, 349, 383sh; +NaOAc 279, 311, 389; $+NaOAc/H_3BO_3$ 272, 344. LC-MS: *m/z* 433 $[M+H]^+$, 431 $[M-H]^-$ (apigenin+1 mol hexose).

Vitexin 7-*O*-glucoside (isosaponarin, **2**). TLC: Rf 0.09 (BAW), 0.10 (BEW), 0.20 (15% HOAc); UV—dark purple, UV/NH_3—dark yellow. HPLC: Rt 6.81 min. UV: λ_{max} (nm) MeOH 269, 332; +NaOMe 275, 384 (inc.); $+AlCl_3$ 275, 303, 349, 377sh; $+AlCl_3$/HCl 276, 302, 343, 375sh; +NaOAc 257sh, 269, 392; $+NaOAc/H_3BO_3$ 270, 342. LC-MS: *m/z* 595 $[M+H]^+$, 593 $[M-H]^-$ (apigenin+2 mol hexose).

Isovitexin 7-*O*-glucoside (saponarin, **3**). TLC: Rf 0.25 (BAW), 0.26 (BEW), 0.60 (15% HOAc); UV—dark purple, UV/NH_3—dark yellow. HPLC: Rt 6.34 min. UV: λ_{max} (nm) MeOH 272, 333; +NaOMe 274, 386 (inc.); $+AlCl_3$ 278, 301, 352, 376sh; $+AlCl_3$/HCl 278, 300, 345, 375sh; +NaOAc 257sh, 270, 391; $+NaOAc/H_3BO_3$ 271, 338. LC-MS: *m/z* 595 $[M+H]^+$, 593 $[M-H]^-$ (apigenin+2 mol hexose).

Scoparin 7-*O*-glucoside (**4**). TLC: Rf 0.21 (BAW), 0.26 (BEW), 0.50 (15% HOAc); UV—dark purple, UV/NH_3—dark yellow. HPLC: Rt 7.18 min. UV: λ_{max} (nm) MeOH 255sh, 272, 342; +NaOMe 281, 398 (inc.); $+AlCl_3$ 264sh, 277, 301, 358; $+AlCl_3$/HCl 260sh, 278, 301, 354; +NaOAc 271, 280sh, 406; $+NaOAc/H_3BO_3$ 274, 285sh, 351. LC-MS: *m/z* 625 $[M+H]^+$, 623 $[M-H]^-$ (chrysoeriol+2 mol hexose).

Isoscoparin 7-*O*-glucoside (**5**). TLC: Rf 0.08 (BAW), 0.06 (BEW), 0.12 (15% HOAc); UV—dark purple, UV/NH_3—yellow. HPLC: Rt 8.03 min. UV: λ_{max} (nm) MeOH 252, 270sh, 348; +NaOMe 250, 264sh, 392 (inc.); $+AlCl_3$ 264, 273sh, 304sh, 361; $+AlCl_3$/HCl 274sh, 305sh, 360; +NaOAc 258, 286sh, 410; $+NaOAc/H_3BO_3$ 267sh, 286sh, 349. LC-MS: *m/z* 625 $[M+H]^+$, 623 $[M-H]^-$ (chrysoeriol+2 mol hexose).

Schaftoside (**6**). TLC: Rf 0.22 (BAW), 0.18 (BEW), 0.51 (15% HOAc); UV—dark purple, UV/NH_3—dark greenish yellow. HPLC: Rt 6.63 min. UV: λ_{max} (nm) MeOH 273, 333; +NaOMe 283, 331, 400 (inc.); $+AlCl_3$ 278, 306, 354, 386sh; $+AlCl_3$/HCl 278, 304, 349, 386sh; +NaOAc 282, 398; $+NaOAc/H_3BO_3$ 276sh, 285, 322, 348sh. LC-MS: *m/z* 565 $[M+H]^+$, 563 $[M-H]^-$ (apigenin+each 1 mol hexose and pentose).

