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Flavonoid glycosides in the shoot system of Okinawa Taumu (*Colocasia esculenta* S.)

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ABSTRACT

Six *C*-glycosylflavonoids and one *O*-glycosylflavonoid have been isolated from the shoot system of Taumu (*Colocasia esculenta* S.). They were identified as schaftoside, isoschaftoside, orientin, isovitexin, isoorientin, vitexin and luteolin 7-*O*-sophoroside. The presence of catechol moiety in the B-ring of isoorientin, orientin and luteolin 7-*O*-sophoroside showed strong antioxidant activity with different mechanisms of action in DPPH radical scavenging, β-carotene bleaching and superoxide radical inhibition assays. The amount of dry weight matter extract of water was higher than methanol for both the leaf and the stem parts. Isovitexin was the main compound in water and methanol extracts of the leaf while schaftoside was the main compound of the water extract of the stem. Methanol extract of the leaf showed higher DPPH radical scavenging activity than water extract while the opposite was observed for the stem. The results of this experiment suggest the potential of the leaf of Taumu as a source of dietary antioxidant.

1. Introduction

Free radicals and reactive oxygen species caused denaturation in foods especially lipids (Yasaei, Yang, Warner, Daniels, & Ku, 1996). They were also known to cause oxidative damage to the body (Aniya, 2002). Natural antioxidants from plants such as flavonoids and polyphenols were found to help in reducing oxidative damage to fish during storage (Ramanathan & Das, 1992). Wiseman (1996) reported that antioxidants from plant foods were also found to have important role in protecting cells from oxidative damage.

Okinawan plants were rich in antioxidants (Nakatani, 2003). These antioxidants had free radical scavenging activity that protected liver from inflammation and preventing diabetes complications (Aniya, Itokazu, & Shimoji, 2002; Aniya, Miyagi et al., 2002).

Taumu or *Colocasia esculenta* Schott var. *aquatilis* Kitamura is one of the traditional plant foods in Okinawa. Taumu was once widely cultivated in various places in Okinawa. Nowadays, the main cultivation areas are in Kin town and Ginowan due to the decrease of paddy field (Kin Town Hall, 1994). Besides the tuber, the Okinawan people also use the stem of Taumu as an important vegetable. Taumu can grow throughout the year although the tuber of Taumu is harvested during December to April. The stem of Taumu is eaten either as salad, stew or as soup.

Nakatani (1992) suggested that the antioxidant compounds contained in the Okinawan plants contributed to the good health of the Okinawans. It is possible that Taumu as one of the important dietary plant foods contains antioxidants that contributes to the good health of the Okinawan people. In this study, we investigated the antioxidant compounds of the shoot system of Taumu using DPPH radical scavenging assay as the antioxidant activity marker.

2. Materials and methods

2.1. Chemicals and reagents

Analytical grade chemicals and reagents were used. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2-morpholinoethanesulphonic (MES), bovine serum albumin (BSA), β-carotene, linoleic acid, methanol, nitro blue tetrazolium (NBT), polysorbate 20 (Tween 20), α-tocopherol and xanthine were from Nacalai Tesque Inc. (Kyoto, Japan). Chloroform, copper (II) chloride, dimethyl sulfoxide-d_6 (DMSO-d_6) for NMR analysis, ethylenediaminetetraacetic acid (EDTA), formic acid and xanthine oxidase (XOD) from buttermilk were from Wako Pure Chemical Industries Ltd. (Osaka, Japan). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was purchased from Sigma–Aldrich (St. Louis, MO, USA) while L(+)-ascorbic acid was purchased from Kanto Chemical Inc. (Tokyo, Japan). Water was purified using a MilliQ-Labo purification system by Millipore Ltd. (Osaka, Japan).

