Allergy-Preventive Flavonoids from *Xanthorrhoea hastilis*

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Allergy-preventive activity was demonstrated for an extract of resins from *Xanthorrhoea hastilis* R. Br. in a search for allergy-preventive substances from natural sources. By bioassay-directed fractionation of this plant extract, a new flavanone, 3′,5′-dihydroxy-7,4′-dimethoxyflavanone (1), and two new chalcones, 3,5,2′-trihydroxy-4,4′-dimethoxychalcone (2) and 5,2′-dihydroxy-3,4,4′-trimethoxychalcone (3), were isolated together with five known compounds, 5′-hydroxy-7,3′,4′-trimethoxyflavanone (4), 3′-hydroxy-7,4′-dimethoxyflavanone (5), liquiritigenin 7-methyl ether (6), 4,2′-dihydroxy-4′-methoxychalcone (7) and sakuranetin (8). The structures of 1, 2 and 3 were elucidated by spectroscopic methods. All of these compounds showed allergy-preventive effects.

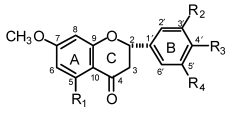
Key words *Xanthorrhoea hastilis*; flavanone; chalcone; allergy-preventive effect; platelet aggregation

Xanthorrhoea hastilis R. Br. (Xanthorrhoeaceae), commonly called grass trees, is an Australian native plant genus. A commercially available resin commonly obtained from *Xanthorrhoea* trees is gum accoroides, but no medical uses have been described for this plant. In our continuing search for allergy-preventive substances from natural sources[1] we found that extracts of *X. hastilis* exhibited allergy-preventive activity. Known extracts from *X. hastilis* include benzoic acid,[2] fragrant oil[3] and C-methylated flavonoid,[4] but no detailed chemical study had been reported. In the present study, we isolated from *X. hastilis*, a new flavanone, 3′,5′-dihydroxy-7,4′-dimethoxyflavanone (**1**) and two new chalcones, 3,5,2′-trihydroxy-4,4′-dimethoxychalcone (**2**) and 5,2′-dihydroxy-3,4,4′-trimethoxychalcone (**3**). We report their structures and also the isolation of five known compounds. Finally, we describe the allergy-preventive effects of these compounds isolated from *X. hastilis*.

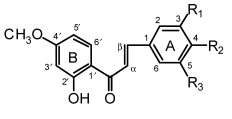
Results and Discussion

Compound **1** was obtained as colorless needles and its HR-EI-MS showed the $[M]^+$ ion at *m*/*z* 316.0942 which established the molecular formula as $C_{17}H_{16}O_6$ (Calcd 316.0947). The IR spectrum indicated the presence of hydroxy (3500—3200 cm^{-1}) and conjugated carbonyl (1670 cm^{-1}) groups. The UV spectrum showed λ_{max} at 237, 272 and 310 nm, indicating a phenolic nature. ^{1}H-NMR of **1** showed the presence of a methylene (δ 2.75, 2.96), an oxymethine (δ 5.32), two methoxy groups (δ 3.81, 3.84) and five aromatic protons, in which two protons appeared as a singlet at δ 6.49, suggesting a symmetrical structure and the other three protons indicated a typical ABX spin system (δ 6.54, 6.62, 7.77). ^{13}C-NMR showed the signals for a carbonyl carbon at δ 193.1, twelve aromatic carbons between δ 102.1 and 168.2, an oxygenated methine at δ 80.9, two methoxy groups at δ 56.3 and 60.8 and a methylene at δ 48.5. These data suggested that **1** is dihydroxy-dimetoxyflavanone. EI-MS showed fragment peaks at *m*/*z* 151, 166, which indicated the presence of one methoxy group in the A ring, one methoxy and two hydroxy groups in the B ring.[5] The methoxy group of the B ring should be placed at position 4′ to form the B ring symmetry. The positions of methoxy groups were established at 7 and 4′ by correlation with the HMBC spectrum (Table 1). The absolute configuration at C-2 was confirmed by a positive Cotton effect at 331 nm and a negative Cotton effect at 304 nm in the CD spectrum, which is characteristic for the 2*S* configuration of flavanones.[6] Based on these observations, the new compound **1** was established to be (2*S*)-3′,5′-dihydroxy-7,4′-dimethoxyflavanone.

