Bull. Natl. Mus. Nat. Sci., Ser. B, 37(3), pp. 133–145, August 22, 2011

# Flavonoid Properties of six *Asplenium* Species in Vanuatu and New Caledonia, and Distribution of Flavonoid and Related Compounds in *Asplenium*

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(Received 7 June 2011; accepted 22 June 2011)

**Abstracts** Flavonoid and related compounds were isolated from six *Asplenium* species, *A. carruthersii*, *A. contiguum*, *A. caudatum*, *A. insiticium*, *A. laserpitiifolium* and *A. subfulexuosum*, in Vanuatu and New Caledonia. The compounds were characterized by UV, acid hydrolysis, and PC and HPLC comparisons with authentic samples. Flavonoid characters of *A. carruthersii* and *A. contiguum* are *C*-glycosylflavones, and xanthones and flavonol glycosides based on kaempferol, respectively. On the other hand, those of *A. insiticium*, *A. laserpitiifolium* and *A. subflexuosum* were kaempferol glycosides. No flavonoid was detected from *A. caudatum*. Though flavonoid diversification in the genus *Asplenium* has been reported until now, it was confirmed by this survey of the species in Vanuatu and New Caledonia that polyglycosylated flavonols, especially kaempferol glycosides are major compounds and flavone *C*- and *O*-glycosides, xanthones, flavones and aurone scatteredly occur in the genus. Moreover, distribution of flavonoids and xanthones in the genus *Asplenium* was reviewed in this paper.

**Key words** : *Asplenium*, flavonoids, New Caledonia, Vanuatu, xanthones.

## Introduction

The genus *Asplenium* consists of ca. 650 species and is distributed in the world (Sasaki, 2008). In South Pacific Islands including Vanuatu and New Caledonia, 33 taxa have been reported (Sasaki, 2008). In the botanical expedition to Vanuatu and adjacent countries and area in 1996 and 1997 (Iwashina *et al*., 1998), authors have collected 14 *Asplenium* species, *A. amboinense* Baker, *A. australasicum* Hook., *A. bipinnatifidum* Baker, *A. carruthersii* Baker, *A. caudatum* G. Forst., *A. contiguum* Kaulf., *A. cuneatum* Lam., *A. excisum* C. Presl (=*Hymenasplenium excisum*), *A. horridum* Kaulf., *A. insiticium* Brack., *A. laserpitiifolium* Lam., *A. nidus* L., *A. polyodon* G. Forst. and *A. unilaterale* Lam. sensu lato (=*Hymenasplenium laterale*) in Vanuatu (Matsumoto *et al*., 1998), and 8 species, *A*. aff. *adiantoides*, *A. amboinense*, *A. australasicum*, *A. bipinnatifidum*, *A. insticium*, *A. laserpitiifolium*, *A. oligolepidum* C. Chr. and *A. subflexuosum* in New Caledonia and Fiji (Hashimoto *et al*., 1998). In 2000 and 2001 expedition (Iwashina *et al*., 2002), 17 species, i.e., *A. amboinense*, *A. australasicum*, *A. bipinnatifidum*, *A. contiguum*, *A. cuneatum*, *A. caudatum*×*laserpitiifolium*, *A. gibberosum* (G. Forst.) Mett., *A. horridum*, *A. insiticium*, *A. laserpitiifolium*, *A. marattioides* (Brack.) C. Chr., *A. nidus*, *A. polyodon*, *A. subflexuosum*, *A. excisum*, *A. subnormale* Copel. (=*Hymenasplenium subnormale*) and *A. unilaterale*, were collected in Vanuatu (Matsumoto *et al*., 2002).

As the chemotaxonomic study of the genus *Asplenium*, authors have been surveyed the flavonoid compounds in *A. normale* D. Don and related species, *A. boreale* (Ohwi ex Sa. Kurata)

Nakaike, *A. shimurae* (H. Ito) Nakaike and *A. oligophlebium* Baker including var. *iezimaense* (Tagawa) Tagawa and their hybrids (Iwashina *et al*., 1990, 1993b, 2010a; Iwashina and Matsumoto, 1990, 1994; Matsumoto *et al*., 2003). An alpine *Asplenium* species, *A. viride* Hudson (=*A. trichomanes-ramosum* L.), which were collected at representative places in the Northern Hemisphere, was surveyed for flavonoids, and it was shown that their flavonoid composition including new and rare substances consist in all samples (Iwashina *et al*., 1995). Three *Asplenium* species belonging to subgenera *Ceterach* and *Ceterachopsis*, *A. ceterach* L., *A. dalhousiae* Hook. and *A. punjabense* Bir., Fraser-Jenkins et Lovis in Pakistan have been isolated for flavonoids (Iwashina *et al*., 1993). The flavonoids of four European and Japanese *Asplenium* species, *A. obovatum* Viv., *A. foreziense* Legrand ex Hérib., *A. fontanum* (L.) Bernh. and *A. incisum* Thunb. have been isolated and their flavonoid characters were shown (Iwashina *et al*., 2000). Some *Asplenium* species in tropical zone, Malaysia, have been surveyed for flavonoids by Umikalsom *et al*. (1994). However, flavonoid characters of *Asplenium* species native to South Pacific area have hardly surveyed.

In this paper, flavonoid composition of six *Asplenium* species in Vanuatu and New Caledonia was surveyed and distribution of flavonoids and related xanthones in the genus *Asplenium* is reviewed.

## Materials and Methods

*Plant materials*

Six *Asplenium* species in Vanuatu and New Caledonia were surveyed for flavonoids. Their collection sites are as follows.

*Asplenium carruthersii* Baker. Along the Pialapa River, Tsureviu, 10–220 m alt., Espiritu Santo Is., Vanuatu, 14 Oct. 1997 (*Matsumoto111*), along the Pialapa River, Tsureviu, 220–540 m alt., Espiritu Santo Is., Vanuatu, 15 Oct. 1997 (*Matsumoto121*).

*Asplenium incisum* Brack. Between Nokovula and Mt. Tabwemasana, on north ridge of Mt. Tabwemasana, 1700 m alt., Espiritu Santo Is., Vanuatu, 7 Nov. 1996 (*Iwashina3070*), Nokovula, 1200 m alt., Espiritu Santo Is., Vanuatu, 7 Nov. 1996 (*Iwashina3100*), east ridge of Mt. Vutimele, 1500 m alt., Espiritu Santo Is., Vanuatu, 23 Nov. 1996 (*Iwashina3218*), and Mts. Koghis, north of Nouméa, 480–800 m alt., Grande Terre Is., New Caledonia, 4 Nov. 1997 (*Matsumoto431*).

*Asplenium laserptiifolium* Lam. Nokovula, 1070 m alt., Espiritu Santo Is., Vanuatu, 6 Nov. 1996 (*Iwashina3039*), Nokovula, 1050 m alt., Espiritu Santo Is., Vanuatu, 9 Nov. 1996 (*Iwashina3115*), Butmas, ca. 500 m alt., Espiritu Santo Is., Vanuatu, 23 Oct. 1997 (*Matsumoto332*), along the Pialapa River, 240–540 m alt., Espiritu Santo Is., Vanuatu, 19 Oct. 1997 (*Matsumoto278*), and Mts. Koghis, north of Nouméa, 480–800 m alt., Grande Terre Is., New Caledonia, 4 Nov. 1997 (*Matsumoto436*).

