Flavonoids from two Parasitic and Achlorophyllous Plants, *Aeginetia indica* and *Orobanche minor* (Orabanchaceae)

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**Abstract**  Two parasitic and achlorophyllous species, *Aeginetia indica* and *Orobanche minor* belonging to the family Orobanchaceae, were surveyed for flavonoids. The flowers and aerial parts of *A. indica* were extracted with MeOH and the flavonoids were isolated by various chromatographical techniques. Two anthocyanins were obtained from the flowers together with other flavonoids, and characterized as cyanidin 3-O-rutinoside and 3-O-glycoside. Other flavonoids were identified as apigenin 7-O-glucuronide, luteolin 7-O-glucuronide, apigenin, luteolin, quercetin 3-O-rutinoside and naringenin 7-O-glucoside. They were also obtained from the aerial parts except for anthocyanins and luteolin. On the other hand, two flavonoids were isolated from the aerial parts of *Orobanche minor* and identified as luteolin 7-O-glucoside and 7-O-glucuronide.

**Key words**: *Aeginetia indica*, anthocyanin, apigenin, flavonoids, luteolin, Orobanchaceae, *Orobanche minor*.

**Introduction**

*Aeginetia indica* L. is parasitic on roots of monocots, especially the Poaceae such as *Saccharum officinarum* L. and *Miscanthus sinensis* Anderss., and is distributed in Indonesia to Japan (Mabberley, 1997). Though the whole plants are achlorophyllous and therefore brownish or yellow brown, the flowers are pale red purple.

On the other hand, *Orobanche minor* Sutton is parasitic on roots of the Leguminosae, *Trifolium* species, and sometimes Asteraceae and Umbelliferae species. Though the species is originally native to Europe and Africa, it was now widely naturalized in the world.

A flavone aglycone, apigenin has been reported from *A. indica* as antihepatotoxic substance (Oshima *et al.*, 1984). The occurrence of phenylethanoids, e.g. acteoside, bandioside, orobanchoside and crenatoside, has been reported in the Orobanchaceae (Serafini *et al.*, 1995; Mølgaard and Ravn, 1988; Cometa *et al.*, 1993; Andary *et al.*, 1982).

In this paper, the flavonoid properties of the flowers and aerial parts of two parasitic plants, *Aeginetia indica* and *Orobanche minor*, are described.

**Materials and Methods**

**Plant materials**

*Aeginetia indica* L. (Fig. 1) which is natively growing in Tsukuba Botanical Garden, National Museum of Nature and Science, Tsukuba, Japan, was collected for flavonoid analysis. It was parasitic on roots of *Miscanthus condensatus* Hack. (Poaceae).

*Orobanche minor* Sutton (Fig. 1) was collected in Tsukuba City, Ibaraki, Japan. The species was parasitic on roots of *Trifolium repens* L. (Legu-
minosae). Voucher specimens were deposited in the herbarium of National Museum of Nature and Science, Tokyo (TNS).

**Extraction and isolation of anthocyanin and flavonoids in *A. indica* flowers**

Fresh flowers (107 g) were extracted with MeOH/HOAc/H₂O (10:1:10). The concentrated extracts were applied to preparative paper chromatography (prep. PC) using solvent system: BAW (n-BuOH/HOAc/H₂O = 4:1:5, upper phase). Anthocyanin and other flavonoid bands were cut out and eluted with 5% HOAc in MeOH (anthocyanins) or MeOH (other flavonoids). Anthocyanins were purified by Sephadex LH-20 column chromatography using solvent system: HOAc/MeOH/H₂O (5:70:25). On the other hand, other flavonoids were applied to prep. PC using solvent systems: 15% HOAc and then BEW (n-BuOH/EtOH/H₂O = 4:1:2.2). The obtained flavonoids were finally purified by Sephadex LH-20 column chromatography using solvent system: 70% MeOH. Two flavonoids (F1 and F2) were obtained as pale yellow powders (ca. 10 and 5 mg, respectively).