Compound **2** was isolated as yellow needles. HR-EI-MS showed the $[M]^+$ ion at *m*/*z* 316.0949 corresponding to the



	R_1	R_2	R_3	R_4
1	H	OH	OCH_3	OH
4	H	OCH_3	OCH_3	OH
5	H	OH	OCH_3	H
6	H	H	OH	H
8	OH	H	OH	H



	R_1	R_2	R_3
2	OH	OCH_3	OH
3	OCH_3	OCH_3	OH
7	H	OH	H

Table 1. ^{1}H- and ^{13}C-NMR Spectroscopic Data of **1**[a)]

Position	**1** (CD_3OD)		HMBC[b)]
	δ_C	δ_H	
2	80.9	5.32 dd (3.2, 12.8)	4, 9, 2′, 6′
3	48.5	2.75 dd (3.2, 17.0)	4, 10, 1′
		2.96 dd (12.8, 17.0)	
4	193.1		
5	129.5	7.77 d (9.2)	4, 7, 9
6	110.0	6.62 dd (2.3, 9.2)	8, 10
7	168.2		
8	102.1	6.54 d (2.3)	6, 7, 9, 10
9	165.3		
10	115.9		
1′	136.4		
2′	106.8	6.49 s	2, 4′, 6′
3′	152.0		
4′	137.0		
5′	152.0		
6′	106.8	6.49 s	2, 2′, 4′
7-OCH_3	56.3	3.84 s	7
4′-OCH_3	60.8	3.81 s	4′

a) Data were recorded at 500 MHz (^{1}H-) or 125 MHz (^{13}C-NMR). Values in parentheses are coupling constants in Hz (500 MHz). *b*) HMBC correlations are proton(s) stated for the indicated carbon.

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Table 2. ^{1}H- and ^{13}C-NMR Spectral Data for Compounds **2** and **3**[a)]

Position	**2** ($CDCl_3$)		**3** ($CDCl_3$)		HMBC[b)]
	δ_C	δ_H	δ_C	δ_H	
1	131.2		130.8		
2	108.5	6.82 s	107.8	6.72 d (2.0)	3, 4, 6, β
3	149.1		152.4		
4	136.7		137.9		
5	149.1		149.6		
6	108.5	6.82 s	105.5	6.96 d (2.0)	2, 4, 5, β
C=O	191.8		191.8		
α	120.4	7.43 d (15.4)	119.4	7.43 d (15.4)	1, C=O
β	143.7	7.70 d (15.4)	144.3	7.75 d (15.4)	2, 6, C=O
1′	114.2		114.2		
2′	166.3		166.3		
3′	101.2	6.47 d (2.6)	101.2	6.47 d (2.5)	1′, 5′
4′	166.8		166.8		
5′	107.7	6.49 dd (2.6, 8.9)	107.7	6.49 dd (2.5, 8.7)	1′, 3′
6′	131.6	7.80 d (8.9)	131.2	7.81 d (8.7)	2′, 4′, C=O
3-OCH_3			56.1	3.93 s	3
4-OCH_3	61.3	3.94 s	61.1	3.97 s	4
4′-OCH_3	55.6	3.87 s	55.6	3.86 s	4′
3-OH		5.38 s			
5-OH		5.38 s		5.81 s	4, 5, 6
2′-OH		13.38 s		13.38 s	1′, 2′, 3′

a) Data were recorded at 500 MHz (^{1}H-) or 125 MHz (^{13}C-NMR). Values in parenthesis are coupling constants in Hz (500 MHz). *b*) HMBC correlations are proton(s) stated for the indicated carbon.

