イチジク（*Ficus carica* L.）葉におけるフェニルプロパノイド類の同定

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Identification of Phenylpropanoids in Fig (*Ficus carica* L.) Leaves

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In this study, the phenylpropanoid composition and antioxidant activity of identified components in fig (*Ficus carica* L.) leaves were examined. Known polyphenols rutin, isoschaftoside, isoquercetin, and chlorogenic acid were identified. Furthermore, caffeoylmalic acid (CMA) was the most abundant polyphenol and was identified for the first time. CMA exhibited antioxidant activity similar to that of vitamin C or catechin. Psoralen and bergapten were identified as known furanocoumarins, with psoralen being the most abundant. Moreover, psoralic acid glucoside (PAG) was identified for the first time. As a precursor of psoralen, PAG content was equivalent to the psoralen content in moles. Notably, the content of these compounds varied between the five fig varieties, and the furanocoumarin and PAG contents varied more than that of the polyphenols. Further investigations concerning the influence of CMA and PAG on human health are necessary to elucidate functionalities of fig leaves.

Key words: fig, *Ficus carica*, leaf, polyphenol, furanocoumarin, antioxidant, radical scavenging activity, caffeoylmalic acid

INTRODUCTION

Fig (*Ficus carica* L.) is a deciduous tree of the Moraceae and is recognized as one of the oldest fruits along with apple and grape.[1,2] The fruits of figs are eaten raw and are processed into some products such as dry fruit and jam, whereas fig leaves have been used in folk medicine or as materials for Chinese medicine.[3–5] Furthermore, in recent research, antioxidant activity,[6,7] inhibition of increased postcibal blood glucose levels in patients with type I diabetes mellitus,[8] reduction of blood glucose and blood cholesterol levels in diabetic rats,[9,10] reduction of blood triglyceride levels in rats,[11] antipyretic effects in rats,[12] and inhibition of inflammation in rats,[13] were reported for fig leaves. However, the exact mechanisms and compounds involved in the aforementioned effects are still unknown.

Phenylpropanoids are secondary metabolites biosynthesized from phenylalanine and include polyphenols, coumarins, and lignans. In particular, polyphenols attract attention in regard to their influence on human health. Polyphenols may exhibit antioxidant activities, cancer suppression, and antiallergic action and may prevent cardiovascular disease[14–16]. Polyphenols including 3-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid, quercetin 3-*O*-rutinoside (rutin), and quercetin 3-*O*-glucoside have been identified in fig leaves.[17–20]

Fig leaves also contain furanocoumarins such as psoralen and bergapten.[21] Furanocoumarins inhibit the activities of detoxification enzymes in the liver[22] and have photosensitization effects[23]. In fact, photodermatitis have been caused by furanocoumarins from fig leaves.[21,24] Therefore, furanocoumarins from fig leaves may have an influence on human health.

As mentioned previously, secondary metabolites from plant materials such as polyphenols or furanocoumarins may have favorable and/or unfavorable influences on human health. The determination of phenylpropanoids in fig leaves is regarded as an important task in the search for new efficacy or for validation of known efficacy.

The objectives of this study were to identify the phenylpropanoids in fig leaves completely and to evaluate the potential of fig leaves to enhance the longevity of human health, and furthermore, to consider the importance of varietal characteristics in the composition and content. In this study, phenylpropanoids were identified by liquid chromatography-mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR). Furthermore, as one of functional index, the antioxidative activity was evaluated by electron spin resonance (ESR) and spectrophotometric methods.

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MATERIALS AND METHODS

Chemicals and Reagents. Chlorogenic acid, rutin, *p*-hydroxybenzoic acid ethyl ester, 2,2′-azobis (2-methylpropionamidine) dihydrochloride (AAPH), glucose, fluorescein, phosphate buffer, acetonitrile, methanol (MeOH), acetone, chloroform, dimethyl sulfoxide (DMSO), acetic acid, and potassium borate were purchased from Wako Pure Chemical Industries (Tokyo, Japan). Caffeoylmalic acid was purchased from ChromaDex (Irvine, CA, USA). Quercetin 3-*O*-(6″-*O*-malonyl)-glucoside, psoralen, bergapten, hypoxanthine (HPX), and superoxide dismutase (SOD, from bovine erythrocytes) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Schaftoside, isoschaftoside, isoquercetin, and astragalin were purchased from Extrasynthese (Genay, France). Trolox was purchased from MP Biomedicals (Solon, OH, USA). 5,5-Dimethyl-1-pyrroline-*N*-oxide (DMPO) and xanthine oxidase (XOD, from cow's milk) were purchased from Labotec (Tokyo, Japan). All reagents were used as received. Ultrapure water prepared by using a Milli-Q Gradient A10 system (Merck Millipore, Billerica, MA, USA) was used throughout this study.

