Profiles of Potentially Antiallergic Flavonoids in 27 Kinds of Health Tea and Green Tea Infusions

Masatake Toyoda,*,† Keiko Tanaka,‡ Kaori Hoshino,‡ Hiroshi Akiyama,† Akio Tanimura,‡ and Yukio Saito†

Division of Foods, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158, Japan, and Showa Woman's University, 1-7 Taishido, Setagaya-ku, Tokyo 154, Japan

Seven flavonoid profiles, some of which were reported to show antiallergic activity *in vitro*, were surveyed in 28 kinds of tea. Flavonoids were extracted with hot water, hydrolyzed to aglycons, and determined by HPLC. The hydrolysis time needed to obtain the maximum for each aglycon was examined. Flavonols quercetin and kaempferol were found in 17 kinds of tea infusions, as well as Japanese green tea infusions. Myricetin was found in jasmine and pu-erh tea infusions. A flavon, luteolin, was found in 5 kinds of tea infusions including great plantain tea. Apigenin was found in 4 kinds of tea infusions including perilla leaf tea, and scutellarein was found in 3 kinds of tea infusions. Scutellarein, which is a potent antiallergic and low cytotoxic flavonoid, is found in great plantain tea and was detected in perilla leaf and safflower tea infusions at concentrations of approximately 2 mg/g and 0.3 mg/g in dried whole tea leaves, respectively. The presence of scutellarein in both samples was confirmed by LC/MS.

Keywords: *Tea; green tea; flavonoids; quercetin; kaempferol; scutellarein*

INTRODUCTION

In Japan, the demand for various dried tea leaves or flower or treated seed such as green tea and other teas including oolong tea, the infusions of which are drunk to keep good health (these are called health teas in Japan), is growing. The nutritional and therapeutic values of green tea have been reviewed (Hara, 1995a). It was found that green tea had a beneficial effect on cardiovascular and cerebrovascular diseases, a hypocholesterolemic effect, antitumor properties, and antioxidant, antigenotoxic, and anticarious activities; it also significantly decreased blood cholesterol levels.

Particular emphasis has been placed on a group of polyphenolic compounds, because polyphenols and their products are responsible for the above-mentioned unique characteristics of teas (Hara, 1995a; Elliott, 1994) such as anti-inflammatory properties and antibacterial properties. The polyphenols in tea are mainly flavanols, flavonols, flavonol glycosides, flavons, and depsides. Fourteen flavonols and flavonol glycosides and 19 flavons and flavon glycosides have been detected in green tea (Hara, 1995b).

The inhibitory effects of hot water tea extracts on histamine release from rat peritoneal mast cells and their hyaluronidase activity has been examined, and tea extracts were shown to have antiallergic effects (Maeda, 1989, 1990). Furthermore, structure−activity studies of flavonoids as inhibitors of hyaluronidase showed that luteolin, apigenin, kaempferol, and silybin have antiallergic activity *in vitro* (Kuppusamy, 1990). Histamine release tests using rat basophilic leukemia (RBL-2H3) cells showed that scutellarein potently inhibits histamine release and has low cytotoxicity (Kawasaki, 1994).

However, there are not many data in the literature on the content of the above-mentioned flavonoids in teas except in widely and commonly consumed black tea and green tea. Our objective is to know the content of those seven well-known flavonoids in health tea that is being sold in Japan. Hertog et al. (1993) examined the contents of three kinds of flavonoids in three kinds of tea including Japanese green tea. Engelhardt et al. (1993) examined the apigenin and luteolin glycoside contents in three kinds of tea.

As five widespread flavonoid aglycons were successfully determined by high-performance liquid chromatography (HPLC) (Hasler and Sticher, 1990), we used HPLC to determine the amounts of the three major flavonols, quercetin, kaempferol, and myricetin, and the four major flavons, scutellarein, apigenin, baicalein, and luteolin. After the most suitable hydrolysis time had been decided for each tea, we compared their levels in 28 kinds of tea infusions.