molecular formula $C_{17}H_{16}O_6$ (Calcd 316.0947). The IR spectrum (1645 cm^{-1}) and the UV spectrum (276, 365 nm) indicated the presence of α,β-unsaturated ketone. ^{1}H-NMR spectra indicated the presence of three hydroxy groups (one of which is chelated), chalcone *trans* double bond [δ 7.70, 7.43 (J=15.4 Hz)], five aromatic protons, in which two protons appeared as a singlet (δ 6.82) and the other three protons indicated a typical ABX spin system (δ 7.80, 6.49, 6.47), and two methoxy groups (δ 3.94, 3.87). ^{13}C-NMR spectra showed a carbonyl carbon at δ 191.8, fourteen olefinic or aromatic carbons between δ 166.8 and 101.2 and two methoxy signals at δ 61.3 and 55.6. Comparison of the ^{1}H- and ^{13}C-NMR data of the A ring of **2** with B ring of **1** indicated the same signal pattern. In the B ring, with the hydroxy group being at C-2′, an ABX spin system in the ^{1}H-NMR spectrum was in agreement with the structure methoxylated at C-4′. All signal assignments of ^{1}H- and ^{13}C-NMR spectra were confirmed by HMQC spectra. Thus, the structure of new compounds **2** was established to be 3,5,2′-trihydroxy-4,4′-dimethoxychalcone.

Compound **3** was isolated as yellow needles. HR-EI-MS showed the $[M]^+$ ion at *m/z* 330.1100 corresponding to the molecular formula $C_{18}H_{18}O_6$ (Calcd 330.1103). The IR spectrum (1640 cm^{-1}) and the UV spectrum (276, 365 nm) indicated the presence of α,β-unsaturated ketone. The ^{1}H- and ^{13}C-NMR spectrum of **3** closely resembled those of **2**, except for an additional methoxy group and the lack of symmetry in the A ring. EI-MS showed fragment peaks at *m/z* 151, 180, which indicated the presence of two methoxy and one hydroxy groups in the A ring and one methoxy group in the B ring. The positions of the methoxy group were established at 3, 4, 4′ by correlation with the HMBC spectrum. Based on these observations, the new compound **3** was established to be 5,2′-dihydroxy-3,4,4′-trimethoxychalcone.

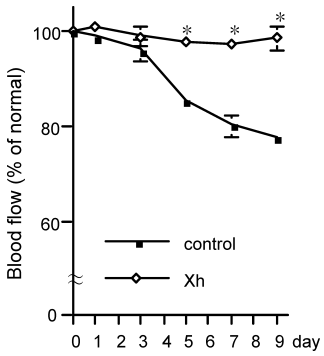

Fig. 1. Allergy-Preventive Effects of the Extract of *Xanthorrhoea hastilis*

■: control (blood flow of HEL sensitized mice), ◇: pretreatment with extract (Xh) of *X. hastilis* at 0 (starting day), 3, 6 and 9 d from sensitization. Each value presents the mean±S.E. (n=5). $*$ $p<0.05$ as compared with control group (Dunnett's test with Bonferroni).

Compound **4** was isolated as colorless needles and EI-MS showed $[M]^+$ ion at *m/z* 330. The ^{1}H- and ^{13}C-NMR spectrum closely resemble those of **1**, except for an additional methoxy group and the lack of symmetry in the B ring. These findings suggested that **4** was additionally methoxylated at C-3′ of **1**. By comparison of this physical and spectroscopic data with the reported values,[7)] the structure of **4** was identified as 5′-hydroxy-7,3′,4′-trimethoxyflavanone. This compound has been isolated from the leaves of *Muntingia calabura* and reported to have cytotoxicity against P-388 cell lines *in vitro*.[7)] This is the first report of its being isolated from *Xanthorrohea*.

Compound **5**, 3′-hydroxy-7,4′-dimethoxyflavanone, was isolated as colorless needles and the EI-MS showed $[M]^+$ ion at *m/z* 300. The structure of **5** was further confirmed by comparison of the ^{1}H- and ^{13}C-NMR spectra data with the reported data.[8)] It was isolated from the stem of *Bauhinia manca*[8)] and reported to have inhibitory activity against aromatase,[9)] but there has been no report on allergy-preventive activity.

In addition to these compounds, we isolated some known flavanons and chalcons, liquiritigenin 7-methyl ether (**6**),[8)] 4,2′-dihydroxy-4′-methoxychalcone (**7**)[10)] and sakuranetin (**8**).[11)] Their structures were established by comparing their spectral data and physical constants. Though various bioactivities have been reported for these compounds,[12—14)] the present findings show an allergy-preventive activity for these compounds for the first time.