*Asplenium subflexuosum* Ros. Mts. Koghis, north of Nouméa, 480–800 m alt., Grande Terre Is., New Caledonia, 4 Nov. 1997 (*Matsumoto434*).

*Asplenium contiguum* Kaulf. West peak of Mt. Tabwemasana, 1800 m alt., Espiritu Santo Is., Vanuatu, 7 Nov. 1996 (*Iwashina3068*), and north ridge of Mt. Tabwemasana, 1700 m alt., Espiritu Santo Is., 7 Nov. 1996 (*Iwashina3083*).

*Asplenium caudatum* G. Forst. Mt. Vutimena, 540–1050 m alt., Espiritu Santo Is., Vanuatu, 18 Oct. 1997 (*Matsumoto225a*).

*Extraction and isolation*

Dry fronds of *A. carruthersii* (11.7 g), *A. insiticium* (15.8 g), *A. laserpitiifolium* (22.9 g), *A. subflexuosum* (8.2 g), *A. contiguum* (35.6 g) and *A. caudatum* (39.5 g) were extracted with MeOH, respectively. The concentrated crude 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 compounds 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 Senshu Pak 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 350 nm and eluent was MeCN/$H_2O$/$H_3PO_4$ (20 : 80 : 0.2).

*Liquid chromatograph-mass spectra (LC-MS)*

LC-MS was performed with Shimadzu LC-MS systems using Senshu Pak PEGASIL ODS column (I.D. 2.0×150 mm, Senshu Scientific Co. Ltd.) at a flow-rate of 0.1 ml $min^{-1}$, $ESI^+$ 4.5 kV and $ESI^-$ 3.5 kV, 250ºC. The eluent was HCOOH/MeCN/$H_2O$ (5 : 20 : 75).

*Acid hydrolysis*

Acid hydrolysis was performed in 12% HCl, 100 ºC, 30 min. After shaking with diethyl ether, aglycones migrated to organic layer, and sugars and *C*-glycosylflavones were left in aqueous layer.

*UV spectral survey*

UV spectra were measured in MeOH according to Mabry *et al*. (1970).

*Identification of flavonoids and xanthones*

Flavonoids and xanthones were identified by UV spectra, acid hydrolysis, LC-MS, and direct PC and HPLC comparisons with authentic samples. PC, UV and LC-MS data of the isolated compounds are as follows.

Kaempferol 3-*O*-rhamnoside-7-*O*-glucoside (**1**). PC: Rf 0.48 (BAW), 0.58 (BEW), 0.63 (15%HOAc), 0.51 (5%HOAc); color UV–dark purple, UV/$NH_3$–greenish yellow. HPLC: t*R* (min) 4.97. UV: λmax (nm) MeOH 266, 350; +NaOMe 274, 390 (inc.); +$AlCl_3$ 235, 275, 300, 352, 395; +$AlCl_3$/HCl 235, 275, 299, 348, 396; +NaOAc 266, 397; +NaOAc/$H_3BO_3$ 266, 352. LC-MS: *m/z* 595 $[M+H]^+$, 593 $[M-H]^-$ (kaempferol+each 1 mol glucose and rhamnose) (molecular ion peak), *m/z* 433 $[M-162+H]^+$ (kaempferol+1 mol rhamnose), and *m/z* 287 $[M-308+H]^+$ (kaempferol) (fragment ion peaks).

Kaempferol 3-*O*-glucoside-7-*O*-rhamnoside (**2a**) and kaempferol 3-*O*-galactoside-7-*O*-rhamnoside (**2b**). PC: Rf 0.29 (BAW), 0.45 (BEW), 0.80 (15%HOAc), 0.71 (5%HOAc); color UV–dark purple, UV/$NH_3$–greenish yellow. HPLC: t*R* (min) 5.09. UV: λmax (nm) MeOH 267, 349; +NaOMe 275, 385 (inc.); +$AlCl_3$ 233sh, 275, 300, 350, 392; +$AlCl_3$/HCl 235, 275, 299, 346, 392; +NaOAc 267, 390; +NaOAc/$H_3BO_3$ 267, 350.

Kaempferol 3,7-*O*-pentaglycoside (**3**). HPLC: t*R* (min) 3.98. UV: λmax (nm) MeOH 243, 267, 331; +NaOMe 273, 388 (inc.); +$AlCl_3$ 272, 301, 352, 386sh; +$AlCl_3$/HCl 236, 277, 300, 338, 390sh; +NaOAc 266, 369; +NaOAc/$H_3BO_3$ 265, 352. LC-MS: *m/z* 1079 $[M-H]^-$ (kaempferol+4 mol hexose and 1 mol rhamnose) (molecular ion peak), *m/z* 595 $[M-486+H]^+$ (kaempferol+each 1 mol hexose and rhamnose), and *m/z* 287 $[M-794+H]^+$ (kaempferol) (fragment ion peaks).

Kaempferol 3,7,4′-*O*-hexaglycoside (**4**). PC: Rf 0.12 (BAW), 0.16 (BEW), 0.69 (15%HOAc), 0.69 (5%HOAc); color UV–dark purple, UV/$NH_3$–dark greenish purple. HPLC: t*R* (min) 3.65. UV: λmax (nm) MeOH 269, 327; +NaOMe 282, 394 (dec.); +$AlCl_3$ 226sh, 275, 299, 344, 378sh; +$AlCl_3$/HCl 233, 279, 298, 333, 393sh; +NaOAc 269, 324; +NaOAc/$H_3BO_3$ 266, 348. LC-MS: *m/z* 1259 $[M+H]^+$, 1257 $[M-H]^-$ (kaempferol+6 mol hexose) (molecular ion peak), *m/z* 935 $[M-324+H]^+$ (kaempferol+4 mol hexose), *m/z* 611 $[M-648+H]^+$ (kaempferol+2 mol hexose), *m/z* 449 $[M-810+H]^+$ (kaempferol+1 mol hexose), and *m/z* 287 $[M-972+H]^+$ (kaempferol) (fragment ion peaks).

Kaempferol 3,7-*O*-tetraglycoside (**5**). PC: Rf 0.46 (BAW), 0.49 (BEW), 0.61 (15%HOAc), 0.55 (5%HOAc); color UV–dark purple, UV/$NH_3$–greenish yellow. HPLC: t*R* (min) 5.15. UV: λmax (nm) MeOH 242sh, 266, 337; +NaOMe 272, 386 (inc.); +$AlCl_3$ 233sh, 275, 301, 350, 396sh; +$AlCl_3$/HCl 237, 277, 301, 338, 395sh; +NaOAc 265sh, 360; +NaOAc/$H_3BO_3$ 262sh, 351. LC-MS: *m/z* 919 $[M+H]^+$, 917 $[M-H]^-$ (kaempferol+3 mol hexose and 1

mol rhamnose) (molecular ion peak), *m/z* 757 $[M-162+H]^+$, 755 $[M-162-H]^-$ (kaempferol+2 mol hexose and 1 mol rhamnose), and *m/z* 287 $[M-632+H]^+$ (kaempferol) (fragment ion peaks).