MATERIALS AND METHODS

Materials. The following teas were used: (1) *Angelica keiskei* leaf tea (ashitabacha is the Japanese name); (2) *Gynostemma pentaphyllum* tea (amachazurucha); (3) gingko leaf (*Gingko biloba*) tea (ichoucha); (4) great plantain (*Plantago major*) tea (oobakocha/shazensou); (5) ground ivy (*Glechoma hederacea*) tea (kakidoushicha); (6) Japanese persimmon (*Diospyros kaki*) leaf tea (kakinohacha); (7) Chrysanthemum flower (*Chrysanthemum morifolium*) tea (kikubanacha); (8) *Gymnema sylvestre Schult.* tea (gimunemacha); (9) guava (*Psidium guajava* L.) tea (guavacha); (10) Chinese matrimony vine (*Lycium chinense* Mill.) tea (kukocha); (11) low striped bamboo leaf (*Sasa albo-marginata*) tea (kumazasacha); (12) mulberry leaf (*Morus bombycis* Koidz.) tea (kuwanohacha); (13) perilla (*Perilla frutescens*) leaf tea (shisonohacha); (14) jasmine (*Jasminum sambac*) flower tea (jasumincha); (15) field horsetail (*Equisetum arvense*) tea (suginacha); (16) *Rubus suavissimus* tea (Chinese tencha); (17) *Houttuynia cordata* tea (dokudamicha); (18) gutta-perca tree (*Eucommia ulmoides)* leaf tea (tochucha); (19) East Indian lotus (*Nelumbo nucifera*) tea (hasucha); (20) adlaly (*Coix lachrymajobi*) tea (hatomugicha); (21) banava (*Legerstroemia speciosa*) tea (banavacha); (22) Japanese medlar (*Eriobotrya japonica*) leaf tea (biwanohacha); (23) pu-erh (*Camellia sinensis*, Chinese microbial-fermented

* Author to whom correspondence should be addressed [e-mail toyoda@nihs.go.jp; fax +81(3)3707 6950].
† National Institute of Health Sciences.
‡ Showa Woman's University.

S0021-8561(97)00024-1 CCC: $14.00

dark tea) tea (puarcha); (24) safflower (*Carthamus tinetorius*) tea (benibanacha); (25) mugwort (*Artemisia princeps*) tea (yomogicha); (26) eucalyptus (*Eucalyptus globulus*) tea (yuukaricha); (27) rooibos (*Aspalathus linearis*) tea (ruibosucha); (28) Japanese green tea (*Camellia sinensis*) (ryokucha).

All tea samples were purchased at retail shops in Tokyo and Yokohama between August and December 1995.

HPLC grade apigenin, quercetin dihydrate, kaempferol, myricetin, luteolin, and baicalein were purchased from Funakoshi Co. Ltd. (Tokyo), and scutellarein was kindly supplied by Dr. Kawasaki, Foods and Drugs Safety Center (1988).

Methods of Analysis. After large tea leaves were cut into small pieces, the tea leaf or powder (5.0 g) was extracted with 200 mL of boiling distilled water at more than 95 °C, gently stirred for 5 min, filtered through glass filter No. 17-G 3 (Maeda Ltd. Co., pore size 40−100 μm, thickness 5 mm, diameter 70 mm), transferred into a 200 mL measuring flask, and made up to 200 mL with water. Then 100 mL of this was concentrated to approximately 10 mL using a rotary evaporator and made up to 10 mL with water, and 10 mL of methanol was added. One milliliter of the mixture was transferred to a 15 mL reaction test tube with a stopper, 0.25 mL of 6 N HCl was added, and the mixture was kept at 90 °C for between 5 min and 7 h. After cooling in an ice bath, the mixture was made up to 10 mL with water and extracted twice with 40 and 30 mL of ethyl acetate. The organic layer was washed with a small amount of water and dried on anhydrous Na_2SO_4, then it was concentrated and dried using a rotary evaporator and dissolved in 500 μL of methanol.

HPLC Analysis. The resulting aglycons were quantified by RP-HPLC in a precolumn of Inertsil ODS-3 (C_{18}, 4.0 × 10 mm, particle size 5 μm) and in a separative column of Inertsil ODS-3 (C_{18}, 4 5 × 250 mm, particle size 5 μm) using 0.5% H_3PO_4/methanol (1/1, v/v) as the mobile phase, injection volume of 20 μL, flow rate of 1 mL/min, oven temperature of 40 °C, and UV detection at 349 nm with the reference at 550 nm. The threshold was 1.0 mAU, and peak width was 0.1 min. Peak identity was confirmed using a photodiode array detector to record the UV spectra of the flavonoids in the samples online between wavelengths of 190 and 500 nm. A Hewlett-Packard HP9000A high-performance liquid chromatograph was used. All determinations were carried out in triplicate, and each flavonoid was identified by comparison with a standard absorption curve. The detection limit for flavonoids was approximately 0.01 mg/g, but the confirmation limit of the UV curve was between 0.03 and 0.05 mg/g depending on the tea.