The allergy-preventive effects of the extracts of *X. hastilis* and the isolated compounds **1**, **4**, **5**, **6**, **7** and **8**, which were obtained in sufficient amounts, were measured using an *in vivo* assay method reported previously,[15)] which uses the blood flow decrease in the tail vein microcirculation of mice subjected to hen egg-white lysozyme (HEL)-sensitization as a monitor. This method can be used to a search for preventive agents against allergy involving NO from inducible NO synthase, COX-1, 2 and PGI_2.[15)] The extract of *X. hastilis* (100 mg/kg, *p.o.*) significantly inhibited the blood flow decrease compared with the control group, as shown in Fig. 1. All compounds (10 mg/kg, *p.o.*) also significantly inhibited the blood flow decrease in a similar manner (Fig. 2).

Further evaluation of the isolated compounds was carried out with respect to platelet aggregation, and the results are

shown in Fig. 3. All the compounds inhibited platelet aggregation compared with the control group. This finding appears to correspond to the effect on the blood flow decrease, and thus we can suppose that the platelet aggregation inhibition activity is enumerated in one of the mechanisms of the blood flow decrease. As described above, the resins of *Xanthorrhoea hastilis* could be useful for preventing allergy development. Further study is being done on the other active compounds.

Experimental

General Experimental Procedures Melting points were determined on a Yanagimoto micro melting-point apparatus. IR spectra were recorded on a Shimadzu 435 spectrometer and the UV absorption spectra with Shimadzu UV-160A spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded with a JEOL JEM-GSX 500 spectrometer (TMS as internal reference). EI-MS spectra were performed on a JMS-700 spectrometer.

Plant Material The resin of *X. hastilis* was purchased from Shinwa Bussan Co., Ltd., Osaka, Japan. A voucher specimen (GPU-2006-S-06) is kept at Gifu Pharmaceutical University.

Extraction and Isolation The dried resin of *X. hastilis* (340 g) was refluxed with AcOEt for 6 h and it was filtered. The filtrate was evaporated *in vacuo* to obtain AcOEt extract (280 g). 40.8 g of the extract was subjected to chromatography on a silica gel column using a $CHCl_3$–AcOEt–MeOH step-gradient to give seven fractions (Fr. Ia—g). Fr. Ic (6.9 g), which was eluted by *n*-hexane–AcOEt (1 : 1), was rechromatographed on silica gel column, again using a $CHCl_3$–MeOH gradient, to obtain nine fractions (Fr. IIa—i). Fr. IIf (727 mg) was eluted by *n*-hexane–AcOEt (1 : 1), followed by flash column chromatography and gave five fractions (Fr. IIIa—e). Fr. IIIb was subjected to recrystallization from *n*-hexane–AcOEt to obtain **1** (95.5 mg). Fr. IIe (1.4 g) eluted by *n*-hexane–AcOEt (1 : 1), followed by silica gel column chromatography and gave four fractions (Fr. IVa—d). Fr. IVb (1.1 g) was subjected to flash column chromatography using a $CHCl_3$–MeOH step-gradient to give four fractions (Fr. Va—d). Fr. Vb (218 mg), which was followed by $CHCl_3$: MeOH = 20 : 1, was obtained by silica gel column using a $CHCl_3$–MeOH gradient to yield six fractions. (Fr. VIa—f). Fr. VIc (23.9 mg) was purified by gel filtration on Sephadex LH-20 using MeOH to yield compounds **2** (0.9 mg).

Fr. Ib (5.18 g) was rechromatographed over silica gel using *n*-hexane–$CHCl_3$ gradient and gave six fractions (Fr. VIIa—f). Fr. VIIc (554 mg), eluted by 1 : 10, was purified by gel filtration on Sephadex LH-20 using MeOH to yield three compounds, **3** (2.2 mg), **4** (27.9 mg), **5** (11.8 mg). Similarly, Fr. VIId was subjected to chromatography on silica gel column using *n*-hexane–$CHCl_3$ gradient and purified by gel filtration on Sephadex LH-20 using MeOH to yield three compounds, **6** (100.2 mg), **7** (52.3 mg), **8** (40.8 mg).