Isoschaftoside (**7**). TLC: Rf 0.22 (BAW), 0.18 (BEW), 0.33 (15% HOAc); UV—dark purple, UV/NH_3—dark greenish yellow. HPLC: Rt 6.47 min. UV: λ_{max} (nm) MeOH 273, 333; +NaOMe 283, 331, 400 (inc.); $+AlCl_3$ 278, 306, 354, 386sh; $+AlCl_3$/HCl 278, 304, 349, 386sh; +NaOAc 282, 398; $+NaOAc/H_3BO_3$ 276sh, 285, 322, 348sh. LC-MS: *m/z* 565 $[M+H]^+$, 563 $[M-H]^-$ (apigenin+each 1 mol hexose and pentose).

Chrysoeriol 7-*O*-rhamnosylglucoside (**8**). TLC: Rf 0.44 (BAW), 0.43 (BEW), 0.08 (15% HOAc); UV—dark purple, UV/NH_3—yellow. UV: λ_{max} (nm) MeOH 251, 268, 345; +NaOMe 264, 300sh, 391 (inc.); $+AlCl_3$ 273sh, 300, 360, 387sh; $+AlCl_3$/HCl 273sh, 301, 358, 387sh; +NaOAc 258, 264sh, 405; $+NaOAc/H_3BO_3$ 267, 348. LC-MS: *m/z* 609 $[M+H]^+$ (chrysoeriol+each 1 mol hexose and rhamnose), 463 $[M-146+H]^+$ (chrysoeriol+1 mol hexose).

Vitexin 7-*O*-diglucoside or 7,X″-di-*O*-glucoside (**9**). TLC: Rf 0.11 (BAW), 0.13 (BEW), 0.75 (15% HOAc); UV—dark purple, UV/NH_3—dark yellow. HPLC: Rt 5.38 min. UV: λ_{max} (nm)

MeOH 269, 332; +NaOMe 275, 384 (inc.); +$AlCl_3$ 275, 303, 349, 377sh; +$AlCl_3$/HCl 276, 302, 343, 375sh; +NaOAc 257sh, 269, 392; +NaOAc/H_3BO_3 270, 342. LC-MS: *m/z* 757 $[M+H]^+$ (apigenin+3 mol hexose), 595 $[M-162+H]^+$ (apigenin+2 mol hexose).

Results and Discussion

Nine flavonoids were isolated from the aerial parts of *Pothos chinensis* from Taiwan. Of their compounds, flavonoids **2** (3 mg) and **3** (7 mg) were obtained as pale yellow powder.

UV spectral properties of flavonoid **1** showed the compound is flavone glycoside having free 5-, 7- and 4′-hydroxyl groups (Mabry *et al*., 1970). By hot acid treatment (in 12% HCl, 100°C, 30 min) of the original compound, another flavonoid was produced by Wessely-Moser rearrangement, showing the original flavonoid is 6- or 8-*C*-glycosylflavone (Markham, 1982). Attachment of 1 mol hexose to 5,7,4′-trihydroxyflavone was shown by the appearance of a molecular ion peak, *m/z* 433 $[M+H]^+$ on LC-MS. Finally, flavonoid **1** was identified as 5,7,4′-trihydroxyflavone 8-*C*-glucoside, i.e., vitexin (Fig. 2) by direct TLC and HPLC comparison with authentic sample from the flowers of *Iris ensata* Thunb. (Iridaceae) (Iwashina *et al*., 1996).

UV spectral survey of flavonoids **2** and **3** showed that they are 7-substituted apigenin (Mabry *et al*., 1970). Since vitexin, isovitexin and glucose were liberated by acid hydrolysis of them, it was indicated that the original glycosides are 7-*O*-glucoside of vitexin and isovitexin, respectively. Of their glycosides, Rf values and retention times of flavonoid **3** agreed with those of authentic saponarin from the flowers of *Strongylodon macrobotrys* A. Gray (Leguminosae) (Iwashina *et al*., 1984). Thus, flavonoid **3** was identified as isovitexin 7-*O*-glucoside (Fig. 4). On the other hand, flavonoid **2** was considered as vitexin 7-*O*-glucoside, i.e., isosaponarin (Fig. 3).