Thermospray Mass Spectrometry. Scutellarein was analyzed in positive mode by an atomic pressure thermospray interface (Finnigan APCI) linked to a mass spectrometer (Finnigan 4600 quadrupole). Analysis was performed at 150 °C of capillary temperature, 500 °C of vaporized temperature, and 1000 V of ion multivoltage in 65:35 methanol/water (v/v) using TSK gel ODS-80Ts (4.6 × 150 mm) column. Both protonated and methanol adducted ions were obtained.

RESULTS AND DISCUSSION

Under our chromatographic conditions, seven kinds of flavonoids were clearly separated as shown in Figure 1. A detection wavelength of 349 nm was selected because rather high responses of kaempferol, apigenin, and baicalein were obtained depending on the molar coefficient of absorption at this wavelength. Two extraction procedures for flavonoids from tea were compared. The contents of three kinds of flavonoid, scutellarein, luteolin, and apigenin, in the extract were compared using methanol, hot water, and great plantain (shazensou) tea leaves (4), as this tea is known to contain these flavonoids (Miyaichi, 1989; Nishibe, 1995). Methanol extraction yielded 3.2, 0.34, and 0.21 mg/g of these flavonoids from these dried tea leaves, respectively, and 2.5, 0.35, and 0.23 mg/g when extracted with hot water. Both methods gave similar results except

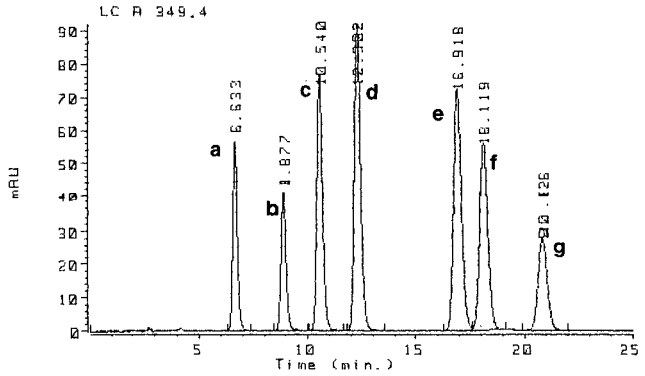

Figure 1. Chromatogram of seven kinds of standard flavonoids monitored by UV detector. Peaks: (a) myricetin, (b) scutellarein, (c) quercetin, (d) luteolin, (e) kaempferol, (f) apigenin, and (g) baicalein. HPLC conditions: precolumn, Inertsil ODS-3 (C_{18}); column, Inertsil ODS-3 (C_{18}); mobile phase, 0.5% H_3PO_4/MeOH (1/1,v/v); column temperature, 40 °C; injection volume, 20 μL; detection wavelength, 349 nm; flow rate, 1 mL/min.

Table 1. Optimum Hydrolysis Time (Hours) for Each of the Aglycon Flavonoids in Tea Infusions

	flavonoids[a]					
tea no.	Myr	Que	Kae	Scu	Lut	Api
1, 2, 3, 8, 17		0.25	0.25			
9, 12, 14, 18, 19, 21, 22, 23, 28	0.5	0.5	0.5			
26		1.0				
16		1.0	0.5			
6, 7, 10, 15		1.0	1.0		1.0	1.0
27		1.0	2.0			
24			0.5	5.0		
4				3.0	3.0	3.0
5					5.0	
11	5.0	5.0	5.0		5.0	
13				5.0	6.0	6.0

[a] Myr, myricetin; Que, quercetin; Kae, kaempferol; Scu, scutellarein; Lut, luteolin; Api, apigenin.

the amount of scutellarein was a little higher in the methanol extract. Accordingly, we used the hot water extraction method as it may provide levels similar to the actual dietary levels of flavonoids taken in from tea.

As the completeness of hydrolysis largely depends on the type of glycoside, we determined the optimum hydrolysis conditions for the teas, as described by Hertog et al. (1993). That is, after the hydrolysis of each tea infusion for 5, 10, 15, 30, and 60 min, and 2, 3, 4, 5, 6, and 7 h, seven kinds of flavonoids were determined by HPLC, the time course of flavonoid peaks appearance on chromatograms were recorded, and the time needed to reach maximum concentration was decided. Table 1 shows the optimum hydrolysis times for each flavonoid observed in 24 kinds of tea. In the case of ashitabacha (1) and 11 other teas, a hydrolysis time of 15 or 30 min was sufficient to obtain the corresponding flavonoids, but the teas containing scutellarein glycoside needed longer hydrolysis times to obtain the corresponding aglycon flavonoids, the reason for which is apparently due to the difference of sugar binding sites in these flavonoid aglycons. The recovery of quercetin, kaempferol, scutellarein, luteolin, and apigenin from great plantain leaf tea infusions spiked at 1.0 mg/g was >90%.