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2.2. Plant material and extraction

The shoots (leaves and stems) of Taumu were collected from Kin town of Okinawa prefecture in April 2008. They were washed and freeze-dried. About 750 g of pulverised shoots were extracted with methanol at room temperature for 8 h. The methanol extract was filtered and the residue was extracted twice again under the same conditions with a total volume of 8 l of methanol. The extracts were pooled and the methanol was evaporated using rotary evaporator. The dried extract was dissolved in 2 l of water. Hexane (1:1, v/v) was added to remove low polarity compounds from the water phase solution. The liquid–liquid separation was conducted five times.

2.3. Chromatographic separation

Waters Sep-Pak C18 solid phase extraction (SPE) cartridge (35 cc. reservoir) was used for fractionation of water phase solution using a stepwise elution from water to methanol. Five fractions were obtained. The fraction eluted with 25% aqueous methanol from SPE was separated by MPLC using Ultra Pack ODS-S-50B (300 × 26 mm i.d. column, 50 μm) (Yamazen Corp., Osaka, Japan) with UV detection at 254 nm. Separation was achieved using the following gradient elution at a flow rate of 7.5 ml/min: 5 min of 40% aqueous methanol, increased to 70% aqueous methanol in 15 min and held for 10 min. Five fractions were obtained.

HPLC was used for further separation of the fourth fraction using Cosmosil 5C18-AR-II (20 × 250 mm, 5 μm) (Nacalai Tesque Inc., Kyoto, Japan) at 40 °C. Flow rate was 4.5 ml/min with monitoring the absorbance at 254 nm. HPLC separation of this fraction using isocratic elution with 1% (v/v) formic acid in 30% aqueous methanol afforded compounds **1**, **2** and **3**.

The fraction eluted with 50% aqueous methanol from SPE was also separated using the same MPLC system. Separation was achieved using the following gradient elution: 10 min of 50% aqueous methanol, increased to 80% aqueous methanol in 20 min and held for 10 min. Four fractions were obtained. The fourth fraction was crystallised during vacuum-evaporation, which afforded compound **4**. The second and third fractions obtained by MPLC were further separated by HPLC system as described above. HPLC separation of the second fraction of MPLC using isocratic elution with 1% (v/v) formic acid in 30% aqueous methanol afforded compounds **1** and **2**. Separation of the third fraction using isocratic elution with 1% (v/v) formic acid in 35% aqueous methanol afforded compounds **5**, **6** and **7**.

All the samples were purified by crystallisation process prior to identification using LC/MS and NMR spectrometer.

2.4. DPPH radical scavenging assay

The DPPH radical scavenging assay by Morishita, Hara, Suda, and Tetsuka (2002) was used with slight modification. Samples in various concentrations up to 0.4 mM were prepared. A reaction mixture contained 0.3 ml of 0.4 mM of DPPH solution in methanol, 0.3 ml of 0.2 M MES buffer (pH 6.0) and 0.3 ml of 50% aqueous methanol. The mixture was added into 0.3 ml of sample. Absorbance was read at 520 nm after 1 h of reaction. The blank used for this assay was 50% aqueous methanol. Trolox in various concentrations of up to 0.02 mM was used as a positive control. The scavenging activity (SC) of the samples was calculated using the following formula: SC (%) = $[1-(A_s/A_0)] \times 100$ where A_s is the absorbance of the sample and A_0 is the absorbance of the blank. The SC_{50} value of each samples was determined from their respective linear regression curves.