(2*S*)-3′,5′-Dihydroxy-7,4′-dimethoxyflavanone (**1**): Colorless needles; mp 182 °C (*n*-hexane–AcOEt); ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz) see Table 1; IR (KBr) ν_{max} 3500—3200, 2920, 1670, 1610 cm^{-1}; UV (MeOH) λ_{max} (log ε): 237 (4.52), 272 (4.52), 310 (4.32) nm; HR-EI-MS *m/z* 316.0942 (Calcd for $C_{17}H_{16}O_6$, 316.0947); EI-MS *m/z* 316 $[M]^+$ (61), 151 $[C_6H_3(OCH_3)(OH)CO]^+$ (100), 166 $[C_6H_2(OH)(OCH_3)(OH)CH{=}CH_2]^+$ (19); $[\alpha]_D$ −64.2° (*c*=0.5, MeOH); CD (*c*=0.27, MeOH) λ nm ($\Delta\varepsilon$) 304 (−13.5), 331 (+7.76).

3,5,2′-Trihydroxy-4,4′-dimethoxychalcone (**2**): Yellow needles; mp 135 °C ($CHCl_3$–AcOEt); ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR (125 MHz, $CDCl_3$) see Table 2; IR (KBr) ν_{max} 3500—3200, 1645, 1580, 1520 cm^{-1}; UV (MeOH) λ_{max} (log ε): 276 (2.47), 365 (3.20) nm; HR-EI-MS *m/z* 316.0949 (Calcd for $C_{17}H_{16}O_6$, 316.0947); EI-MS *m/z* 316 $[M]^+$ (100), 151 $[C_6H_3(OCH_3)(OH)CO]^+$ (82), 166 $[C_6H_2(OH)(OCH_3)(OH)CH{=}CH_2]^+$ (12).

5,2′-Dihydroxy-3,4,4′-trimethoxychalcone (**3**): Yellow needles; mp 178 °C (*n*-hexane–AcOEt); ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR (125 MHz, $CDCl_3$) see Table 2; IR (KBr) ν_{max} 3300—3200, 1640, 1580, 1510 cm^{-1}; UV (MeOH) λ_{max} (log ε): 276 (2.47), 365 (3.20) nm; HR-EI-MS *m/z* 330.1100 (Calcd for $C_{18}H_{18}O_6$, 330.1103); EI-MS *m/z* 330 $[M]^+$ (100), 151 $[C_6H_3(OCH_3)(OH)CO]^+$ (37), 180 $[C_6H_2(OH)(OCH_3)(OCH_3)CH{=}CH_2]^+$ (17).

(2*S*)-5′-Hydroxy-7,3′,4′-trimethoxyflavanone (**4**): Colorless needles; mp 123 °C ($CHCl_3$–AcOEt); ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 2.80 (1H, dd, *J*=16.9, 3.2 Hz, H_{eq}-3), 3.00 (1H, dd, *J*=16.9, 13.3 Hz, H_{ax}-3), 3.84 (3H, s, 7-OCH_3), 3.89 (3H, s, 3′-OCH_3), 3.91 (3H, s, 4′-OCH_3), 5.35 (1H, dd, *J*=13.3, 3.2 Hz, H-2), 6.50 (1H, d, *J*=2.3 Hz, H-8), 6.59 (1H, d, *J*=1.9 Hz, H-2′), 6.61 (1H, dd, *J*=9.2, 2.3 Hz, H-6), 6.72 (1H, d, *J*=1.9 Hz, H-6′), 7.86 (1H, d, *J*=9.2 Hz, H-5); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 44.4 (C-3), 55.7 (7-OMe), 56.0 (3′-OMe), 61.0 (4′-OMe), 80.0 (C-2), 102.1 (C-8), 102.3 (C-2′), 106.3 (C-6′), 110.3 (C-6), 114.9 (C-10), 128.8 (C-5), 134.9 (C-1′), 135.8 (C-4′), 149.6 (C-3′), 152.7 (C-5′), 163.5 (C-9), 166.3 (C-7), 190.5 (C-4); EI-MS *m/z* 330 $[M]^+$ (100), 151 $[C_6H_3(OCH_3)(OH)CO]^+$ (57), 180 $[C_6H_2(OH)(OCH_3)(OCH_3)CH{=}CH_2]^+$ (48); $[\alpha]_D$ −94.8° (*c*=0.88, MeOH).