Kaempferol 3,7-*O*-tetraglycoside (**6**). PC: Rf 0.38 (BAW), 0.44 (BEW), 0.71 (15%HOAc), 0.68 (5%HOAc); color UV–dark purple, UV/$NH_3$–greenish yellow. HPLC: t*R* (min) 4.06. UV: λmax (nm) MeOH 245, 268, 330; +NaOMe 273, 383 (inc.); +$AlCl_3$ 273, 301, 352, 393sh; +$AlCl_3$/HCl 236, 277, 300, 338, 392; +NaOAc 267, 382; +NaOAc/$H_3BO_3$ 266, 350. LC-MS: *m/z* 919 $[M+H]^+$, 917 $[M-H]^-$ (kaempferol+3 mol hexose and 1 mol rhamnose) (molecular ion peak), *m/z* 771 $[M-146-H]^-$ (kaempferol+3 mol hexose), *m/z* 433 $[M-486+H]^+$ (kaempferol+1 mol rhamnose), and *m/z* 287 $[M-632+H]^+$ (kaempferol) (fragment ion peaks).

Kaempferol 3,7-*O*-triglycoside (**7**). PC: Rf 0.37 (BAW), 0.43 (BEW), 0.79 (15%HOAc), 0.77 (5%HOAc); color UV–dark purple, UV/$NH_3$–bright yellow. HPLC: t*R* (min) 3.70. UV: λmax (nm) MeOH 266, 347; +NaOMe 243sh, 270, 383 (inc.); +$AlCl_3$ 234sh, 273, 300, 352, 390; +$AlCl_3$/HCl 232sh, 274, 298, 345, 390; +NaOAc 266, 394; +NaOAc/$H_3BO_3$ 266, 350. LC-MS: *m/z* 755 $[M-H]^-$ (kaempferol+2 mol hexose and 1 mol rhamnose) (molecular ion peak), *m/z* 595 $[M-162+H]^+$ (kaempferol+ each 1 mol hexose and rhamnose), *m/z* 433 $[M-324+H]^+$ (kaempferol+1 mol rhamnose), and *m/z* 287 $[M-470+H]^+$ (kaempferol) (fragment ion peaks).

Kaempferol 3-*O*-sophoroside (**8**). PC: Rf 0.52 (BAW), 0.62 (BEW), 0.63 (15%HOAc), 0.47 (5%HOAc); color UV–dark purple, UV/$NH_3$–dark greenish yellow. HPLC: t*R* (min) 4.28. UV: λmax (nm) MeOH 267, 332; +NaOMe 275, 392 (inc.); +$AlCl_3$ 227sh, 273, 305, 350, 387sh; +$AlCl_3$/HCl 232, 276, 303, 339, 391; +NaOAc 274, 382; +NaOAc/$H_3BO_3$ 266, 351. LC-MS: *m/z* 609 $[M-H]^-$ (kaempferol+2 mol glucose) (molecular ion peak), *m/z* 449 $[M-162+H]^+$ (kaempferol+1 mol glucose), and *m/z* 287 $[M-324+H]^+$ (kaempferol) (fragment ion peaks).

Kaempferol 3-*O*-(caffeoylsophoroside) (**9**). PC: Rf 0.52 (BAW), 0.62 (BEW), 0.63 (15%HOAc), 0.47 (5%HOAc); color UV–dark purple, UV/$NH_3$–dark greenish yellow. HPLC: t*R* (min) 4.95. UV: λmax (nm) MeOH 270, 313; +NaOMe 274sh, 364 (inc.); +$AlCl_3$ 275, 303, 311sh, 359, 380sh; +$AlCl_3$/HCl 277, 302, 347, 380sh; +NaOAc 268, 326, 350sh; +NaOAc/$H_3BO_3$ 269, 314. LC-MS: *m/z* 771 $[M-H]^-$ (kaempferol+2 mol glucose+1 mol caffeic acid) (molecular ion peak), and *m/z* 287 (kaempferol) (fragment ion peak).

Mangiferin (**10**). PC: Rf 0.49 (BAW), 0.55 (BEW), 0.42 (15%HOAc), 0.31 (5%HOAc); color UV–orange, UV/$NH_3$–bright yellow. HPLC: t*R* (min) 3.70. UV: λmax (nm) MeOH 241, 257, 315, 364; +NaOMe 236sh, 273, 303sh, 339sh, 392; +$AlCl_3$ 236, 269, 287sh, 355, 417; +$AlCl_3$/HCl 233, 266, 278sh, 338, 404; +NaOAc 246sh, 271, 303, 339sh, 387; +NaOAc/$H_3BO_3$ 262, 288sh, 322, 362, 416sh. LC-MS: *m/z* 423 $[M+H]^+$, 421 $[M-H]^-$ (mangiferin) (molecular ion peak).

Isomangiferin (**11**). PC: Rf 0.30 (BAW), 0.31 (BEW), 0.24 (15%HOAc), 0.16 (5%HOAc); color UV–orange, UV/$NH_3$–bright yellow. HPLC: t*R* (min) 4.34. UV: λmax (nm) MeOH 241, 257, 314, 365; +NaOMe 247sh, 273, 302sh, 340sh, 391; +$AlCl_3$ 235, 268, 287sh, 355, 418; +$AlCl_3$/HCl 232, 265, 279sh, 337, 406; +NaOAc 271, 303, 336sh, 386; +NaOAc/$H_3BO_3$ 261, 288sh, 321, 359, 415sh. LC-MS: *m/z* 423 $[M+H]^+$, 421 $[M-H]^-$ (isomangiferin) (molecular ion peak).

Mangiferin X″-*O*-glucoside (**12**). PC: Rf 0.17 (BAW), 0.20 (BEW), 0.63 (15%HOAc), 0.58 (5%HOAc); color UV–orange, UV/$NH_3$–bright yellow. HPLC: t*R* (min) 3.66. UV: λmax (nm) MeOH 243, 259, 316, 365; +NaOMe 238, 275, 303sh, 338sh, 392; +$AlCl_3$ 237, 271, 290sh, 355, 418; +$AlCl_3$/HCl 233, 267, 280sh, 337, 407; +NaOAc 249sh, 275, 305, 336sh, 389; +NaOAc/$H_3BO_3$ 263, 279sh, 324sh, 364, 418. LC-MS: *m/z* 585 $[M+H]^+$, 583 $[M-H]^-$ (mangiferin+1 mol glucose) (molecular ion peak), and *m/z* 421 $[M-162-H]^-$ (mangiferin) (fragment ion peak).