2.5. β-carotene bleaching assay

The assay by Kumaran and Joel karunakaran (2006) was used with modifications. A mixture containing 0.5 ml of β-carotene (1 mg/ml), 0.2 ml of linoleic acid (0.1 g/ml) and 1 ml of Tween 20 (0.2 g/ml) was prepared in chloroform. The chloroform was removed under a stream of nitrogen. Then, 90 ml of water and 8 ml of sodium phosphate buffer (0.2 M, pH 6.8) were added into the dried mixture. The solution mixture was thoroughly mixed and 4.8 ml of the mixture were withdrawn into individual test tubes. After adding 0.2 ml of samples, the zero time absorbance of the resulting mixture (t = 0 min) was read at 470 nm. The tubes were placed in a water bath at 50 °C for 2 h. After that, the absorbance was read again. Inhibitory activity was calculated using the following formula: inhibitory activity (%) = $[1-(A_s^0-A_s^{120})/(A_c^0-A_c^{120})] \times 100$ where A_s^0 is the test sample absorbance at 0 min, A_s^{120} is the test sample absorbance at 120 min, A_c^0 is the control sample absorbance at 0 min and A_c^{120} is the control sample absorbance at 120 min. Methanol was used as a blank while α-tocopherol was used as a positive control.

2.6. Superoxide radical inhibition assay

The assay described in Leong, Tako, Hanashiro, and Tamaki (2008) was used. Briefly, 2.8 ml of reaction mixture contained 0.1 ml of 0.05% (w/v) BSA, 0.1 ml of 1 mM EDTA, 0.1 ml of 0.25 mM NBT, 0.1 ml of 1 mM xanthine and 2.4 ml of sodium carbonate buffer (0.05 M, pH 10.2). Then, 0.1 ml of sample prepared in various concentrations up to 0.5 mM was added into the reaction mixture before preincubation at room temperature for 10 min. After that, 0.1 ml of 0.1 U/ml of XOD was added to initiate the reaction. The solution mixture was incubated at room temperature for 30 min. Reaction was terminated by adding 0.1 ml of 6 mM copper (II) chloride. Absorbance was read at 560 nm. Water was used as a blank while L(+)-ascorbic acid was used as a reference compound. The inhibitory activity (IC) of the samples was calculated using the following formula: IC (%) = $[1-(A_s/A_0)] \times 100$ where A_s is the absorbance of the sample and A_0 is the absorbance of the blank. The IC_{50} value of each samples was determined from the linear regression of curves.

2.7. Quantification

Clean weighing bottles (40 × 40 mm) were weighed and dried in an oven at 90 °C for an hour. The bottles were transferred into a desiccator containing silica gel and left for about an hour to cool down. The weight of each bottle was measured and the procedure was repeated until there was no significant weight change (±0.0003 g) on each bottle.

Methanol and water were used to extract 2 g of pulverised dried Taumu leaves and stems, separately. The extraction was conducted one time. The extracts were filtered and filled up to 100 ml.

Each extracts (5 ml) were withdrawn and placed separately into weighing bottles. The bottles with their contents were dried in an oven and transferred into the desiccator. The weight of each bottle was measured and the procedure was repeated until a constant weight was attained.

Isolated compounds were quantified using HPLC as described by Leong et al. (2008). Cosmosil 5C18-AR-II (4.6 × 150 mm, 5 μm) column purchased from Nacalai Tesque Inc. (Kyoto, Japan) was used. The column temperature was set at 40 °C. Mobile phase A was 1% (v/v) formic acid in water while mobile phase B was 1% (v/v) formic acid in methanol. The column was developed at a flow rate of 0.8 ml/min using the gradient system as follow: one min of 5% B then ascended to 100% B in 55 min. The column under this concentration was held for 5 min before reconditioning in 5% of

B for 20 min prior to the next injection. UV detection was set at 254 nm.

The compounds isolated from the shoots of Taumu were used as standard compounds (>95% purity by HPLC). Each standard compound was prepared in 0.06, 0.1, 0.18, 0.3, 0.52 mg/ml. Five microlitres of the compounds were subjected to HPLC separately. The same gradient system, as described above, was used for column development. Chromatographic analysis of each standard compound was performed three times. The calibration curve was plotted as the peak area (arbitrary units) of the chromatogram against the standard compound (mg). The data points were fitted into a best fit line by linear regression method.