Table 2 shows the amount of 6 flavonoids in 27 kinds of health tea and Japanese green tea. Numbers in the same samples represent samples purchased from different companies. No baicalein was found in any of the teas. No flavonoid peaks tested were detected in mugwort tea (26) and adlay tea (20) infusions, although the

Table 2. Amount (Milligrams per Gram) of 6 Flavonoids in 27 Kinds of Health Tea and Green Tea

	flavonols[a,b]			flavons[a,b]		
tea	Myr	Que	Kae	Scu	Lut	Api
Angelica keiskei leaves (ashitabacha)		0.03 ± 0.04[c]				
Gynostemma pentaphyllum leaves (amachazurucha)		1.80 ± 0.31	0.90 ± 0.17			
gingko leaves (ichoucha)		0.18 ± 0.03	0.17 ± 0.01			
great plantain leaves (oobakocha)			0.02 ± 0	2.08 ± 0.11		
ground ivy leaves (kakidoushicha)					0.08 ± 0	
Japanese persimmon leaves (kakinohacha) 1		1.05 ± 0.12	1.27 ± 0.13			
2		0.60 ± 0.04	0.56 ± 0.08			
chrysanthemum flower (kikubanacha)					2.51 ± 0.67	
gymnema (gimunemacha) 1		1.79 ± 0.21	1.45 ± 0.27			
2		1.73 ± 0.22	0.72 ± 0.09			
3		3.23 ± 0.12	1.52 ± 0.08			
guava (guabacha)		1.09 ± 0.08	0.04 ± 0.03			
Chinese matrimony vine (kukocha)		1.41 ± 0.08	0.62 ± 0.01			
low striped bamboo leaves (kumazasacha) 1						
2	0.03 ± 0.04	0.03 ± 0.01	0.03 ± 0.01		0.02 ± 0.01	
mulberry leaves (kuwanohacha)		2.67 ± 0.12	0.76 ± 0.04			
perilla leaves (shisonohacha) 1				2.25 ± 0.49	1.08 ± 0.08	1.17 ± 0.16
2				2.22 ± 0.99	1.35 ± 0.57	0.39 ± 0.10
jasmine (jasumincha)	0.72 ± 0.12	1.15 ± 0.22	0.95 ± 0.22			
field horsetail leaves (suginacha)			1.05 ± 0			0.76 ± 0.04
Rubus suavissimus (Chinese tenncha) 1		1.64 ± 0.19	0.46 ± 0.04			
2		1.06 ± 0.01	0.38 ± 0.03			
Houttuynia cordata (dokudamicha) 1		3.81 ± 0.63	0.08 ± 0.01			
2		3.01 ± 0.40	0.06 ± 0.01			
gutta-percha (tochucha) 1		1.43 ± 0.20	0.23 ± 0.04			
2		1.19 ± 0.12	0.16 ± 0.01			
3		0.28 ± 0.04	0.05 ± 0			
East Indian lotus (hasucha)		6.00 ± 0.21	0.12 ± 0.04		0.08 ± 0.03	
adlay (hatomugicha)						
banava (banavacha) 1		0.96 ± 0.12	0.34 ± 0.05			
2		1.03 ± 0.07	0.30 ± 0.03			
Japanese medlar (biwanohacha) 1		0.39 ± 0.05	0.13 ± 0.01			
2		0.09 ± 0.02	0.04 ± 0.02		0.08 ± 0.03	0.18 ± 0.10
pu-erh (puarcha)	0.40 ± 0.22	0.52 ± 0.18	0.23 ± 0.09		0.05 ± 0.09	
safflower (benibanacha)			0.38 ± 0.03	0.29 ± 0.04		
eucalyptus (yukaricha)		0.22 ± 0.03				
mugwort (yomogicha)						
rooibos (ruiboscha)		0.92 ± 0.10	0.16 ± 0.32			
Japanese green (sencha)	1.31 ± 0.14	1.56 ± 0.18	1.49 ± 0.17			

[a] Myr, myricetin; Que, quercetin; Kae, kaempferol; Scu, scutellarein; Lut, luteolin; Api, apigenin. [b] Milligrams of flavonoids per gram of dried tea. [c] Means of triplicate determination and the standard deviations.