Fig Leaves. Five fig varieties (Masui Dauphine, Horaishi, Kadota, Violette de Solies, and Negronne) cultivated in our orchard (Kawanishi, Hyogo, Japan) were used in this study. From the middle of June to early July, leaves at the nodes from the tip of the shoot to the fifth node were obtained. After washing with water, the leaves were cut with a ceramic kitchen knife into 1.5 cm squares. The cut leaves were packaged in polyethylene bag with nitrogen gas and stored in an MDF-393AT freezer (Panasonic Healthcare, Tokyo, Japan) at −80 ºC.

Preparation of Leaf Extract. The frozen leaves were freeze-dried by using an FDU-2100 freeze-dryer equipped with a DRC-3L dry chamber (Tokyo Rikakikai, Tokyo, Japan). Immediately after freeze-drying, leaves were pulverized to powder with a food mill (IFM-300SG, Iwatani, Osaka, Japan) four times for 10 s. Methanol or a mixture of water/methanol/acetone = 1:1:1 (v/v) was used as the extractant. Leaf powder (0.2 g) was placed in an Erlenmeyer flask, and 30 mL of extractant was added. The flask was shaken in a circular motion at 120 rpm using an NR-30 rotary shaker (Taitec, Saitama, Japan) for 3 h at room temperature. The atmosphere in the chamber of the food mill and in the flask was replaced by nitrogen. After centrifugation at 17000 *g* for 10 min with a CR21G II centrifuge (Hitachi High-Technologies, Tokyo, Japan), the supernatant was collected. The residue was resuspended in 10 mL of extractant and centrifuged again under the same conditions. This process was repeated and the collected supernatant was combined and made to be the volume of 50 mL by adding the extractant. The extract was filtered using a syringe filter (PTFE membrane, pore size = 0.45 μm) and stored at −20 ˚C.

Identification of Phenylpropanoids. *LC-DAD-MS/MS Analysis***.** Analysis was carried out according to the method described by Kammerer et al.[25] with some modifications in equipment, column and gradient program to reduce analysis time. An LC20ADXR HPLC with a diode array detector (DAD) system (Shimadzu, Kyoto, Japan) and a micrOTOF-Q II quadrupole-time-of-flight tandem mass spectrometer fitted with an ESI ion source (Bruker Daltonics, Billerica, MA, USA) were utilized. The UV–vis spectrum was recorded from 200 to 800 nm by DAD. A Synergi Hydro-RP column (particle size = 2.5 μm; 100 mm × 3 mm i.d., Phenomenex, Torrance, CA, USA) was employed for the separation of the phenylpropanoids. The mobile phase was (A) 2% acetic acid and (B) 0.5% acetic acid/acetonitrile = 1:1. The gradient began with 10% B and was varied to 24% B at 8 min, 30% B at 16 min, 55% B at 24 min, 100% B at 30 min, 100% B isocratic from 30 to 33.2 min, and 10% B from 34 to 36 min. The flow rate of the mobile phase was 0.4 mL/min. The temperature of the column oven was 40˚C, and the sample injection volume was 5 μL. Mass spectrometry was carried out using the following conditions: mass range, *m/z* 50–1000; spectra rate, 1 Hz; nebulizing gas, nitrogen (1.6 bar); drying gas, nitrogen (180˚C, 7 L/min); capillary voltage, −4500 V for positive ion and +2800 V for negative ion; hexapole RF, 100 Vpp; quadrupole ion energy, 5 eV; collision gas, nitrogen (1.6 Bar); collision energy, 10 eV; collision RF, 100 Vpp.

Separation of HPLC Peak Fractions. A semipreparative HPLC system equipped with a Delta 600 solvent pump (Waters, Milford, MA, USA) and AQUA C18 column (particle size = 5 μm; 250 × 10 mm i.d., Phenomenex) was used. The mobile phase was the same as that utilized for LC-DAD-MS/MS. The gradient started with 10% B and was varied to 24% B at 20 min, 30% B at 40 min, 55% B at 60 min, 100% B at 75 min, 100% B isocratic from 75 to 83 min, and 10% B from 84 to 90 min. The flow rate of the mobile phase was 4.7 mL/min. The temperature of the column oven was 30˚C. The extract was concentrated by evaporation, and 250 μL was injected into the system. The peaks that adsorbed at 320 nm were collected. The separated peak fractions were evaporated to remove the organic solvent. The dried fractions were dissolved in water and freeze-dried.

NMR Measurements. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz), distortionless enhancement by polarization transfer (DEPT), double quantum filtered correlated spectroscopy (DQF-COSY), heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple-bond connectivity (HMBC) NMR spectra were recorded with a JNM-ECA400 spectrometer (JEOL, Tokyo, Japan) in deuterated methanol (CD_3OD) using a 5 mm NMR tube. The chemical shifts were given on a δ (parts per million) scale with tetramethylsilane as an internal standard.