Mangiferin X″-acetate (**13**). PC: Rf 0.70 (BAW), 0.68 (BEW), 0.52 (15%HOAc), 0.42 (5%HOAc); color UV–orange, UV/$NH_3$–bright yellow. HPLC: t*R* (min) 5.29. UV: λmax (nm) MeOH 241, 258, 315, 365; +NaOMe 237sh, 273, 302sh, 337sh, 394; +$AlCl_3$ 236, 269, 287sh, 355, 417; +$AlCl_3$/HCl 233, 266, 278sh, 338, 403; +NaOAc 270, 303, 336sh, 386; +NaOAc/$H_3BO_3$ 263, 287sh, 322, 360, 414sh. LC-MS: *m/z* 465 $[M+H]^+$, 463 $[M-H]^-$ (mangiferin+1 mol acetic acid) (molecular ion peak).

Schaftoside (**14**). PC: Rf 0.24 (BAW), 0.31 (BEW), 0.47 (15%HOAc), 0.45 (5%HOAc); color UV–dark purple, UV/$NH_3$–dark yellow. HPLC: t*R* (min) 3.84. UV: λmax (nm) MeOH 273, 332; +NaOMe 283, 333, 400 (inc.); +$AlCl_3$ 280, 305, 352, 384sh; +$AlCl_3$/HCl 280, 304, 345, 382sh; +NaOAc 283, 313sh, 337, 396; +NaOAc/$H_3BO_3$ 277, 284sh, 323, 347sh, 413sh. LC-MS: *m/z* 565 $[M+H]^+$, 563 $[M-H]^-$ (apigenin+each 1 mol glucose and arabinose) (molecular ion peak).

Vicenin-2 (**15**). PC: Rf 0.21 (BAW), 0.28 (BEW), 0.47 (15%HOAc), 0.45 (5%HOAc); color UV–dark purple, UV/$NH_3$–dark yellow. HPLC: t*R* (min) 3.67. UV: λmax (nm) MeOH 273, 333; +NaOMe 283, 332, 400 (inc.). LC-MS: *m/z* 595 $[M+H]^+$, 593 $[M-H]^-$ (apigenin+2 mol glucose) (molecular ion peak).

Apigenin 6-*C*-hexoside-8-*C*-pentoside (**16**). PC: Rf 0.28 (BAW), 0.31 (BEW), 0.49 (15%HOAc), 0.45 (5%HOAc); color UV–dark purple, UV/$NH_3$–dark yellow. HPLC: t*R* (min) 4.18. UV: λmax (nm) MeOH 273, 332; +NaOMe 283, 333, 400 (inc.); +$AlCl_3$ 280, 305, 352, 384sh; +$AlCl_3$/HCl 280, 304, 345, 381sh; +NaOAc 283, 313sh, 338, 396; +NaOAc/$H_3BO_3$ 277, 285sh, 323, 345sh, 410sh. LC-MS: *m/z* 565 $[M+H]^+$, 563 $[M-H]^-$ (apigenin+each 1 mol pentose and hexose) (molecular ion peak).

Apigenin 6-*C*-pentoside-8-*C*-hexoside (**17**). PC: Rf 0.36 (BAW), 0.42 (BEW), 0.50 (15%HOAc), 0.45 (5%HOAc); color UV–dark purple, UV/$NH_3$–dark greenish yellow. HPLC: t*R* (min) 4.41. UV: λmax (nm) MeOH 274, 333; +NaOMe 281, 331, 400 (inc.); +$AlCl_3$ 280, 308, 357, 387sh; +$AlCl_3$/HCl 281, 306, 349, 385sh; +NaOAc 281, 316, 336, 395; +NaOAc/$H_3BO_3$ 277, 326, 342, 410sh. LC-MS: *m/z* 565 $[M+H]^+$, 563 $[M-H]^-$ (apigenin+each 1 mol pentose and hexose) (molecular ion peak).

Table 1. Flavonoids and xanthones isolated from six *Asplenium* species used as plant materials

| Species | Classes | Compounds |
|---|---|---|
| *A. carruthersii* | *C*-Glycosylflavone | Schaftoside |
| | | Vicenin-2 |
| | | Apigenin 6-*C*-pentoside-8-*C*-hexoside |
| | | Apigenin 6-*C*- hexoside-8-*C*-pentoside |
| *A. insiticium* | Flavonol | Kaempferol 3-rhamnoside-7-glucoside |
| | | Kaempferol 3-glucoside-7-rhamnoside |
| | | Kaempferol 3-galactoside-7-rhamnoside |
| *A. laserpitiifolium* | Flavonol | Kaempferol 3,7-pentaglycoside |
| | | Kaempferol 3,7,4′-hexaglycoside |
| *A. subflexuosum* | Flavonol | Kaempferol 3,7-tetraglycoside |
| | | Kaempferol 3,7-triglycoside |
| *A. contiguum* | Flavonol | Kaempferol 3-sophoroside |
| | | Kaempferol 3-caffeoylsophoroside |
| | Xanthone | Mangiferin |
| | | Isomangiferin |
| | | Mangiferin X″-glucoside |
| | | Mangiferin X″-acetate |
| *A. caudatum* | | No flavonoids and xanthones |

## Results and Discussion

*Characterization of the compounds*

In this survey, some flavonols based on kaempferol, *C*-glycosylflavones and *C*-glycosylxanthones from the fronds of five *Asplenium* species in Vanuatu and New Caledonia (Table 1). Two flavonol glycosides, **1** and **2**, were isolated from *A. insiticium*. Kaempferol, glucose and rhamnose were obtained by acid hydrolysis of both compounds. UV spectral properties of their glycosides showed that **1** and **2** are kaempferol 3,7-*O*-glycosides (Mabry *et al.*, 1970). However, mild acid hydrolysis [in 1.2% HCl:MeOH (1:1), 100ºC, 15-20 min] of **2** liberated kaempferol 3-*O*-glucoside and also kaempferol 3-*O*-galactoside as intermediates, together with kaempferol and original glycoside, showing that the compound is a mixture. LC-MS survey of **2** was appeared the molecular ion peak, *m/z* 595 $[M+H]^+$ and 593 $[M-H]^-$, showing the attachment of each 1 mol hexose and rhamnose. Finally, flavonoid **2** was characterized as a mixture of kaempferol 3-*O*-glucoside-7-*O*-rhamnoside (**2a**) (Fig. 1) and kaempferol 3-*O*-galactoside-7-*O*-rhamnoside (**2b**) (Fig. 2). HPLC properties of the former compound agreed with those of authentic sample from the fronds of *Cyrtomium falcatum* (L.f) C. Presl (Dryopteridaceae) (Iwashina *et al*., 2006). By the similar manners, another flavonoid **1** was identified as kaempferol 3-*O*-rhamnoside-7-*O*-glucoside (Fig. 3). Of their glycosides, **1** has been reported from two *Asplenium* species, *A. bulbiferum* (Imperato, 1985) and *A. kaulfussii* (Imperato, 1989a), **2a** from *A. scolopendrium* (Mizuno *et al*., 1990a), *A. kaulfussii* (Imperato, 1989a) and *A. viride* (Iwashina *et al*., 1995). Kaempferol 3-*O*-galactoside-7-*O*-rhamnoside (**2b**) was found in *Asplenium* species for the first time.