For quantification of the solvent extracts of Taumu, a concentration of 20 mg/ml of each extract was prepared and 100 μl of it were subjected to HPLC analysis. Column development was the same as that described above. The amount of the isolated compounds extracted by these two solvents was determined based on the peak area of the chromatogram of the extract with the calibration curve.

2.8. Solvent extraction capacity

Evaluation of the extraction capacity of water and methanol was based on the DPPH radical scavenging assay as described in Section 2.4. Two grams of each leaf and stem of Taumu were extracted separately with 100 ml of water and methanol for 3 h. Extraction was conducted once. Ekuseru-Toukei 2006 by Social Survey Research Information Co. Ltd. (Tokyo, Japan) was used for statistical analysis. One-way ANOVA and Scheffé's method of multiple comparison were applied with statistical significance at $p < 0.05$.

3. Results and discussion

The fractions eluted from SPE were tested for DPPH radical scavenging activity. The 25% and 50% aqueous methanol eluted fractions showed scavenging activity in which the SC_{50} values were 0.68 and 0.94 mg/ml, respectively. Therefore, both fractions were further isolated prior to identification. The physical appearance of all isolated compounds was yellow amorphous powder.

Analysis of these compounds by LC/ESI-MS with combinations of 1D (^{1}H and ^{13}C), and 2D (^{1}H-^{1}H COSY, HMQC and HMBC) NMR spectroscopy showed that these compounds were flavonoid glycoside. Compound **1** and compound **2** were identified as schaftoside (apigenin 6-*C*-β-D-glucopyranosyl-8-*C*-α-L-arabinopyranosyl) and isoschaftoside (apigenin 6-*C*-α-L-arabinopyranosyl-8-*C*-β-D-glucopyranosyl), respectively. The confirmation of spectral data was made by comparison with literature (Agrawal & Bansal, 1989).

Compound **3** was identified as orientin (luteolin 8-*C*-β-D-glucopyranosyl) while compound **4** was identified as isovitexin (apigenin 6-*C*-β-D-glucopyranosyl). Spectral data of both compounds **3** and **4** corresponded to the results obtained by Mun'im, Negishi and Ozawa (2003) and Peng, Fan, Hong, Chai, and Wu (2005), respectively. Compound **5** was identified as isoorientin (luteolin 6-*C*-β-D-glucopyranosyl) and this was confirmed upon comparison with Mun'im et al. (2003).

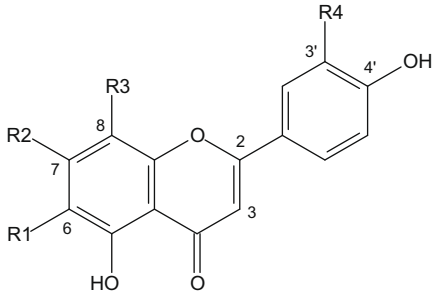
NMR spectral analysis showed that compound **6** was luteolin with diglucoside being at position 7. The data were compared with the data reported by Imperato and Nazzaro (1996) and identified this compound as luteolin 7-*O*-β-D-glucopyranosyl (1→2) β-D-glucopyranoside or luteolin 7-*O*-sophoroside.

Compound **7** was identified as vitexin (apigenin 8-*C*-β-D-glucopyranosyl), corresponded to the results by Zhou, Peng, Fan, and Wu (2005). Fig. 1 shows the molecular structures of these compounds isolated from the shoots of Taumu. The MS data and the ^{1}H NMR data of the flavonoid glycosides are summarised in Table 1 while the ^{13}C NMR data are listed in Table 2.

The antioxidants activities of the isolated flavonoid glycosides were studied using DPPH radical scavenging, β-carotene bleaching inhibition, and superoxide radical inhibition assays. These assays were also used to evaluate the mechanisms of action of these compounds. The results of these three assays are shown in Table 3.