Analysis of Sugar in Glycosides. The freeze-dried HPLC peak fraction (1 mg) was dissolved in 5 mL of methanol, and HCl was added to reach 6 N. The mixture was heated at 90˚C

for 4 h to hydrolyze the glycosides. After neutralization with NaOH, the sugar released from the glycoside was labeled by an ABEE(4-amino benzoic acid ethyl ester) labeling kit (J-oil mills, Tokyo, Japan) and was analyzed by HPLC. An LC-10A HPLC system (Shimadzu) and XBridge Phenyl (particle size = 5 μm; 150 mm × 4.6 mm i.d., Waters) were used for the analysis of the ABEE-labeled sugar. The mobile phase was 0.2 M potassium borate buffer (pH 8.9)/acetonitrile = 93:7 (v/v). The flow rate was 1.0 mL/min. The column oven temperature was 30˚C. The injection volume was 10−30 μL, and the peak was detected by monitoring the absorbance at 305 nm.

Quantitative Determination of Phenylpropanoids. An 1100 system HPLC-DAD (Agilent Technologies, Santa Clara, CA, USA) was used for determination of the phenylpropanoids. The column, mobile phase, and analytical conditions for HPLC were the same as those utilized for LC-DAD-MS/MS. Caffeoylmalic acid was detected by absorbance at 320 nm. Rutin, psoralen, bergapten, and psoralic acid glucoside were detected by absorbance at 250 nm. The calibration curves were made from standard chemicals, except for that of psoralic acid glucoside, which was made from the separated material from the fig leaves. All measurements were performed in triplicate.

Evaluation of Antioxidative Activities. *Superoxide Anion Radical ($O_2^{\cdot-}$) Scavenging Activity.* Following the method described by Saito et al.,[26] $O_2^{\cdot-}$ generated from the reaction of HPX and XOD was measured by ESR spin trapping with DMPO as the spin trapping agent. The ESR measurement conditions (JES-FA100, JEOL) were as follows: microwave power, 4 mW; field sweep, 330.5-340.5 mT; field modulation frequency, 100 kHz; field modulation width, 0.07 mT; sweep time, 2 min; time constant, 0.1 s. Fifty microliters of 2 mM HPX was placed in a test tube, and 30 μL of DMSO, 50 μL of sample, 20 μL of 4.45 M DMPO were added. The mixture was added to 50 μL of 0.4 U/mL XOD and vortexed for 20 s. The mixture was transferred to an ESR spectrometry liquid cell, and the DMPO−OOH spin adduct was quantified 100 s after the addition of XOD. The measurements were performed in duplicate. The $O_2^{\cdot-}$ scavenging activity was expressed as superoxide dismutase (SOD)-like activity from the calibration curve of standard SOD. Additionally, IC_{50} values of samples were measured and the reaction rate constants with $O_2^{\cdot-}$ of samples (k_2) were calculated using eq 1, according to the method described by Mitsuta et al.[27]

$$k_2\ (M^{-1}s^{-1}) = k_1 \times [DMPO]/IC_{50} \quad (1)$$

k_1 is the reaction rate constant with DMPO and $O_2^{\cdot-}$. The literature value used for the calculation was 15.7 $M^{-1}s^{-1}$.[28] [DMPO] indicates the final concentration of DMPO, and the utilized value was 445 mM.

Oxygen Radical Absorbance Capacity (ORAC). The assay was carried out according to the method described by Dávalos et al.[29] with some modifications. In brief, 25 μL of the sample and 150 μL of phosphate buffer (1/15 M, pH 7.4) containing 100 nM fluorescein were placed in each well of a 96-well black, flat-bottom microwell plate (Thermo Fisher Scientific NUNC, Roskilde, Denmark). After preincubation at 37˚C for 30 min, 25 μL of 480 mM AAPH was added to the mixture using an onboard pipettor of plate reader (FLUOstar OPTIMA, BMG Labtech, Offenburg, Germany). From 270 s before the AAPH addition, the emission at 520 nm of each well was measured using a plate reader every 90 s for 60 intervals, with an excitation of 485 nm. The microwell plate was incubated at 37˚C during the measurements and was stirred prior to each measurement. The measurements were performed in triplicate. The area under curve (AUC; calculated as an integral of the fluorescence intensity during measurement) of each sample was calculated, and activity was expressed as the trolox equivalent (μmol TE/g).

RESULTS AND DISCUSSION

Identification of Phenylpropanoids in Fig Leaves. HPLC chromatograms of the MeOH and water/MeOH/acetone = 1:1:1 (v/v) extracts from Masui Dauphine leaves are shown as an example in **Figure 1**.