(2*S*)-3′-Hydroxy-7,4′-methoxyflavanone (**5**): Colorless needles; mp 136 °C; ^{1}H-NMR (CD_3OD, 500 MHz) δ 2.75 (1H, dd, *J*=17.0, 3.0 Hz, H_{eq}-3), 3.03 (1H, dd, *J*=17.0, 13.0 Hz, H_{ax}-3), 3.84 (3H, s, 7-OCH_3), 3.86 (3H, s, 4′-OCH_3), 5.40 (1H, dd, *J*=13.0, 3.0 Hz, H-2), 6.54 (1H, d, *J*=2.3 Hz, H-8), 6.62 (1H, dd, *J*=9.0, 2.3 Hz, H-6), 6.91 (1H, dd, *J*=8.2, 1.9 Hz, H-6′), 6.93 (1H, d, *J*=8.2 Hz, H-5′), 6.97 (1H, d, *J*=1.9 Hz, H-2′), 7.78 (1H, d, *J*=9.0 Hz, H-5); ^{13}C-NMR (CD_3OD, 125 MHz) δ 45.0 (C-3), 56.3 (7-OMe), 56.6 (4′-OMe), 81.1 (C-2), 102.1 (C-8), 111.2 (C-6), 112.8 (C-5′), 114.7(C-2′), 115.9 (C-10), 119.0 (C-6′), 129.5 (C-5), 133.4 (C-1′), 147.9 (C-3′), 149.4 (C-4′), 165.5 (C-9), 168.2 (C-7), 193.4 (C-4); EI-MS *m/z* 300 $[M]^+$ (100), 151 $[C_6H_3(OCH_3)(OH)CO]^+$ (61), 150 $[C_6H_3(OH)(OCH_3)CH{=}CH_2]$ (42); $[\alpha]_D$ −28.5° (*c*=0.6, MeOH).

Liquiritigenin 7-Methyl Ether {(2*S*)-4′-Dihydroxy-7-methoxyflavanone} (**6**): Colorless needles; mp 175 °C ($CHCl_3$–AcOEt); ^{1}H-NMR ($CDCl_3$, 500 MHz); δ 2.81 (1H, dd, *J*=16.9, 2.8 Hz, H_{eq}-3), 3.05 (1H, dd, *J*=16.9, 13.3 Hz, H_{ax}-3), 3.83 (3H, s, 7-OCH_3), 5.40 (1H, dd, *J*=13.3, 2.8 Hz, H-2), 5.95 (1H, s, 4′-OH), 6.48 (1H, d, *J*=2.3 Hz, H-8), 6.61 (1H, dd, *J*=9.2, 2.3 Hz, H-6), 6.90 (2H, d, *J*=8.7 Hz, H-3′, 5′), 7.33 (2H, d, *J*=8.7 Hz, H-2′,

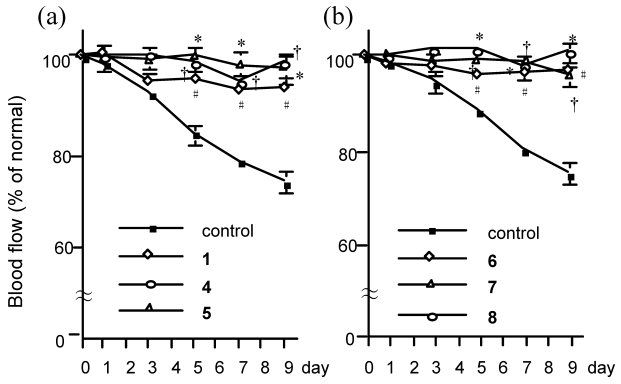

Fig. 2. Allergy-Preventive Effects of Isolated Compounds

■: control (blood flow of HEL-sensitized mice); (a), (b); ◇, △ or ○: pretreatment with isolated compounds at 0 (starting day), 3, 6 and 9 d from sensitization. Each value represents the mean±S.E. (*n*=5). ＊$p<0.05$, $^{\#}p<0.05$, $^{\dagger}p<0.05$ as compared with control group (Dunnett's test with Bonferroni).