DPPH radical scavenging assay showed that orientin, isoorientin and luteolin 7-*O*-sophoroside had stronger DPPH radical scavenging activity than trolox. The results suggested that DPPH free radicals were reduced by the electron transfer from these compounds (Prior, Wu, & Schaich, 2005). Among all the compounds, isoorientin showed the strongest radical scavenging activity (SC_{50} = 0.031 mM) followed by luteolin 7-*O*-sophoroside, orientin, trolox, vitexin and isovitexin.

The isolated flavonoid glycosides at a concentration of 1 mM were also tested for their effectiveness in inhibiting decolorization of β-carotene coupled with the oxidation of linoleic acid. In this assay, α-tocopherol showed the highest inhibitory activity (81.8%)



Compound	R1	R2	R3	R4
1 (Schaftoside)	*C*-β-D-glucopyranosyl	OH	*C*-α-L-arabinopyranosyl	H
2 (Isoschaftoside)	*C*-α-L-arabinopyranosyl	OH	*C*-β-D-glucopyranosyl	H
3 (Orientin)	H	OH	*C*-β-D-glucopyranosyl	OH
4 (Isovitexin)	*C*-β-D-glucopyranosyl	OH	H	H
5 (Isoorientin)	*C*-β-D-glucopyranosyl	OH	H	OH
6 (Luteolin 7-*O*-sophoroside)	H	*O*- β-D-glucopyranosyl (1 →2) glucopyranoside	H	OH
7 (Vitexin)	H	OH	*C*-β-D-glucopyranosyl	H

Fig. 1. Molecular structures of the flavonoid glycosides isolated from the shoot system of *Colocasia esculenta* S.

Table 1
Mass and ^{1}H [δ values (ppm) and J values (Hz), 500 MHz, DMSO-d_6] NMR spectra data of **1** (schaftoside), **2** (isoschaftoside), **3** (orientin), **4** (isovitexin), **5** (isoorientin), **6** (luteolin 7-*O*-sophoroside) and **7** (vitexin).

	1	**2**	**3**	4	**5**	**6**	**7**
m/z	$[M–H]^-$ 563.1	$[M–H]^-$ 563.2	$[M–H]^-$ 447.3	$[M–H]^-$ 431.1	$[M–H]^-$ 447.1	$[M+H]^+$ 611.1	$[M–H]^-$ 431.1
H-3	6.74 (1H, s)	6.81 (1H, s)	6.58 (1H, s)	6.82 (1H, s)	6.66 (1H, s)	6.66 (1H, s)	6.83 (1H, s)
H-5	13.68 (OH, s)	13.67 (OH, s)	13.11 (OH, s)	13.60 (OH,s)	13.54 (OH, s)	12.89 (OH, s)	13.22 (OH, s)
H-6	–	–	–	–	6.47 (1H, s)	6.74 (1H, d, J = 2.0 Hz)	6.31 (1H, s)
H-8	–	–	6.20 (1H, s)	6.53 (1H, s)	–	6.41 (1H, d, J = 2.0 Hz)	–
H-2′	7.89 (2H, d, J = 8.4 Hz)	8.02 (2H, d, J = 8.8 Hz)	7.41 (1A, d, J = 1.6 Hz)	7.96 (2H, d, J = 8.8 Hz)	7.33 (1H, d, J = 2.0 Hz)	7.32 (1H, d, J = 2.0 Hz)	8.06 (2H, d, J = 8.5 Hz)
H-3′	6.88 (2H, d, J = 8.4 Hz)	6.88 (2H, d, J = 8.5 Hz)	–	6.95 (2H, d, J = 8.8 Hz)	–	–	6.92 (2H, d, J = 8.5 Hz)
H-5′	6.88 (2H, d, J = 8.4 Hz)	6.88 (2H, d, J = 8.5 Hz)	6.79 (1H, d, J = 8.3 Hz)	6.95 (2H, d, J = 8.8 Hz)	6.88 (1H, d, J = 8.5 Hz)	6.81 (1H, d, J = 8.1 Hz)	6.92 (2H, d, J = 8.5 Hz)
H-6′	7.89 (2H, d, J = 8.4 Hz)	8.02 (2H, d, J = 8.8 Hz)	7.47 (1H, dd, J = 8.0, 2.0 Hz)	7.96 (2H, d, J = 8.8 Hz)	7.36 (1H, dd, J = 8.0, 2.2 Hz)	7.35 (1H, dd, J = 8.3, 1.8 Hz)	8.06 (2H, d, J = 8.5 Hz)
H-1″	4.49 (1H, d, J = 9.9 Hz)	4.71 (1H, d, J = 9.6 Hz)	4.61 (1H, d, J = 9.9 Hz)	4.60 (1H, d, J = 9.9 Hz)	4.56 (1H, d, J = 9.6 Hz)	5.13 (1H, d, J = 7.3 Hz)	4.71 (1H, d, J = 9.9 Hz)
H-1‴	4.69 (1H, d, J = 9.9 Hz)	4.75 (1H, d, J = 9.9 Hz)	–	–	–	4.39 (1H, d, J = 7.9 Hz)	-