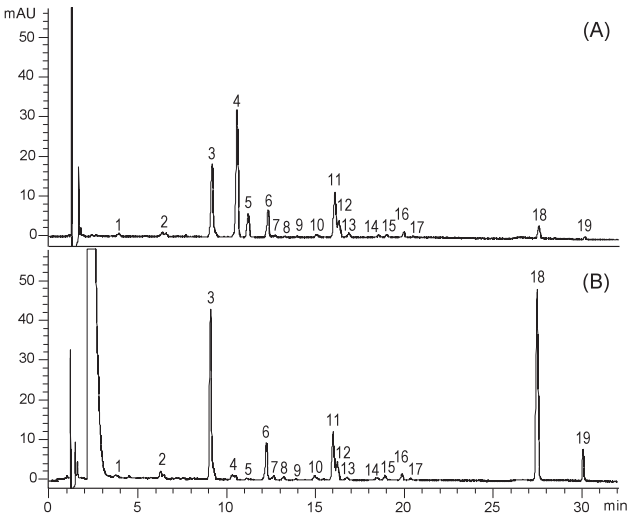

Figure 1. HPLC separation of phenylpropanoids from fig leaves: (A) MeOH extract; (B) water/MeOH/acetone = 1:1:1 extract of Masui Dauphine leaves. Peak detection was carried out at 280 nm. For peak assignment see Table 1.

The chromatogram profile differed depending on the extractant. Peaks 4 and 5 were reduced in the water/MeOH/acetone extract. Peaks 3, 18, and 19 of the water/MeOH/acetone extract were bigger than those in the MeOH extract. The LC-DAD-MS/MS results and corresponding identification of each peak are shown in **Table 1**. These peaks were detected from the five varieties examined. The intensities of the precursor ion (molecular ion)

of peak 3, 4, and 5 detected in negative ion mode were >10 times than those detected in the positive ion mode. On the other hand, the intensities of the precursor ions of peaks 18 and 19 in the positive ion mode were about 30-fold higher than in the negative mode. In other peaks, the differences of the intensity of precursor ion were 0.7- to 1.2-fold between positive ion mode and negative ion mode.

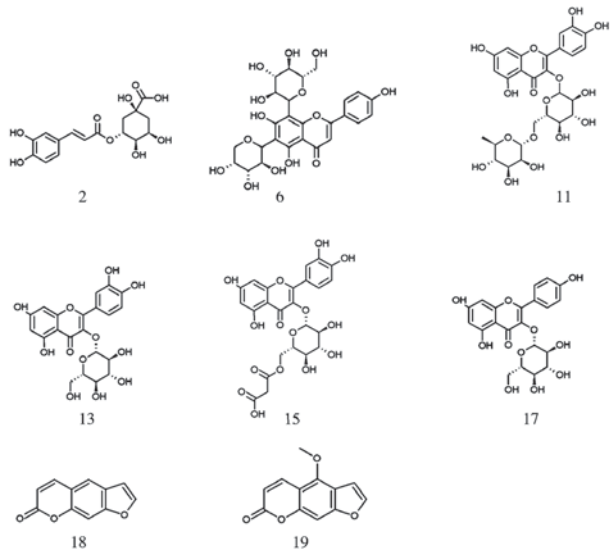

Figure 2. Identified compounds in fig leaf extract (except compounds 3 and 4): compound 2, chrologenic acid; compound 6, isoschaftoside; compound 11, rutin; compound 13, isoquercetin; compound 15, quercetin 3-O-(6”-O- malonyl)-glucoside; compound 17, astragalin; compound 18, psoralen; compound 19, bergapten.

Compound 2 showed a retention time (RT) of 6.5 min in HPLC, a molecular ion with *m/z* 353 $[M-H]^-$, and a product ion with *m/z* 191 in MS/MS. The formula of the molecular ion was calculated to be $C_{16}H_{17}O_9$. By matching the data with those of the standard chemical, compound 2 was identified as chlorogenic acid (**Figure 2**).

Compound 3 afforded a RT of 9.3 min in HPLC, a molecular ion with *m/z* 295 $[M-H]^-$, and product ions with *m/z* 133 (100%), 115 (85%), and 135 (75%) in MS/MS. The formula of the molecular ion was calculated to be $C_{13}H_{12}O_8$. These values matched with those of caffeoylmalic acid (CMA). Furthermore, NMR was used to confirm the planar structure (**Figure 3**; **Table 2**). Therefore, compound 3 was identified as CMA.

Compound 4 afforded a RT of 10.3 min in HPLC analysis, a molecular ion with *m/z* 365 $[M-H]^-$, and product ion with *m/z* 159 in MS/MS. The formula of the molecular ion was calculated to be $C_{17}H_{17}O_9$. From the data obtained by NMR (**Table 3**), compound 4 was found to have the planar structure shown in

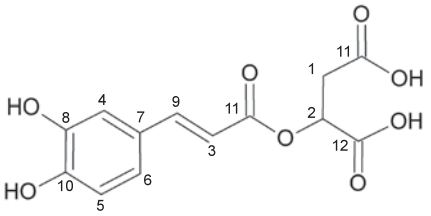

Figure 3. Chemical structure of compound 3. For atom numbering see Table 2.