Two polyglycosylated kaempferol, **3** and **4**, were isolated from *A. laserptiifolium*. By UV spectral survey, **3** and **4** were shown to be kaempferol 3,7- and 3,7,4′-glycosides, respectively. Since molecular ion peaks, *m/z* 1079 $[M-H]^-$, and *m/z* 1259 $[M+H]^+$, appeared on

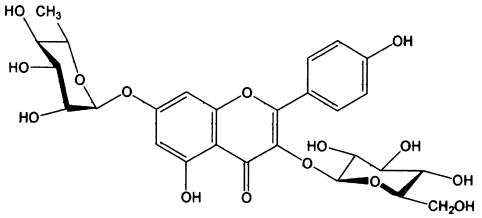

Fig. 1. Kaempferol 3-*O*-glucoside-7-*O*-rhamnoside (**2a**).

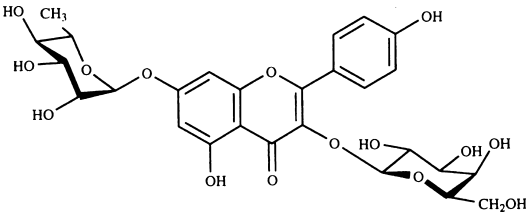

Fig. 2. Kaempferol 3-*O*-galactoside-7-*O*-rhamnoside (**2b**).

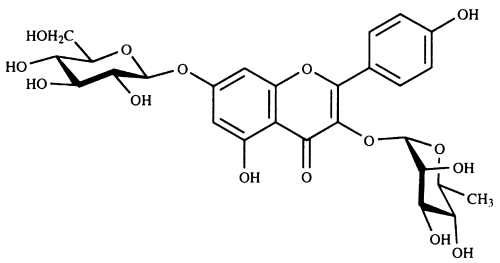

Fig. 3. Kaempferol 3-*O*-rhamnoside-7-*O*-glucoside (**1**).

LC-MS, it was determined that **3** and **4** were kaempferol tetrahexosyl-monorhamnoside and hexahexoside.

Three flavonol glycosides, **5**, **6** and **7**, from *A. subflexuosum* were also polyglycosylated kaempferol and characterized as kaempferol 3,7-*O*-glycosides which attached 3 mol hexose and 1 mol rhamnose (**5** and **6**) and 2 mol hexose and 1 mol rhamnose (**7**), by UV spectra and LC-MS. Kaempferol polyglycosides have been reported from some Malaysian *Asplenium* species, e.g., *A. longissimum* Blume, *A. pellucidum* Lam., *A. scortechinii* Bedd., *A. tenerum* G. Forst. etc (Umikalsom *et al*., 1994). However, their flavonol glycosides could not be identified for

their complicated chemical structures.

Two flavonol glycosides, **8** and **9**, and four xanthones, **10**-**13**, were isolated from the fronds of *A. contiguum*. Of their compounds, **8** and **9** liberated kaempferol and glucose by acid hydrolysis. Additionally, caffeic acid was also produced as hydrolysate of **9**. The attachment of glucose to 3-position of kaempferol was shown by UV spectral survey. The attachment of 2 mol glucose, and 1 mol caffeic acid and 2 mol glucose to kaempferol were shown by appearance of molecular ion peaks, *m/z* 609 $[M-H]^-$ (**8**) and *m/z* 771 $[M-H]^-$ (**9**) on LC-MS. Thus, they were characterized as kaempferol 3-*O*-diglucoside and 3-*O*-caffeoyldiglucoside. Finally, flavonoid **8** was identified as kaempferol 3-*O*-glucosyl-(1→2)-glucoside by HPLC comparison with authentic kaempferol 3-*O*-sophoroside (Fig. 4) from the flowers of carnation, *Dianthus caryophyllus* L. (Iwashina *et al*., 2010b). Another **9** was characterized as kaempferol 3-*O*-(caffeoylsophoroside). Their kaempferol glycosides were reported from *Asplenium* species for the first time.

UV spectral properties (appearance of four major absorption maxima) of **10** and **11** showed the compounds are xanthones. By hot acid treatment, their compounds were not hydrolysable, showing that they are *C*-glycosylxanthones. LC-MS survey of the compounds indicated the molecular ion peaks, *m/z* 423 $[M+H]^+$ and 421 $[M-H]^-$ without the appearance of fragment ion peak, showing that the compounds are tetrahydroxyxanthone mono-*C*-hexosides. Finally, **10** was identified as 1,3,6,7-tetrahydroxyxanthone 2-*C*-glucoside by direct PC and HPLC comparison with authentic mangiferin (Fig. 5) from the flowers and leaves of *Iris lossii* Baker (Iridaceae) (Hayashi *et al*., 1980). On the other hand, **11** was identified as 1,3,6,7-tetrahydroxyxanthone 4-*C*-glucoside (Fig. 6) by direct PC and HPLC comparison with authentic sample from the leaves of *Iris setosa* Pallas var. *canadensis* M. Foster (Iwashina and Ootani, 1995). Other two compounds, **12** and **13**, were also mangiferin derivatives. Since molecular ion peaks, *m/z* 585 $[M+H]^+$ and 583 $[M-H]^-$, and *m/z* 465 $[M+H]^+$ and 463 $[M-H]^-$, appeared by LC-MS survey of their compounds, it was shown that the attachment of 1 mol glucose and acetic acid to mangiferin, respectively. Thus, they were characterized as mangiferin X″-*O*-glucoside (**12**) and mangiferin X″-acetate (**13**). Mangiferin and its derivatives have been reported from *Asplenium adiantum-nigrum*, *A. balearicum* (Richardson and Lorenz-Liburnau, 1982), *A. montanum*, *A. bradley*, *A. pinnatifidum*, *A. stotleri* (Smith and Harborne, 1971) and *A. ceterach* (Imperato, 1983b).

Four flavonoid glycosides, **14**–**17**, were isolated from *A. carruthersii*. They were shown to be apigenin 6,8-di-*C*-glycosides by hot acid treatment, UV spectral survey and LC-MS. Of their compounds, HPLC properties of **14** and **15** agreed with authentic schaftoside (apigenin 6-*C*-

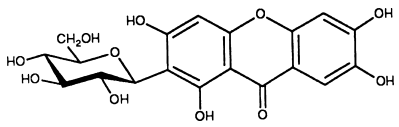

Fig. 5. Mangiferin (**10**).

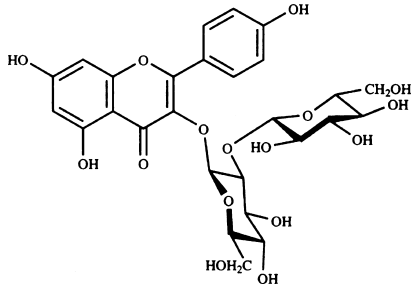

Fig. 4. Kaempferol 3-*O*-sophoroside (**8**).

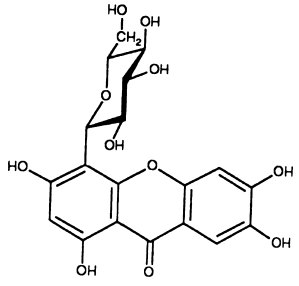

Fig. 6. Isomangiferin (**11**).