Table 2
^{13}C [δ (ppm), 125.65 MHz, DMSO-d_6] NMR spectral data of **1** (schaftoside), **2** (isoschaftoside), **3** (orientin), **4** (isovitexin), **5** (isoorientin), **6** (luteolin 7-*O*-sophoroside), and **7** (vitexin).

Position	**1**	**2**	**3**	**4**	**5**	**6**	**7**
C-2	164.12	164.14	164.05	163.39	163.78	164.45	163.95
C-3	102.64	102.64	102.39	102.73	102.91	103.11	102.47
C-4	182.45	182.35	182.00	181.87	182.01	181.92	182.14
C-4a	103.32	103.76	104.01	103.18	103.51	105.33	104.04
C-5	159.16	160.97	160.35	160.70	160.79	161.08	160.41
C-6	108.04	108.14	98.09	108.91	108.95	99.65	98.14
C-7	160.83	158.22	162.51	–	163.38	162.75	162.60
C-8	104.06	105.14	104.50	93.68	93.62	94.74	104.61
C-8a	153.77	155.12	155.98	156.27	156.32	156.85	156.01
C-1′	121.45	121.57	122.01	121.09	121.52	121.33	121.62
C-2′	129.14	129.09	114.06	128.47	113.36	113.52	129.01
C-3′	116.05	115.86	145.79	115.94	145.86	145.79	115.82
C-4′	161.28	161.24	149.57	160.70	149.83	149.98	161.16
C-5′	116.05	115.86	115.60	115.94	116.17	115.97	115.82
C-6′	129.14	129.09	119.36	128.47	119.11	119.15	129.01
C-1″	73.64	73.83	73.37	73.08	73.13	104.65	73.39
C-2″	70.97	68.44	70.74	70.64	70.72	82.71	70.82
C-3″	78.56	74.19	78.73	78.97	79.04	75.65[a]	78.65
C-4″	69.98	69.63	70.66	70.15	70.27	69.15[b]	70.51
C-5″	81.14	70.91	81.98	81.62	81.68	76.94[c]	81.88
C-6″	61.27	–	61.61	61.49	61.60	60.55[d]	61.27
C-1‴	74.96	73.29	–	–	–	98.35	–
C-2‴	68.86	70.56	–	–	–	74.69	–
C-3‴	75.21	78.89	–	–	–	76.19[a]	–
C-4‴	69.60	70.14	–	–	–	69.56[b]	–
C-5‴	70.97	81.94	–	–	–	76.94[c]	–
C-6‴	–	61.22	–	–	–	60.46[d]	–

[a–d]Assignments with the same superscripts may be interchanged.

Table 3
Antioxidant activities of the flavonoid glycosides isolated from the shoots of *Colocasia esculenta* S.