**Extraction and isolation of flavonoids in *A. indica* and *O. minor* aerial parts**

Fresh aerial parts of *A. indica* (280 g) and *O. minor* (367 g) were extracted with MeOH, respectively. The concentrated extracts were washed with petroleum ether for removal of lipophilic compounds, and then applied to prep. PC using solvent systems: BAW, 15% HOAc and then BEW. The obtained flavonoids were finally purified by Sephadex LH-20 column chromatography using solvent system: 70% MeOH.

**High performance liquid chromatography (HPLC)**

Qualitative HPLC was performed with Shimadzu HPLC systems using Shim-pack CLC-ODS [I.D. 6.0×150 mm (Shimadzu)], at a flow-rate of 1.0 ml min⁻¹; detection wavelength was 190–350 nm and eluents were MeCN/H₂O/H₃PO₄ (20:80:0.2 for glycosides, and 35:65:0.2 for aglycones).

**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
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Identification of anthocyanins

The anthocyanins from *A. indica* flowers were identified by characterization of the products obtained by acid hydrolysis (in 12% HCl, 100°C, 3 min), visible spectroscopy, and direct PC comparison with authentic sample. PC and UV spectral data of two isolated anthocyanins were as follows.

Cyanidin 3-O-rutinoside (keracyanin, A1). PC: RF 0.22 (BAW), 0.35 (BuH, n-BuOH/conc.HCl/H₂O=7 : 2 : 5), 0.17 (1% HCl), 0.79 (Forestal, HOAc/conc.HCl/H₂O=5 : 3 : 30); UV—purplish red. Visible spectra: \( \lambda_{\text{max}} \) (nm) 0.1% MeOH–HCl 530 nm.

Cyanidin 3-O-glycoside (A2). PC: RF 0.28 (BAW), 0.30 (BuH), 0.07 (1% HCl), 0.71 (Forestal); UV—purplish red. Visible spectra: \( \lambda_{\text{max}} \) (nm) 0.1% MeOH–HCl 528 nm.

Identification of other flavonoids

Other flavonoids from *A. indica* and *O. minor* were identified by UV spectroscopy according to Mabry et al. (1970), LC-MS, acid hydrolysis (in 12% HCl, 100°C, 30 min), direct HPLC and TLC comparisons with authentic specimens. Their TCL, UV and LC-MS data were as follows.

Apigenin 7-O-glucuronide (F1). TLC: RF 0.64 (BAW), 0.54 (BEW), 0.19 (15% HOAc); UV—dark purple, UV/NH₃—dark greenish yellow. UV: \( \lambda_{\text{max}} \) (nm) MeOH 267, 333; +NaOMe 274, 378 (inc.); +AlCl₃ 275, 299, 348, 376; +AlCl₃/HCl 275, 298, 341, 374; +NaOAc 266, 389; +NaOAc/H₂BO₃ 267, 340. LC-MS: molecular ion peak, \( m/z \) 447 [M+H]+ (apigenin+1 mol glucuronic acid), fragment ion peak, \( m/z \) 269 [M–176+H]+ (apigenin).

Luteolin 7-O-glucuronide (F2). TLC: RF 0.40 (BAW), 0.40 (BEW), 0.11 (15% HOAc); UV—dark purple, UV/NH₃—yellow. UV: \( \lambda_{\text{max}} \) (nm) MeOH 256, 266, 348; +NaOMe 266, 272, 392 (inc.); +AlCl₃ 274, 426; +AlCl₃/HCl 265, 273, 295sh, 360, 384sh; +NaOAc 257sh, 265, 402; +NaOAc/H₂BO₃ 257sh, 262, 374. LC-MS: molecular ion peak, \( m/z \) 463 [M+H]+ (luteolin+1 mol glucuronic acid), fragment ion peak, \( m/z \) 287 [M–176+H]+ (luteolin).

Apigenin (F3). TLC: RF 0.93 (BAW), 0.96 (BEW), 0.80 (Forestal); UV—dark purple, UV/NH₃—dark greenish yellow. UV: \( \lambda_{\text{max}} \) (nm) MeOH 267, 339; +NaOMe 275, 326, 393 (inc.); +AlCl₃ 274, 302, 355, 376sh; +AlCl₃/HCl 276, 299, 348, 375sh; +NaOAc 275, 311, 388; +NaOAc/H₂BO₃ 267, 348. LC-MS: molecular ion peak, \( m/z \) 271 [M+H]+ (apigenin).