Table 1. LC-MS Data of Phenylpropanoids from Fig Leaf Extract.

Peak	compound	retention time (min)	MS precursor ion *m/z*	MS/MS product ions *m/z* (%)
		Negative Ion Mode $[M-H]^-$		
1	unknown	4.0	343	137 (100), 181 (47)
2	chlorogenic acid	6.5	353	191 (100)
3	caffeoylmalic acid (CMA)	9.3	295	133 (100), 115 (85), 135 (75)
4	psoralic acid-glucoside (PAG)	10.3	365	159 (100)
5	unknown	11.3	395	201 (100), 189 (65)
6	isoschaftoside	12.4	563	353 (100), 383 (78), 443 (56)
7	unknown	12.7	447	357 (100), 327 (90)
8	unknown	13.3	163	119 (100), 98 (60)
9	unknown	14.0	423	201 (100), 89 (25)
10	unknown	15.0	193	134 (100)
11	rutin (quercetin 3-*O*-rutinoside)	16.1	609	300 (100)
12	unknown	16.3	365	159 (100)
13	isoquercetin (quercetin 3-*O*-glucoside)	16.9	463	300 (100)
14	unknown	18.3	467	ND
15	quercetin 3-*O*-(6″-*O*-malonyl)-glucoside	19.1	549	300 (100), 505 (30)
16	unknown	20.1	593	285 (100)
17	astragalin (kaempferol 3-*O*-glucoside)	20.5	447	284 (100)
		Positive Ion Mode $[M+H]^+$		
18	psoralen	27.7	187	131 (100)
19	bergapten (5-methoxypsoralen)	30.3	217	202 (100), 174 (45)

Table 2. NMR Spectroscopic Data of Compound 3 (δ in CD_3OD)

	^1H		^{13}C
	δ	multiplicity (*J* in Hz)	δ
1	2.911	m	37.47
2	5.453	dd (3.7, 8.7)	70.64
3	6.313	d (15.6)	114.49
4	7.055	d (2.3)	115.19
5	6.776	d (8.2)	116.55
6	6.958	dd (1.8, 8.2)	123.20
7			127.73
8			146.87
9	7.586	d (16.0)	147.75
10			149.79
11			168.24
12			173.42

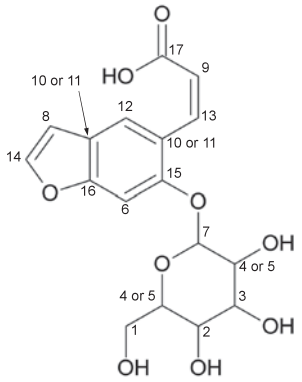

Figure 4. Chemical structure of compound 4. For atom numbering see Table 3.

Figure 4. Psoralen and sugar were generated by the hydrolysis of the compound 4 with hydrochloric acid. This sugar was identified as glucose by HPLC analysis using the ABEE labeling method. As such, compound 4 was determined to be (2*Z*)-3-[6-(*β*-D-glucopyranosyloxy)-1-benzofuranyl]-2-propenoic acid (psoralic acid -glucoside).

Compound 5 exhibited a RT of 11.3 min in HPLC, a molecular ion with *m/z* 395 $[M-H]^-$, and product ions with *m/z* 201 (100%) and 189 (65%) in MS/MS. The formula of the molecular ion was calculated to be $C_{18}H_{19}O_{10}$. The chemical composition was equivalent to psoralic acid glucoside (PAG) with an additional methoxy group (CHO_3−, FW 31.03). Furthermore, bergapten and glucose were generated by hydrolysis of the compound 5 fraction with hydrochloric acid. Thus, compound 5 was determined to be a PAG derivative with an additional methoxy group in the aglycone.

Compound 6 showed a RT of 12.4 min in HPLC, a molecular ion with *m/z* 563 $[M-H]^-$, and product ions with *m/z* 353 (100%), 383 (78%), and 443 (56%) in MS/MS. The formula of the molecular ion was calculated to be $C_{26}H_{27}O_{14}$. By matching the data to that of a standard chemical, compound 6 was identified as isoschaftoside (**Figure 2**). Schaftoside, the structural isomer of isoschaftoside, afforded a different product ion pattern (*m/z* 353, 100%; 383, 83%; 443, 72%), but the same RT. Thus, it is possible that a small amount of schaftoside was present in fig leaves.