Sample	SC_{50} of DPPH radical scavenging activity (mM)	Inhibitory activity of β-carotene bleaching at 1 mM (%)	IC_{50} of superoxide radical inhibition activity (mM)
L(+)-Ascorbic acid	–	–	>0.5
Trolox	0.104	–	–
α-Tocopherol	–	81.8	–
Schaftoside	>0.4	18.4	>0.5
Isoschaftoside	>0.4	11.1	>0.5
Orientin	0.038	17.6	0.171
Isovitexin	0.325	3.3	>0.5
Isoorientin	0.031	17.0	0.067
Luteolin 7-*O*-sophoroside	0.032	52.0	0.037
Vitexin	0.129	2.6	>0.5

followed by luteolin 7-*O*-sophoroside (52.0%). The rest of the flavonoid glycosides also showed inhibition activity but with much lower effect compared with luteolin 7-*O*-sophoroside. The results suggested that these compounds donated hydrogen atoms to quench the peroxyl radicals induced by heat that was necessary to oxidize β-carotene (Prior et al., 2005).

As for the superoxide radical inhibition assay, orientin, isoorientin and luteolin 7-*O*-sophoroside showed inhibitory activity against the formation of formazan caused by the superoxide radical generated by xanthine oxidase. Ascorbic acid did not show any inhibitory activity on superoxide radical even at a concentration of 0.5 mM. Luteolin 7-*O*-sophoroside showed the highest inhibitory activity followed by isoorientin and orientin. The rest of the compounds did not show any inhibition activity.

Based on the results obtained, the antioxidant activities of the isolated flavonoid glycosides were affected by the number of the hydroxyl groups at the B-ring of the aglycone. Orientin, isoorientin and luteolin 7-*O*-sophoroside have two hydroxyl groups, which explain their greater antioxidant activities than the other flavonoid glycosides that have only one hydroxyl group at the B-ring. Matsuda, Wang, Managi, and Yoshikawa (2003) reported similar results using a wide variety of flavonoids.

The antioxidant strength of these flavonoid glycosides was also affected by the different bonding position of glycosyl groups to the aglycone. As shown in Table 3, DPPH radical scavenging activity of vitexin was greater than isovitexin while isoorientin showed greater superoxide radical scavenging activity than orientin. Besides the different mechanisms of actions of these compounds, the bonding position of glucosyl to an aglycone is expected to have an important effect towards its antioxidant activity. We could not evaluate other compounds as they exceeded the maximum concentrations used in the respective assays.

Although isoorientin and orientin showed stronger DPPH radical scavenging and superoxide radical scavenging activities than the other compounds, they do not scavenge peroxyl radicals as effectively as compared to luteolin 7-*O*-sophoroside. Luteolin

Table 4
Quantification of the dry matter and flavonoid glycosides contents in the water and the methanol extracts of the shoots of *Colocasia esculenta* S.

Plant part	Extracted by	Dry matter of extract (mg/g)	mg/g of dry matter of extract					
			Schaftoside	Isoschaftoside	Orientin	Isovitexin	Isoorientin	Luteolin 7-*O*-sophoroside and Vitexin
Leaf	Water	28.8	0.14	0.06	0.12	0.28	0.19	0.06
	MeOH	21.7	0.21	0.09	0.22	0.51	0.37	0.09
Stem	Water	33.5	0.06	0.02	0.01	Trace	0.01	Trace
	MeOH	24.1	Trace	0.01	0.02	Trace	0.02	Trace

Values are means of triplicate tests.

7-*O*-sophoroside has an *O*-glycoside bonding to the aglycone while the other compounds have *C*-glycosyl bonding to the aglycone. This type of glycosidic bonding seems to have effect on the antioxidant activities of these compounds.