Since the molecular ion peak, *m/z* 625 $[M+H]^+$ appeared on LC-MS of flavonoids **4** and **5**, their compounds were presumed as trihydroxy-monomethoxyflavone dihexosides. Their UV spectral survey of them showed the presence of free 5- and 4′-hydroxyl and substituted 7- and 3′-hydroxyl groups. Each one product was liberated by acid hydrolysis of them, together with glucose. Their hydrolysates were proved to be 3′-substituted 5,7,4′-trihydroxyflavones by UV spectral survey, showing the attachment of glucose to 7-hydroxyl group and the presence of methoxyl group. Thus, flavonoids **4** and **5** were estimated as scoparin (chrysoeriol 8-*C*-glucoside) and isoscoparin (chrysoeriol 6-*C*-glucoside) 7-*O*-glucosides, respectively (Fig. 5 and 6).

Flavonoids **6** and **7** were also shown to be *C*-glycosylflavones by hot acid treatment, and to be 5,7,4′-trihydroxyflavones by UV spectral survey.

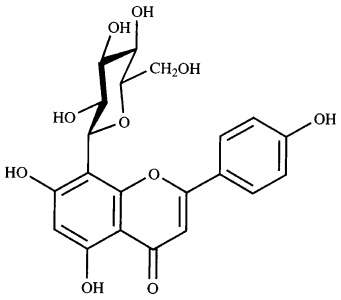

Fig. 2. Vitexin (**1**).

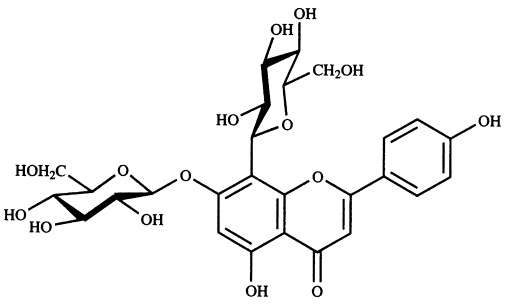

Fig. 3. Vitexin 7-*O*-glucoside (Isosaponarin, **2**).

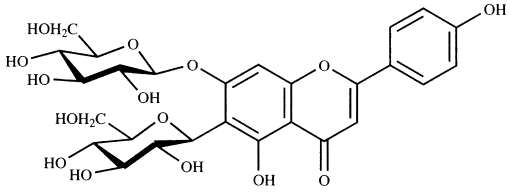

Fig. 4. Isovitexin 7-*O*-glucoside (Saponarin, **3**).

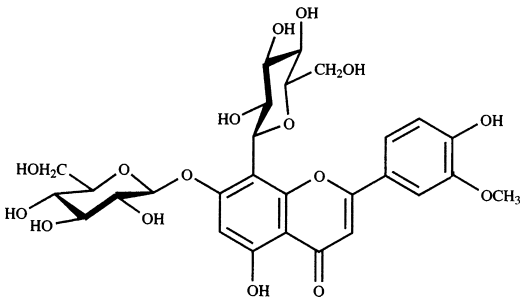

Fig. 5. Scoparin 7-*O*-glucoside (**4**).

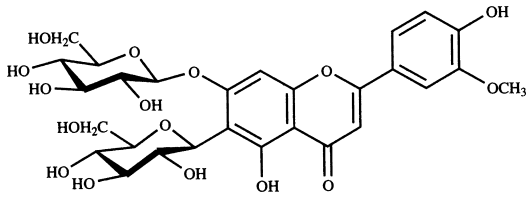

Fig. 6. Isoscoparin 7-*O*-glucoside (**5**).