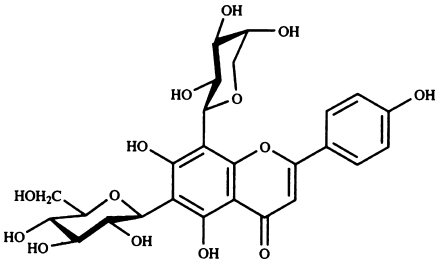

Fig. 7. Schaftoside (**14**).

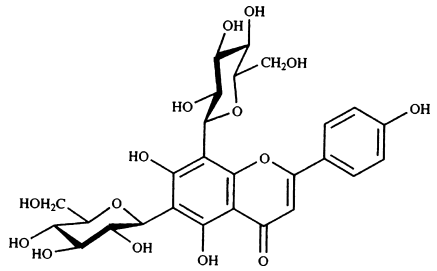

Fig. 8. Vicenin-2 (**15**).

glucosyl-8-*C*-arabinoside) (Fig. 7) from *Osyris alba* L. (Santalaceae) (Iwashina *et al*., 2008), and vicenin-2 (apigenin 6,8-di-*C*-glucoside) (Fig. 8) from *Asplenium normale* (Iwashina *et al*., 2010). Though **16** and **17** were characterized as apigenin 6-*C*-hexoside-8-*C*-pentoside and 6-*C*-pentoside-8-*C*-hexoside, their HPLC properties did not agreed with those of authentic isoschaftoside (apigenin 6-*C*-arabinoside-8-*C*-glucoside). Flavone *C*-glycosides have been reported from *A. viviparum* and identified as luteolin 6,8-di-*C*-rhamnoside and apigenin 3,6,8-tri-*C*-xyloside (Imperato, 1992, 1993). A common flavone *C*-glycoside, vicenin-2, have been reported from *A. normale* and related species, *A. boreale*, *A. shimurae* and *A. oligophlebium* (Iwashina *et al*., 1990, 2010a; Iwashina and Matsumoto, 1994).

One population (Vanuatu) of A. *caudatum* was surveyed for flavonoid. Though three compounds were isolated, they were characterized as simple phenolics by LC-MS and UV spectra, and flavonoids could not be found. However, Malaysian population of this species has been surveyed and kaempferol 3,7,4′-tri-*O*-glycoside and various kaempferol 3,7-di-*O*-glycosides and uncharacterized quercetin glycosides were found (Umikalsom *et al*., 1994).

*Distribution of flavonoids and xanthones in* Asplenium

Flavonoids of *Asplenium* have been reported from some species (Table 2). In almost species, kaempferol and its derivatives, especially polyglycosylated compounds have been isolated from many species, e.g., kaempferol 3-*O*-glucosyl-(1→3)-(2″-caffeoylglucoside)-7-*O*-rhamnoside, 3-*O*-glucosyl-(1→3)-glucoside-7-*O*-rhamnoside and 3-*O*-(2″-caffeoylglucoside)-7-*O*-rhamnoside from *A. scolopendrium* (Mizuno *et al*., 1990a), kaempferol 3-*O*-sophorotrioside-7-*O*-glucoside (Imperato, 1984a), 3-*O*-sophoroside-4′-*O*-glucoside (Imperato, 1990a) and 3-*O*-(3″- or 6″-sulphate-glucoside) together with quercetin 3-*O*-glucoside and 3-*O*-(3″-sulphate-glucoside) (Imperato, 1983a) from *Asplenium septentrionale* (L.) Hoffm., kaempferol 3-*O*-rhamnoside-7-*O*-[6″-feruloylglucosyl-(1→3)-rhamnoside] from *A. prolongatum* Hook. (Mizuno *et al*., 1990b), kaempferol 3,7-di-*O*-glucoside, 3-*O*-rhamnoside-7-*O*-glucoside and 3-*O*-glucoside-7-*O*-galactoside (Imperato, 1985), kaempferide 3-*O*-rhamnoside-7-*O*-(6″-succinylglucoside) (Imperato, 1987a), 3-*O*-arabinoside-7-*O*-glucoside (Imperato, 1987b), 3,7-di-*O*-rhamnoside (Imperato, 1987c), 3-*O*-glucoside-7-*O*-rhamnoside (Imperato, 1984b), 3-*O*-rhamnoside-7-*O*-glucoside and 3,7-di-*O*-glucoside from *A. bulbiferum* G. Forst. (Imperato, 1984c). Three kaempferol glycosides, kaempferol 3,7-di-*O*-rhamnoside, 3-*O*-rhamnoside-7-*O*-arabinoside and 3-*O*-arabinoside-7-*O*-rhamnoside were obtained from *A. trichomanes* L. (Imperato, 1979a). From the same species, quercetin 3-*O*-rutinoside was also found (Bhardwaj *et al*., 1982). Kaempferol 3-*O*-gentiobioside-7,4′-di-*O*-glucoside (Imperato, 1986) and 3-*O*-vicianoside were isolated from *A. nidus* L. (Imperato, 1987d). Kaempferol 3-*O*-(6″-sulphate-glucoside) and 3-*O*-$\alpha$-glucoside were detected from *A. filix-foemina* Bernh. (Imperato, 1979b).

Kaempferol 3-*O*-gentiobioside and 3-*O*-(gentiobioside-6″-sulphate) were isolated from *A.*