Table 3. NMR Spectroscopic Data of Compound 4 (δ in CD_3OD)

	^1H		^{13}C
	δ	multiplicity (*J* in Hz)	δ
1	3.713	dd (119, 5.0)	62.53
	3.905	dd (11.9, 1.8)	
2	3.407−3.430	m	71.35
3	3.455−3.536	m	74.96
4	3.455−3.536	m	78.14
5	3.455−3.536	m	78.27
6	7.380	s	99.98
7	4.960	d (7.3)	103.31
8	6.754	d (1.4)	107.55
9	5.971	d (12.8)	120.82
10			122.96
11			123.13
12	7.859	s	123.63
13	7.371	d (12.4)	139.67
14	7.664	d (2.3)	146.58
15			155.04
16			157.31
17			170.76

Compound 11 showed a RT of 16.1 min in HPLC, a molecular ion with *m/z* 609 $[M-H]^-$, and product ion with m/z 300 in MS/MS. The formula of the molecular ion was calculated to be $C_{27}H_{29}O_{16}$. By comparison to the standard, compound 11 was identified as rutin (quercetin 3-*O*-rutinoside, **Figure 2**).

Compound 12 showed a RT of 16.3 min in HPLC and the same molecular ion as PAG in MS/MS. The formula of the molecular ion was the same as that of PAG ($C_{18}H_{19}O_{10}$). Therefore, compound 12 was thought to be an isomeric form of PAG.

Compound 13 showed a RT of 16.9 min in HPLC, a molecular ion with *m/z* 463 $[M-H]^-$, and product ion with *m/z* 300 in MS/MS. The formula of the molecular ion was calculated to be $C_{21}H_{19}O_{12}$. By comparison with the standard, compound 13 was identified as isoquercetin (quercetin 3-*O*-glucoside, **Figure 2**).

Compound 15 presented a RT of 19.1 min in HPLC, a molecular ion with *m/z* 549 $[M-H]^-$, and product ions with *m/z* 300 (100%) and 505 (30%) in MS/MS. The formula of the molecular ion was calculated to be $C_{24}H_{21}O_{15}$. Compound 15 was identified as quercetin 3-*O*-(6″-*O*-malonyl)-glucoside (**Figure 2**) by comparison with the standard.

Compound 17 exhibited a RT of 20.5 min in HPLC, a molecular ion with *m/z* 447 $[M-H]^-$, and a product ion with *m/z* 284 in MS/MS. The formula of the molecular ion was calculated

Table 4. Major Phenylpropanoid Content (Milligrams per Gram DW)[a] from Leaves of 5 Fig Varieties

variety	CMA[b]	PAG[b]	rutin	psoralen	bergapten
Masui Dauphine	16.6 ± 0.6	18.0 ± 1.4	9.7 ± 0.4	10.2 ± 0.5	1.4 ± 0.1
Horaishi	17.6 ± 0.3	6.7 ± 0.2	11.3 ± 0.2	3.8 ± 0.0	0.8 ± 0.0
Kadota	18.7 ± 0.1	14.0 ± 0.6	8.7 ± 0.1	7.0 ± 0.1	1.1 ± 0.0
Violette de Solies	20.6 ± 0.6	42.2 ± 0.9	11.4 ± 0.4	23.0 ± 0.5	5.0 ± 0.1
Negronne	14.4 ± 0.7	15.4 ± 0.6	7.2 ± 0.4	9.2 ± 0.3	1.1 ± 0.0

[a] Mean ± standard deviation (n=3). [b] CMA, caffeoylmalic acid, PAG, psoralic acid-glucoside.

to be $C_{21}H_{19}O_{11}$. By matching to the standard, compound 17 was identified as astragalin (kaempferol 3-*O*-glucoside, **Figure 2**).

Compound 18 presented a RT of 27.7 min in HPLC, a molecular ion with *m/z* 187 $[M+H]^+$, and product ion with *m/z* 131 in MS/MS. The formula of the molecular ion was calculated to be $C_{11}H_7O_3$. Compound 18 was identified as psoralen (**Figure 2**) by comparison to the standard.

Compound 19 showed a RT of 30.3 min in HPLC, a molecular ion with *m/z* 217 $[M+H]^+$, and product ions with *m/z* 202 (100%) and 174 (45%) in MS/MS. The formula of the molecular ion was calculated to be $C_{12}H_9O_4$. By matching to the standard, compound 19 was identified as bergapten (5-methoxypsoralen, **Figure 2**).

Phenylpropanoid Composition in Fig Leaves. The amounts of some identified phenylpropanoids with comparatively large peak areas in the UV chromatograms were measured for five varieties including Masui Dauphine and Horaishi, the major varieties in Japan (**Table 4**).