Table 4 shows the dry weight matter of water and methanol extracts of Taumu as well as the content of the flavonoid glycosides in these extracts. Water has a higher extraction capacity for both the leaf and stem of Taumu compared to the methanol extract in which 28.8 mg/g of dry weight of the leaf and 33.5 mg/g of dry weight of the stem were extracted. The retention times for schaftoside, isoschaftoside, orientin, isoorientin, luteolin 7-*O*-sophoroside, vitexin and isovitexin by HPLC were 24.50, 24.87, 25.20, 25.59, 26.76, 26.77 and 28.02 min, respectively. The linear equations and the R^2 values of these individual standard compounds used for quantification were as follow: schaftoside ($y = 112{,}766\times + 4672.8$, $R^2 = 0.9995$), isoschaftoside ($y = 73{,}284\times + 1978.2$, $R^2 = 0.9994$), orientin ($y = 115{,}035\times -955.35$, $R^2 = 0.9989$), isovitexin ($y = 91{,}286\times + 11{,}320$, $R^2 = 0.9955$), isoorientin ($y = 136{,}877\times -3987.1$, $R^2 = 0.9998$), luteolin 7-*O*-sophoroside ($y = 77{,}199\times + 2392.9$, $R^2 = 0.9998$), and vitexin ($y = 109{,}338\times + 3327$, $R^2 = 0.9990$).

Quantification by HPLC on each flavonoid glycosides showed that isovitexin was the main compound in both water (0.28 mg/g of dry weight extract) and methanol (0.51 mg/g of dry weight extract) extracts of the leaf of Taumu. However, only a trace amount of isovitexin was detected in the water and methanol extracts of the stem. The main compound in the stem was schaftoside (0.06 mg/g of dry weight extract) although a trace amount was detected in the methanol extract. Luteolin 7-*O*-sophoroside and vitexin could not be quantified individually because the difference of the retention times was very small. The two compounds were quantified together as one based on the calibration curve of vitexin. The overall quantification results showed that the flavonoid glycosides content in the leaf was higher than in the stem. Considerable amount of flavonoid glycosides can be extracted with water as shown in Table 4.

The extraction capacity of both water and methanol on the shoots was evaluated using DPPH radical scavenging assay. Based on the results in Table 5, methanol extract of the leaf had higher DPPH radical scavenging activity than the water extract. However, the DPPH radical scavenging activity of the water extract of the stem was higher than the methanol extract. The higher DPPH radical scavenging activity of the leaf extract than the stem extract might be due to the antioxidant content as shown in Table 4. This suggested the consumption of the stem by the Okinawans is sufficient to provide antioxidants for good health.

Table 5
Antioxidant activity of the water and the methanol extracts of the shoots of *Colocasia esculenta* S.

Plant part	Weight (mg)	DPPH radical scavenging activity (%)	
		Methanol extract	Water extract
Leaf	0.03	15.8 ± 2.9^{a}	12.1 ± 0.6^{a}
	0.3	83.9 ± 3.2^{b}	65.0 ± 2.5^{c}
Stem	0.03	4.7 ± 0.8^{d}	4.1 ± 1.1^{d}
	0.3	14.4 ± 1.1^{e}	17.8 ± 1.4^{f}

Values are means ± SD of triplicate analyses. Values with a different letter are significantly different ($p < 0.01$).

Flavonoids widely occur in foods as *O*-glycosides and *C*-glycosides. Hollman and Arts (2000) reported that an aglycone was easily absorbed into the body than a flavonoid glycoside. However, their same report, a flavonoid glycoside could be absorbed and metabolized in the body. Hattori et al. (1998) reported that *C*-glycosylflavones could metabolize in our body. Hence, it is possible that the flavonoid glycosides in the shoots of Taumu can be absorbed into the body. Epidemiological studies had shown that the increased consumption of flavonoid-containing food was associated with health maintenance and the prevention of chronic and degenerative diseases (Havsteen, 2002; Knekt et al. 2002).

In conclusion, the antioxidants in the shoot of Taumu are flavonoid glycosides. The leaf contained more antioxidants than the stem. We propose that besides the stem, the leaf is to be used in the diet.

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