Table 2. Reports of flavonoids and xanthones from *Asplenium* species

| Species | Classes | Compounds | References |
|---|---|---|---|
| *A. adiantum-nigrum* | Xanthone | Mangiferin | (1, 40) |
| | | Isomangiferin | (1, 40) |
| | | Mangiferin X″-glucoside | (1, 40) |
| | | Mangiferin X″-rhamnoside | (4, 40) |
| | | 1,3,7,8-Tetrahydroxyxanthone 1-cellobioside | (15) |
| | | 1,3,7,8-Tetrahydroxyxanthone 1-laminaribioside | (16) |
| *A. antiquum* | Flavonol | Mearnsetin 3,7-dirhamnoside | (22) |
| *A. balearicum* | Xanthone | Mangiferin | (1) |
| | | Isomangiferin | (1) |
| *A. belangeri* | Flavone | Scutellarein 6-glucoside | (40) |
| *A. boreale* | Flavone | Apigenin 7,4′-dirhamnoside | (37) |
| | | Vicenin-2 | (36, 37) |
| *A. bradley* | Xanthone | Mangiferin | (8) |
| | | Isomangiferin | (8) |
| *A. bulbiferum* | Flavonol | Kaempferide 3-arabinoside-7-glucoside | (9) |
| | | Kaempferide 3,7-dirhamnoside | (10) |
| | | Kaempferide 3-rhamnoside-7-(6″-succinylglucoside) | (11) |
| | | Kaempferide 3,7-diglucoside | (12) |
| | | Kaempferide 3-rhamnoside-7-glucoside | (12) |
| | | Kaempferide 3-glucoside-7-rhamnoside | (13) |
| | | Kaempferol 3,7-diglucoside | (13) |
| | | Kaempferol 3-rhamnoside-7-glucoside | (13) |
| | | Kaempferol 3-glucoside-7-galactoside | (13) |
| *A. ceterach* | Flavonol | Kaempferol 3-glucuronide | (31) |
| | | Kaempferol 3-digalactoside | (31) |
| | | Quercetin 3-glucuronide | (31) |
| | | Quercetin 3-digalactoside | (31) |
| | | Kaempferol 3-(6″-malonylglucoside) | (32) |
| | | Quercetin 3-glucoside | (32) |
| | | Quercetin 3-gentiobioside | (32) |
| | | Quercetin 3-(6″-malonylgalactoside) | (32) |
| | Flavanone | Naringenin 7-neohesperidoside | (32) |
| | | Naringenin 7-arabinosyl-(1→6)- glucoside | (33) |
| *A. dalhousiae* | Flavonol | Kaempferol 3-glucuronide | (31) |
| | | Quercetin 3-glucuronide | (31) |
| *A. filix-foemina* | Flavonol | Kaempferol 3-$\alpha$-glucoside | (42) |
| | | Kaempferol 3-(6″-sulphate-glucoside) | (42) |
| *A. fontanum* | Flavonol | Kaempferol 3-(6″-sulphate-gentiobioside) | (27) |
| | | Kaempferol 3-gentiobioside | (27, 28) |
| *A. foreziense* | Flavonol | Kaempferol 3-gentiobioside | (28) |
| *A. incisum* | Flavonol | Kaempferol 3-gentiobioside | (28) |
| | | Kaempferol 3-gentiobioside-4′-glucoside | (28) |
| | | Kaempferol 3-glucoside | (28) |
| | | Quercetin 3-diglucoside | (28) |
| *A. kaulfussii* | Flavonol | Kaempferol 3-sophoroside-7-rhamnoside | (24) |
| | | Kaempferol 3-rhamnoside-7-glucoside | (24) |
| | | Kaempferol 3-glucoside-7-rhamnoside | (24) |
| | Aurone | Bracteatin | (24) |
| *A. marinum* | Flavonol | Kaempferol 3-methyl ether 7-glucoside | (40) |
| | | Kaempferol 3,4′-dimethyl ether 7-glucoside | (40) |
| *A. montanum* | Flavonol | Kaempferol glycosides | (7) |
| | Xanthone | Mangiferin | (8) |
| | | Isomangiferin | (8) |
| *A. nidus* | Flavonol | Kaempferol 3-gentiobioside-7,4′-diglucoside | (29) |
| | | Kaempferol 3-vicianoside | (30) |
| *A. normale* | Flavone | Apigenin 7-rhamnosyl-(1→3)-rhamnoside | (36, 37, 38) |
| | | Apigenin 7-rhamnosyl-(1→4)-rhamnoside | (41) |
| | | Apigenin 7-rhamnoside-4′-glucosyl-(1→3)-rhamnoside | (37, 38, 39) |
| | | Apigenin 7-rhamnosyl-(1→4)-rhamnoside-4′-rhamnoside | (41) |
| | | Luteolin 7-rhamnosyl-(1→3)-rhamnoside | (36, 37, 38) |
| | | Genkwanin 4′-glucosyl-(1→3)-rhamnoside | (36, 37, 38) |
| | | Acacetin 7-glucosylrhamnoside | (40) |
| | | Vicenin-2 | (36, 37) |
| | | Lucenin-2 | (41) |
| *A. obovatum* | Flavonol | Kaempferol 3-gentiobioside | (28) |

Table 2. Continued

| Species | Classes | Compounds | References |
|---|---|---|---|
| *A. oligophlebium* | Flavone | Genkwanin 4′-glucosyl-(1→3)-rhamnoside | (36, 37) |
| | | Vicenin-2 | (36, 37) |
| *A. pinnatifida* | Xanthone | Mangiferin | (8) |
| | | Isomangiferin | (8) |
| *A. platyneuron* | Flavonol | Kaempferol 3,4′-dimethyl ether 7-glucoside | (7) |
| | | Kaempferol 3,7-diglucoside | (7) |
| *A. prolongatum* | Flavonol | Kaempferol 3-rhamnoside-7-[6″-feruloylglucosyl-(1→3)-rhamnoside] | (23) |
| *A. punjabense* | Flavonol | Kaempferol 3-glucuronide | (31) |
| | | Kaempferol 3-digalactoside | (31) |
| | | Quercetin 3-glucuronide | (31) |
| | | Quercetin 3-digalactoside | (31) |
| *A. rhizophyllum* | Flavonol | Kaempferol 7-glucoside | (7) |
| | | Kaempferol 3,7-diglucoside | (7) |
| | | Kaempferol 3-sophoroside-7-glucoside | (7) |
| | | Kaempferol 3,4′-diglucoside | (7) |
| *A. scolopendrium* | Flavonol | Kaempferol 3-glucosyl-(1→3)-(2″-caffeoylglucoside)-7-rhamnoside | (19) |
| | | Kaempferol 3-glucoside-(1→3)-glucoside-7-rhamnoside | (19) |
| | | Kaempferol 3-(2″-caffeoylglucoside)-7-rhamnoside | (19) |
| | | Kaempferol 3-glucoside-7-rhamnoside | (19) |
| | | Kaempferol 7-rhamnoside | (19) |
| *A. septentrionale* | Flavonol | Kaempferol 3-sophorotrioside-7-glucoside | (2) |
| | | Kaempferol 3-sophoroside-4′-glucoside | (5) |
| | | Kaempferol 3-(sulphate-glucoside) | (3) |
| | | Quercetin 3-glucoside | (3) |
| | | Quercetin 3-(3″-sulphate-glucoside) | (3) |
| *A. shimurae* | Flavone | Apigenin 7-glucosylrhamnoside | (36, 37) |
| | | Luteolin 7-glucosylrhamnoside | (36, 37) |
| | | Vicenin-2 | (36, 37) |
| *A. stotleri* | Xanthone | Mangiferin | (8) |
| | | Isomangiferin | (8) |
| *A. trichomanes* | Flavonol | Kaempferol 3,7-dirhamnoside | (25) |
| | | Kaempferol 3-rhamnoside-7-arabinoside | (25) |
| | | Kaempferol 3-arabinoside-7-rhamnoside | (25) |
| | | Quercetin 3-rutinoside | (26) |
| *A. viride* | Flavonol | Kaempferol 3-arabinoside-7-rhamnoside | (35) |
| | | Kaempferol 3-glucoside-7-rhamnoside | (35) |
| | | Kaempferol 3,7-dirhamnoside | (35) |
| | | Kaempferol 3-glucoside | (35) |
| | | Kaempferol 3-arabinoside | (35) |
| | | Quercetin 3-methyl ether 5-glucoside | (35) |
| | | Quercetin 3-rhamnoside-7-arabinoside | (35) |
| | | Quercetin 3-glucoside-7-rhamnoside | (35) |
| | | Quercetin 3-glucoside | (35) |
| | | Quercetin 3-arabinoside | (35) |
| | | Quercetin 3-methyl ether | (32) |
| *A. viviparum* | Flavone | Apigenin 3,6,8-tri-*C*-xyloside | (21) |
| | | Luteolin 6,8-di-*C*-rhamnoside | (20) |