Among the polyphenols, the CMA content ranged from 14.4 (Negronne) to 20.6 mg/g dry weight (DW) (Violette de Solies). The rutin content ranged from 7.2 (Negronne) to 11.4 mg/g DW (Violette de Solies), which was around 50−60% of the CMA content. CMA was the most abundant polyphenol in the fig leaves. CMA was reported to be found in plants such as *Chelidonium majus* L., *Corydalis lutea* L.,[30] lettuce (*Lactuca sativa L.*),[31] and nettle (*Urtica dioica* L.).[32] However, we found that fig leaves contained CMA for the first time. The second most abundant polyphenol, rutin, has been identified in many other plants. Additionally, some studies detected rutin in fig leaves.[17,18,20] Isoschaftoside was also previously identified in fig leaves,[33] and it was also considered a major polyphenol in addition to CMA and rutin. The HPLC-UV peak areas of other polyphenols were considerably small.

Among the furanocoumarins, the psoralen content ranged from 3.8 (Horaishi) to 23.0 mg/g DW (Violette de Solies). The bergapten content was only 12−22% of the psoralen content in the five varieties. The PAG content ranged from 6.7 (Horaishi) to 42.2 mg/g DW (Violette de Solies), which was approximately twice the psoralen content. Psoralen was regarded as the primary furanocoumarin in fig leaves. Because psoralen and glucose were generated in the hydrolysis of PAG, PAG may be a precursor of psoralen in fig leaves. PAG was identified in *Psoralea plicata* L.[34] and *Psoralea corylifolia* L.[35] We found that fig leaves contained PAG for the first time. One mole of psoralen (molecular weight (MW) 186) and glucose (MW 180) was generated by the hydrolysis of one mole of PAG (MW 366).[35] In fig leaves, the PAG content was approximately equivalent to the psoralen content by mole. Therefore, the potential psoralen content in fig leaves may be increased by approximately 2 times. Furthermore, a precursor of bergapten was also detected. Kasajima et al. reported a similar compound from methanol extracts of fig leaves.[18]

Varietal Characteristics of Phenylpropanoid Composition. Violette de Solies contained the greatest amounts of CMA, rutin, psoralen, bergapten, and PAG in the leaves among the five varieties of fig investigated in this study. The CMA and rutin contents differed between 1.4−1.6 times among the five fig varieties. Moreover, the furanocoumarin and PAG contents of the Violette de Solies leaves were more than double those of Masui Dauphin.

It was reported that large doses (15 mg/person) of furanocoumarins administered orally induced photodermatitis in humans.[36] Therefore, caution should be exercised with oral ingestion of furanocoumarins from fig leaves. The leaves of Violette de Solies may have more risks for human health than other varieties with high concentrations of furanocoumarin and PAG. Horaishi is thought to be a low-risk variety because the leaves contain a lower amount of furanocoumarins and PAG.

There are a large number of fig varieties, so investigation of other varieties is necessary to elucidate the varietal characteristics of phenylpropanoid composition in fig leaves. If a variety with a low furanocoumarin-related compound content and high polyphenol content is found, it may be suitable for consumption.

Antioxidant Activity of Phenylpropanoids in Fig Leaves. Because CMA and PAG were the most abundant phenylpropanoids in the fig leaves, their $O_2^{\cdot-}$ scavenging activities were measured by ESR spin trapping. The SOD-like activities of CMA and PAG separated from fig leaves were 70604 and 3776 U/g, respectively.

The $O_2^{\cdot-}$ scavenging activities at various concentrations and IC_{50} values were measured, and the reaction rate constants

Table 5. $O_2^{\cdot-}$ Scavenging Activity and ORAC of CMA, Rutin, and PAG from Fig Leaves

	$O_2^{\cdot-}$ scavenging activity[a]		ORAC[b]	
compound	IC_{50} (μM)	k_2 ($M^{-1}s^{-1}$)	μmol TE[c]/g DW	μmol TE/μmol
CMA (standard)	12.0	5.8×10^5	12043 ± 258	3.57 ± 0.08
CMA (separated[d])	33.2	2.1×10^5	11130 ± 558	3.30 ± 0.17
rutin (standard)	17.4	4.0×10^5	14856 ± 2229	9.07 ± 0.36
PAG (separated)	–[e]	–	2156 ± 452	0.79 ± 0.17

[a]Mean (n=2). [b]Mean ± standard deviation (n=2). [c]Trolox equivalent. [d]Separated from fig leaves of Masui Dauphine variety . [e]Not measured because of low activity.

with $O_2^{\cdot-}$ (k_2) were calculated for CMA (standard chemical and separated material) and rutin (standard chemical) (**Table 5**).

The IC_{50} values of standard and separated CMA were 12 and 33.2 μM respectively. The k_2 values of standard and separated CMA were 5.8×10^5 and 2.1×10^5 $M^{-1}s^{-1}$ respectively. Impurities in the CMA separated from the fig leaves may have led to the lower k_2 value as compared to the standard compound. The $O_2^{\cdot-}$ scavenging activity of rutin (standard chemical; IC_{50} = 17.4 μM and $k_2 = 4.0\times10^5$ $M^{-1}s^{-1}$) was equivalent to that of CMA.