The molecular ion peaks, *m/z* 565 $[M+H]^+$ appeared on LC-MS of their compounds, showing the attachment of each 1 mol hexose and pentose to apigenin nucleus. Finally, they were identified as apigenin 6-*C*-glucoside-8-*C*-arabinoside (**6**) (Fig. 7) and apigenin 6-*C*-arabinoside-8-*C*-glucoside (**7**) (Fig. 8) by direct TLC and HPLC comparisons with authentic schaftoside and isoschaftoside from the aerial parts of *Osyris alba* L. (Santalaceae) (Iwashina *et al*., 2008).

Acid hydrolysis of flavonoid **8** liberated chrysoeriol (5,7,4′-trihydroxy-3′-methoxyflavone), rhamnose and glucose. Since the presence of free 5- and 4′-hydroxyl groups was shown by UV spectra, it was proved that the sugars are attached to 7-position of chrysoeriol. A molecular ion peak, *m/z* 609 $[M+H]^+$ appeared on LC-MS, showing the attachment of each 1 mol glucose and rhamnose to chrysoeriol. In addition, a fragment ion peak, *m/z* 463 $[M-146+H]^+$ appeared, showing that the original glycoside is 7-*O*-rhamnosylglucoside but not 7-*O*-glucosylrhamnoside. Thus, flavonoid **8** was characterized as chrysoeriol 7-*O*-rhamnosylglucoside.

It was proved by UV spectra that flavonoid **9** was also 7-substituted *C*-glycosylapigenin. Vitexin and glucose were liberated by acid hydrolysis. Moreover, the presence of an additional glucose was proved by LC-MS, i.e., the appearance of a molecular ion peak, *m/z* 757 $[M+H]^+$, showing the attachment of 3 mol glucose to apigenin. However, the attached position of an additional glucose could not be determined. From the results described above, flavonoid **9** was presumed as vitexin 7-*O*-diglucoside or 7,*X*″-di-*O*-glucoside.

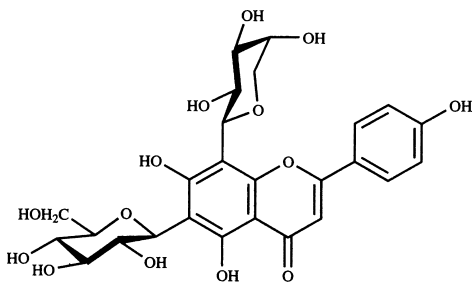

Fig. 7. Schaftoside (**6**).

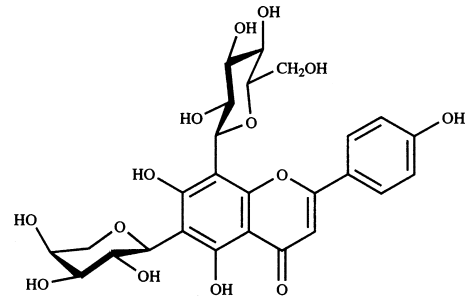

Fig. 8. Isoschaftoside (**7**).

C-Glycosylflavones have been known as characteristic flavonoids in the family Araceae (Williams *et al*., 1981). In this survey, *C*-glycosylflavones and their *O*-glycosides were isolated from the aerial parts of *Pothos chinensis* as major flavonoids. Of their compounds, relatively rare *C*-glycosylflavones, schaftoside and isoschaftoside, have been reported from other Araceous species, *Anthurium bellum* and *Philodendron eichleri* (Williams *et al*., 1981). In spite of *Pothos* is relatively large genus including 50 species, their flavonoid information has only been reported from *Pothos scandens* L. (Williams *et al*., 1981). However, no flavonoid was found in this species. It was shown for the first time that

flavonoid character of the genus was *C*-glycosylflavones as well as almost other Araceae genera.

References

Chan Jr., H. T., Kao-Jao, T. H. C. and Nakayama, T. O. M. 1977. Anthocyanin composition of taro. Journal of Food Science 42: 19–21.