(1) Richardson and Lorenz-Liburnau (1982), (2) Imperato (1984a), (3) Imperato (1983a), (4) Imperato (1990b), (5) Imperato (1990a), (6) Imperato (1983), (7) Harborne *et al*. (1973), (8) Smith and Harborne (1971), (9) Imperato (1987b), (10) Imperato (1987c), (11) Imperato (1987a), (12) Imperato (1984c), (13) Imperato (1984b), (14) Imperato (1985), (15) Imperato (1980b), (16) Imperato (1980c), (17) Imperato (1990a), (18) Imperato (1991), (19) Mizuno *et al*. (1990a), (20) Imperato (1992), (21) Imperato (1993), (22) Mizuno *et al*. (1991), (23) Mizuno *et al*. (1990b), (24) Imperato (1989a), (25) Imperato (1979a), (26) Bhardwaj *et al*. (1982), (27) Imperato (1980a), (28) Iwashina *et al*. (2000), (29) Imperato (1986), (30) Imperato (1987d), (31) Iwashina *et al*. (1993), (32) Imperato (1981), (33) Imperato (1983b), (34) Voirin and Jay (1974), (35) Iwashina *et al*. (1995), (36) Iwashina *et al*. (1990), (37) Iwashina and Matsumoto (1994), (38) Matsumoto *et al*. (2003), (39) Iwashina *et al*. (1993), (40) Umikalsom *et al*. (1994), (41) Iwashina *et al*. (2010) and (42) Imperato (1979b).

*fontanum* (L.) Bernh. (Imperato, 1980a; Iwashina *et al*., 2000). The former compound was reported from other *Asplenium* species, *A. foreziense* Legrand ex Hérib., *A. obovatum* Viv. and *A. incisum* Thunb. (Iwashina *et al*., 2000). In *A. incisum*, kaempferol 3-*O*-glucoside and 3-*O*-gentiobioside-4′-*O*-glucoside, and quercetin 3-*O*-diglucoside were accompanied.

Kaempferol 3-*O*-sophoroside-7-*O*-rhamnoside, 3-*O*-rhamnoside-7-*O*-glucoside and 3-*O*-glucoside-7-*O*-rhamnoside were isolated from *A. kaulfussii* Schleft together with an aurone, bracteatin, which has been found in Aspleniaceae for the first time (Imperato, 1989a). Kaempferol 3,7-di-*O*-glucoside and 7,4′-di-*O*-glucoside, and kaempferol 3,4′-dimethyl ether 7-*O*-glucoside were reported from Appalachian *Asplenium* complex, *A. platyneuron* (L.) Oakes, *A. rhyzophyllum* and *A. montanum* (Harborne *et al*., 1973). Some kaempferol glycosides including 3-*O*-arabinoside-7-*O*-rhamnoside have been isolated from alpine *Asplenium*, *A. viride* Hudson (=*A. trichomanes-ramosum* L.) together with some quercetin and quecetin 3-methyl ether glycosides (Iwashina *et al*., 1995). Kaempferol and quercetin glycosides were also reported from three Pakistani *Asplenium*, *A. dalhousiae* Hook., *A. officinalum* DC. and *A. punjabense* (Iwashina *et al*., 1993a). Rare flavonol glycoside, mearnsetin 3,7-di-*O*-rhamnoside was isolated from *A. antiquum* Makino (Mizuno *et al*., 1991). Distribution of flavonol glycosides including kaempferol has been reviewed by Imperato (1989b). Thus, flavonol glycosides based on kaempferol have been frequently reported from the genus *Asplenium*.

On the other hand, flavone glycosides were found in a few species. Rare apigenin 3,6,8-tri-*C*-xyloside and luteolin 6,8-di-*C*-rhamnoside were isolated from *A. viviparum* L. (Imperato, 1992, 1993). Flavone *O*-glycosides were reported from *A. normale* and related species. Thus, apigenin 7-*O*-rhamnosyl-(1→3)-rhamnoside, apigenin 7-*O*-rhamnosyl-(1→4)-rhamnoside, 7-*O*-rhamnoside-4′-*O*-glucosyl-(1→3)-rhamnoside, 7-*O*-rhamnosyl-(1→4)-rhamnoside-4′-*O*-rhamnoside and 7,4′-di-*O*-rhamnoside, and luteolin 7-*O*-rhamnosyl-(1→3)-rhamnoside were isolated from *A. normale*, *A. shimurae*, *A. boreale* and *A. oligophlebium* together with *C*-glycosylflavones, vicenin-2 and lucenin-2 in various combination (Iwashina *et al*., 1990, 1993b, 2010a; Iwashina and Matsumoto, 1990, 1994; Matsumoto *et al*., 2003). More recently, a molecular phylogenetic analysis based on chloroplast *rbcL* sequences of Aspleniaceae, especially Japanese species, were performed, and flavone *O*-glycoside producing species described above were contained in a same clade (Ebihara, 2011). Another flavone *O*-glycoside, scutellarein 6-*O*-glucoside, has been reported from *A. belangeri* (Bory) Kze. (Umikalsom *et al*., 1994). However, the phylogenetic position of this species is uncertain.

Two flavanone glycosides, naringenin 7-*O*-neohesperidoside and 7-*O*-arabinosyl-(1→6)-glucoside, were isolated from *A. ceterach* (Imperato, 1983b).

Xanthones which biosynthetically relate to flavonoids have also been found in some *Asplenium* species. *C*-Glycosylxanthones, mangiferin and isomangiferin were reported from *A. adiantum-nigrum*, *A. balearicum* (Richardson and Lorenz-Liburnau, 1982), *A. montanum*, *A. bradley*, *A. pinnatifidum* and *A. stotleri* (Smith and Harborne, 1971). They were newly found in *A. contiguum* in this survey. In *A. adiantum-nigrum*, 1,3,7,8-tetrahydroxyxanthone 1-*O*-cellobioside (Imperato, 1980b), and 1,3,7,8-tetrahydroxyxanthone 1-*O*-laminaribioside (Imperato, 1980c) and 1-hydroxy-3,6,7-trimethoxyxanthone 2,4-di-*C*-glucoside (Imperato, 1991) were also isolated together with mangiferin and isomangiferin. Their xanthones have been reported from many ferns (Richardson, 1984).

Thus, flavonols, flavone *O*- and *C*-glycosides, flavanone, aurone and *C*-glycosylxanthones have been reported from *Asplenium* species. Major compounds were flavonol *O*-glycosides based on kaempferol, and flavanones and aurone were minor compounds in *Asplenium*.

Fourty-two *Asplenium* species have been surveyed for flavonoids (Table 2). The survey of

flavonoid and related compounds in other species of the genus is now in progress.

## Acknowledgments

Collection of plant materials in Vanuatu and New Caledonia of 1996 and 1997, and 2000 and 2001 were supported by Monbusho International Scientific Research Program (No. 08041165) (representative: T. Iwashina) from the Ministry of Education, Science Sports and Culture, Japan, and the Monbu-Kagakusho International Sicentific Program (No. 12575013) (representative: T. Iwashina) from Ministry of Education, Culture, Sports, Science and Technology, Japan.

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