CMA is the compound that coupled with caffeic acid and malic acid. The k_2 values of caffeic acid and chlorogenic acid (caffeoylquinic acid) were reported to be 9.6×10^5 and 16.7×10^5 $M^{-1}s^{-1}$, respectively.[37] Rutin is the glycoside of quercetin with rutinose. The $O_2^{\cdot-}$ scavenging activity of rutin is approximately equivalent to that of quercetin.[38] The k_2 values of catechin, epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate were reported to be $(0.4-0.6) \times 10^5$, $(0.5-0.7) \times 10^5$, $(3.8-4.3) \times 10^5$, $(4.1-7.7) \times 10^5$ and $(7.3-14.2) \times 10^5$ $M^{-1}s^{-1}$, respectively.[38,39] Vitamin C has a high $O_2^{\cdot-}$ scavenging activity with a reported reaction rate constant of $(2.7-3.5) \times 10^5$ $M^{-1}s^{-1}$.[40–42] Therefore, the $O_2^{\cdot-}$ scavenging activities of CMA and rutin were higher than or equivalent to those of other catechins and vitamin C, but lower than those of chlorogenic acid and epigallocatechin gallate.

ORAC is one method that is used to evaluate the suppression of peroxy radicals in *vitro*. The ORAC values by weight of CMA (standard chemical and separated material), rutin, and PAG were 12043, 11130, 14856, and 2156 μmol TE/g, respectively (**Table 5**). The ORAC values by mole of the aforementioned compounds were 3.57, 3.30, 9.07, and 0.79 μmol TE/μmol respectively. The ORAC value of PAG was <25% that of CMA, and it was a tendency similar to the SOD-like activity.

The value of the separated CMA from fig leaves was moderately lower than that of the standard CMA. Impurities in the separated CMA may have led to the lower activity. CMA and rutin showed equivalent ORAC values per weight. However, rutin exhibited an ORAC value that was approximately 3 times higher than that of CMA per mole. The difference in molecular structure, including the number or placement of binding hydroxyl groups, may have led to a different affinity with the radical derived from AAPH among the compounds. Ou et al. reported the ORAC values of caffeic acid, chlorogenic acid, quercetin, rutin, and catechin to be 4.37, 3.14, 7.28, 6.01, and 6.76 μmol TE/μmol, respectively.[43] The ORAC level of CMA was equivalent to those of caffeic acid and chlorogenic acid and was moderately lower than those of quercetin, rutin, and catechin. The ORAC level of rutin was higher than those of caffeic acid and chlorogenic acid and was equivalent to those of quercetin and catechin.

In fig leaves, CMA and rutin were the major polyphenols and were found in relatively high concentrations. Therefore, both compounds are thought to contribute to the antioxidant activity. PAG is the most abundant phenylpropanoid in fig leaves, but the $O_2^{\cdot-}$ scavenging activity and ORAC of PAG were significantly lower in comparison with CMA and rutin. Isoschaftoside was the third most abundant compound, but the contribution to antioxidant activity was small because apigenin, the aglycon of isoschaftoside, showed low $O_2^{\cdot-}$ scavenging activity (data abbreviated). Similarly, because the contents of other polyphenols (chlorogenic acid and quercetin glycosides) were significantly less than that of CMA and rutin, the contribution to $O_2^{\cdot-}$ antioxidant activity was thought to be small.

Potential of Fig Leaves as Functional Food Material. Many papers have described the various physiological functions of polyphenols. The reduction effect of rheumatalgia was reported in CMA-rich nettle extracts.[44] Other caffeic acid derivatives, such as chlorogenic acid, were shown to reduce obesity, diabetes, and inflammation in animals.[45–48] The palliation of cerebral ischemia disorder in rats[49] and mitigation of convulsions induced by kainic acid in mice[50] were reported as effects of rutin. The fruit of *Psoralea corylifolia* L. containing PAG composes “Buguzhi”, a euphoriant in Chinese medicine. Ossiferous promotion was reported with Buguzhi extracts,[51] but the exact effect of PAG on human health is unknown. When fig leaf extract is taken orally, the amount of furanocoumarins may increase in the stomach. When using fig leaves as food, it is

necessary to examine how PAG will be hydrolyzed in the human body.

Fig leaf extracts exhibited high antioxidant activities, but the activity was less than that of green tea or coffee according to the phenylpropanoid content found in this investigation. To elucidate the functionalities of fig leaves, further studies in the influence of CMA and PAG on human health are necessary. Moreover, a greater number of fig varieties should be evaluated in regard to phenylpropanoid content and antioxidant activity of the extracts. Similarly, the influence of various processing and extraction methods on the residual rate of the components in fig leaves should also be investigated.

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