Environment Agency of Japan (ed.) 2000. Threatened Wildlife of Japan, Vol. 8, Vascular Plants. pp. 491. Japan Wildlife Research Center, Tokyo (in Japanese).

Forsyth, W. G. C. and Simmonds, N. W. 1954. A survey of the anthocyanins of some tropical plants. Proceedings of the Royal Society, London, Series B 142: 549–564.

Franke, K., Hoffmann, M., Schmidt, J., Porzel, A., Arnold, N. and Wessjohann, L. 2006. 2″-*O*-Glucosylvitexin, a chemotaxonomic marker for the genus *Cryptocoryne* (Araceae). Biochemical Systematics and Ecology 34: 546–548.

Hatusima, S. and Amano, T. 1995. Flora of the Ryukyus, South of Amami Island. 2nd. ed., Enlarged and Revised by Hatusima, S. pp. 136. The Biological Society of Okinawa, Okinawa (in Japanese).

Hong Van, N. T., Minh, C. V., De Leo, M., Siciliano, T. and Alessandra, B. 2006. Secondary metabolites from *Lasia spinosa* (L.) Thw. (Araceae). Biochemical Systematics and Ecology 34: 882–884.

Huang, T.-C. 2000. Araceae. In: Editorial Committee of the Flora of Taiwan (ed.), Flora of Taiwan, 2nd. ed. Volume 5. Angiosperms–Monocotyledons [Alismataceae–Triuridaceae], pp. 664–694. National Taiwan University, Taipei.

Iwashina, T., Ootani, S. and Hayashi, K. 1984. Pigment components in the flower of *Strongylodon macrobotrys* and spectrophotometric analysis of fresh petal and intact cells. Science Reports of the Research Institute of Evolutionary Biology 2: 67–74.

Iwashina, T., Kamenosono, K. and Yabuya, T. 1996. Isolation and identification of flavonoid and related compounds as co-pigments from the flowers of *Iris ensata*. The Journal of Japanese Botany 71: 281–287.

Iwashina, T., Konishi, T., Takayama, A., Fukada, M. and Ootani, S. 1999. Isolation and identification of the flavonoids in the leaves of taro. Annals of the Tsukuba Botanical Garden 18: 71–74.

Iwashina, T., López-Sáez, J. A. and Kitajima, J. 2008. Flavonoids from *Osyris alba*. Biochemical Systematics and Ecology 36: 146–147.

Mabberley, D. J. 1997. The Plant-Book. 2nd. ed. pp. 581. Cambridge University Press, Cambridge.

Mabry, T. J., Markham, K. R. and Thomas, M. B. 1970. The Systematic Identification of Flavonoids. Springer, Berlin.

Markham, K. R. 1982. Techniques of Flavonoid Identification. p. 56. Academic Press, London.

Sosa, V. E., Oberti, J. C. and Juliani, H. R. 1978. Flavonoids of *Synandrospadix vermitoxicus*. Lloydia 41: 277–278.

Ueno, N., Takemura, E. and Hayashi, K. 1969. Additional data for the paperchromatographic survey of anthocyanins in the flora of Japan (IV). Studies on anthocyanins, LXI. The Botanical Magazine, Tokyo 82: 155–161.

Williams, C. A., Harborne, J. B. and Clifford, H. T. 1971. Flavonoid patterns in the monocotyledons. Flavonols and flavones in some families associated with the Poaceae. Phytochemistry 10: 1059–1063.

Williams, C. A., Harborne, J. B. and Mayo, S. J. 1981. Anthocyanin pigments and leaf flavonoids in the family Araceae. Phytochemistry 20: 217–234.

Yoshitama, K., Ishii, K. and Yasuda, H. 1980. A chromatographic survey of anthocyanins in the flora of Japan, I. Journal of the Faculty of Science, Shinshu University 15: 19–26.