Luteolin (F4). TLC: RF 0.84 (BAW), 0.87 (BEW), 0.55 (Forestal); UV—dark purple, UV/NH₃—yellow. UV: \( \lambda_{\text{max}} \) (nm) MeOH 257sh, 265, 360; +NaOMe 267sh, 274, 331, 411 (inc.); +AlCl₃ 275, 431; +AlCl₃/HCl 266, 274, 300, 362, 395sh; +NaOAc 267sh, 274, 326, 397; +NaOAc/H₂BO₃ 265, 380. LC-MS: molecular ion peak, \( m/z \) 285 [M–H]– (luteolin).

Luteolin 7-O-glucoside (F5). TLC: RF 0.42 (BAW), 0.43 (BEW), 0.07 (15% HOAc); UV—dark purple, UV/NH₃—dark yellow. UV: \( \lambda_{\text{max}} \) (nm) MeOH 255, 268sh, 347; +NaOMe 268, 392 (inc.); +AlCl₃ 273, 426; +AlCl₃/HCl 266, 274sh, 295, 359, 384sh; +NaOAc 260, 405; +NaOAc/H₂BO₃ 259, 373.

Acteoside. TLC: RF 0.60 (BAW), 0.52 (BEW), 0.74 (15% HOAc); UV—blue, UV/NH₃—blue green. UV: \( \lambda_{\text{max}} \) (nm) MeOH 246sh, 291, 332; +NaOMe 258, 384 (inc.); +AlCl₃ 262, 300, 363; +AlCl₃ 245sh, 290sh, 332; +NaOAc 279, 289sh, 380; +NaOAc/H₂BO₃ 256sh, 295, 355. LC-MS: molecular ion peak, \( m/z \) 623 [M–H]– (acteoside).

Results

Anthocyanins and flavonoids in Aeginetia indica

Two anthocyanins, four flavones, and each one flavonol and flavanone were found in the flowers of *A. indica*. Of their compounds, major anthocyanin A1 was identified as cyanidin 3-O-rutinoside (Fig. 2) by acid hydrolysis, visible spectral properties and direct PC comparison with authentic keracyanin from the flowers of *Canna generalis* L.H. Bailey (Cannaceae) (Hayashi et
Another minor anthocyanin was characterized as cyanidin glycoside.

UV spectral properties of two flavone glycosides F1 and F2 showed that they are apigenin and luteolin 7-O-glycosides, respectively. Acid hydrolysis of them liberated apigenin and glucuronic acid, and luteolin and glucuronic acid. Finally, F1 and F2 were identified as apigenin 7-O-glucuronide (Fig. 3) and luteolin 7-O-glucuronide (Fig. 4) by direct TLC and HPLC comparisons with authentic samples from the leaves of Uncarina grandiflora (Baill.) Stapf. (Pedaliaceae) (Yamazaki et al., 2007).

UV spectra of flavonoids F3 and F4 showed that they are flavones having free 5-, 7- and 4'-hydroxyl, and free 5-, 7-, 3'- and 4'-hydroxyl groups, respectively. Moreover, they were soluble in diethyl ether, showing that the compounds are flavone aglycones. Their Rf values of TLC and retention times of HPLC completely agreed with those of authentic apigenin (Fig. 5) and luteolin (Fig. 6) from the pubescence of Glycine max (L.) Merr. (Leguminosae) (Iwashina et al., 2006), respectively.

Flavonoid F6 was obtained from the flowers of A. indica as a minor compound. Its UV spectral properties clearly showed that F6 is 3-substituted quercetin. The compound was directly compared with authentic rutin (Fig. 7) from the leaves of Begonia formosana (Hayata) Masam. (Begoniaceae) (Iwashina et al., 2008), and their retention times agreed with that of each other.