reason was not clear, and small amounts of myricetin, quercetin, kaempferol, and luteolin were detected in one sample of low striped bamboo leaf tea (11) infusions. Japanese green tea infusions showed both quercetin and kaempferol. Nakazawa (1995) summarized studies on the function of gutta-perca tea (18) and reported the presence of quercetin and kaempferol. Our results concurred with this. Shimoi et al. (1996) reported the presence of luteolin as an antioxidative plant flavonoid in rooibos tea (27) infusions; however, it was not found in our samples. Rabe et al. (1994) reported the presence of luteolin, quercetin, and isoquecitrin as phenolic metabolites in rooibos tea (27) leaves and stems. Snyckers and Salemi (1974) reported that the antiallergic effect of rooibos tea was mainly due to the flavonol quercetin. We found that the main flavonols were quercetin and kaempferol in rooibos tea infusions.

The most prevalent flavonols, quercetin and kaempferol, were found in 17 kinds of tea infusions including Japanese green tea as described above. Myricetin, found in green tea infusions, was found in only jasmine tea and pu-erh teas. The flavon luteolin, which was not confirmed in our green tea infusions because of the trace content, was found in ground ivy leaves tea (5), chrysanthemum flower tea (7), and perilla leaf tea (13) infusions, apigenin was found in great plantain tea (4), perilla leaf tea (13), and field horsetail tea (suginacha) (15) infusions, and scutellarein was found in great plantain tea (oobakocha) (4), perilla leaf tea (shisocha)

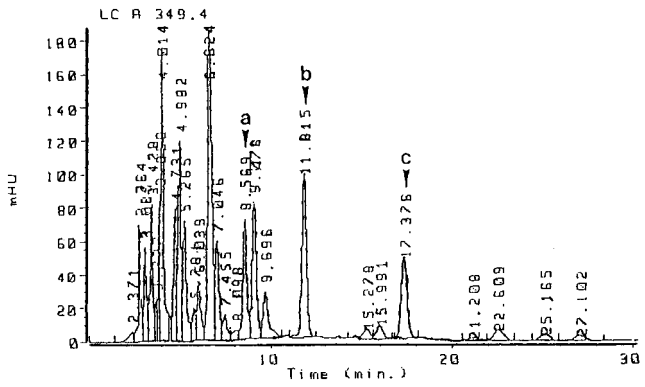

Figure 2. Typical chromatogram of a perilla tea extract. Peaks: (a) scutellarein, (b) luteolin, and (c) apigenin. HPLC conditions were the same as shown in the legend of Figure 1 except the ratio of mobile phase is 5.5:4.5 (v/v).

(13), and safflower flower (benibana) tea (24) infusions. Scutellarein, which was reported to be the strongest antiallergic flavonoid *in vitro* among the many flavonoids tested by Kawasaki (1994), is one of the main components in great plantain (oobako) tea (4) (Miyaichi, 1989; Nishibe, 1995). It was also detected in perilla leaf tea (13) and safflower tea (24) at concentrations of 2 and 0.3 mg/g in whole dried tea leaves, respectively. Typical chromatogram of perilla leaf tea infusions is shown in Figure 2. The presence of scutellarein in the perilla leaf

tea infusions and safflower tea infusions was confirmed by comparing the mass spectrum peaks of scutellarein standard peak (*m/z* 287 $[M + H]^+$, *m/z* 319 $[M + CH_3\text{-}OH + H]^+$)with those of an extract from perilla leaf tea and an extract from safflower tea.

Scutellarein is usually contained in and is a functional component of great plantain (oobako). It has been used since ancient times as a diuretic and as an anti-inflammatory and antiasthmatic drug in China and Japan (Mitsuhashi, 1988). We confirmed that it is also contained in perilla leaves, which are a very popular food in Japan, and in safflower, which is eaten salted with vinegar in a local area of Japan.

ACKNOWLEDGMENT

We thank Dr. Masaru Kawasaki (Foods and Drugs Safety Center, Hadano-shi, Japan) for a sample of scutellarein and valuable suggestions and Dr. Yukihiro Goda (National Institute of Health Sciences, Tokyo, Japan) for kind help for our study.

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Received for review January 10, 1997. Revised manuscript received April 18, 1997. Accepted April 28, 1997.[⊗] This project is financially supported by the Human Health Sciences Foundation.

JF970024Y

[⊗] Abstract published in *Advance ACS Abstracts,* June 15, 1997.