Flavonoid F7 was also isolated from the flowers of A. indica. UV spectral properties of the compound was those of flavanone, dihydroflavonol or dihydrochalcone (Mabry et al., 1970). Finally, flavonoid F7 was identified as flavanone, naringenin 7-O-glucoside (Fig. 8) by direct HPLC comparison with commercial authentic sample (Carl Roth). Though the presence of another flavanone was shown by HPLC survey, its retention time did not agree with those of authentic naringenin 7-O-neohesperidoside and eri-

al., 1953). Another minor anthocyanin was characterized as cyanidin glycoside.

UV spectral properties of two flavone glycosides F1 and F2 showed that they are apigenin and luteolin 7-O-glycosides, respectively. Acid hydrolysis of them liberated apigenin and glucuronic acid, and luteolin and glucuronic acid.

Fig. 2. Cyanidin 3-O-rutinoside (keracyanin, A1).

Fig. 3. Apigenin 7-O-glucuronide (F1).

Fig. 4. Luteolin 7-O-glucuronide (F2).

Fig. 5. Apigenin (F3).

Fig. 6. Luteolin (F4).
Flavonoids in Orobanche minor

Two flavonoids were isolated from the aerial parts of *O. minor*. They were shown to be luteolin 7-O-glycosides by their UV spectral survey. They liberated luteolin and glucose, and luteolin and glucuronic acid by acid hydrolysis, respectively. Finally, they were identified as luteolin 7-O-glucoside (F5) (Fig. 9) and luteolin 7-O-glucuronide (F2) by direct TLC and HPLC comparisons with authentic samples from the leaves of *Schmalhausenia nidulans* Petrak (*Asteraceae*) (Iwashina and Kadota, 1999) and *Uncarina* spp. (Yamazaki *et al.*, 2007), respectively. Apart from their flavonoids, much amount of colorless powder, which was presumed as caffeoyl derivative by UV spectral properties, was obtained (ca. 100 mg). This was identified as phenylethanoid, acteoside, by LC-MS and direct HPLC comparison with authentic sample from the leaves of *Plantago asiatica* (*Plantaginaceae*) (Murai *et al.*, 2009).

Discussion

In this experiment, two anthocyanins, four flavones, and each one flavonol and flavanone were found in the flowers and aerial parts of *Aeginetia indica* and completely or partially characterized. On the other hand, two flavone glycosides were isolated from the aerial parts of *Orobanche minor*. Only one flavonoid, apigenin has been reported from *Aeginetia* species including *A. indica* until now (Oshima *et al.*, 1984). Though the aerial parts of this species is achlorophyllous and therefore brownish or yellow brown, the flower is pale red purple. In this survey, it was shown that the flower color is due to anthocyanin, cyanidin 3-O-rutinoside, together with another cyanidin glycoside. Four flavones, apigenin and luteolin, and their 7-O-gluconides, a flavonol, quercetin 3-O-rutinoside, and a flavanone, naringenin 7-O-glucoside, were accompanied with anthocyanins in flowers. They were detected in the aerial parts except for two anthocyanins and luteolin. Their flavonoids except for apigenin were reported in *Aeginetia* species for the first time.

A flavone, tricin has been reported from seeds of *Orobanche* species as allelopathy compound (Rice, 1984). In this survey, two glycosides, luteolin 7-O-glucoside and 7-O-glucuronide, were found in the aerial parts of *Orobanche* species for the first time.

The flavonoids have been reported from a few achlorophyllous plants, e.g. quercetin 3-O-glucoside and 3-O-glucuronide from *Monotropa uniflora* L. (*Pyrolaceae*) (Bohm and Averett, 1989), 3-hydroxyphloretin and its 4’-O-glucoside from
Balanophora tobiracola Makino (Balanophoraceae) (Ito et al., 1980), vitexin, isovitexin and vicenin-2 from Petrosavia sakuraii (Makino) J.J. Sm. ex van Steenis (Petrosaviaceae, Liliaceae s.l.) (Iwashina et al., unpublished data). However, their flavonoid composition was comparatively simple. One of major function of flavonoids is UV shields (Bohm, 1998), so that achlorophyllous plants, which do not perform the photosynthesis, may be not synthesize many kinds and large amount of flavonoids. The study of the flavonoid function in achlorophyllous plants is in progress.

References