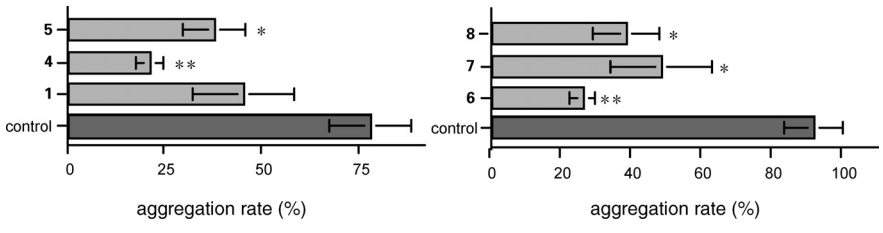

Fig. 3. Platelet Aggregation Effects of Isolated Compounds

Results are the means±S.E. (*n*=5). ＊$p<0.05$, ＊＊$p<0.01$, as compared with control group (Dunnett's test).

6′), 7.87 (1H, d, J=9.2 Hz, H-5); ^{13}C-NMR ($CDCl_3$, 125 MHz); δ 44.1 (C-3), 55.7 (7-OMe), 77.3 (C-2), 101.0 (C-8), 110.3 (C-6), 114.7 (C-10), 115.7 (C-3′, 5′), 127.9 (C-2′, 6′), 128.8 (C-5), 130.0 (C-1′), 156.4 (C-4′), 163.8 (C-9), 166.4 (C-7), 191.4 (C-4); EI-MS m/z 270 $[M]^+$ (95), 151 $[C_6H_3(OCH_3)(OH)CO]^+$ (100), 120 $[C_6H_4(OH)CH{=}CH_2]$ (52); $[\alpha]_D$ −33.0° (c=0.1, MeOH).

4,2′-Dihydroxy-4′-methoxychalcone (**7**): Yellow needles; mp 148 °C; ^{1}H-NMR (500 MHz, $CDCl_3$); δ 3.86 (3H, s, 4′-OCH_3), 6.47 (1H, d, J=2.6 Hz, H-3′), 6.48 (1H, dd, J=7.7, 2.6 Hz, H-5′), 6.87 (2H, d, J=8.9 Hz, H-3, 5), 7.44 (1H, d, J=15.4 Hz, H-α), 7.57 (2H, d, J=8.9 Hz, H-2, 6), 7.82 (1H, d, J=7.7 Hz, H-6′), 7.86 (1H, d, J=15.4 Hz, H-β), 13.48 (1H, s, 2′-OH); ^{13}C-NMR ($CDCl_3$, 125 MHz); δ 55.6 (4′-OMe), 101.2 (C-3′), 107.6 (C-5′), 114.2 (C-1′), 116.0 (C-3, 5), 118.2 (C-α), 127.9 (C-1), 130.6 (C-2, 6), 131.1 (C-6′), 144.1 (C-β), 157.9 (C-4), 166.2 (C-2′), 166.7 (C-4′), 191.9 (C=O); EI-MS m/z 270 $[M]^+$ (66), 151 $[C_6H_3(OCH_3)(OH)CO]^+$ (100), 119 $[C_6H_4(OH)CHCH]^+$ (30).

Sakuranetin {(2*S*)-5,4′-Dihydroxy-7-methoxyflavanone} (**8**): Colorless needles; mp 150 °C; ^{1}H-NMR ($CDCl_3$, 500 MHz); δ 2.79 (1H, dd, J=17.4, 3.3 Hz, H_{eq}-3), 3.08 (1H, dd, J=17.4, 13.1 Hz, H_{ax}-3), 3.80 (3H, s, –OCH_3), 5.08 (1H, s, 4′-OH), 5.35 (1H, dd, J=13.1, 3.3 Hz, H-2), 6.04 (1H, d, J=2.3 Hz, H-6), 6.06 (1H, d, J=2.3 Hz, H-8), 6.88 (2H, d, J=8.7 Hz, H-3′, 5′), 7.33 (2H, d, J=8.7 Hz, H-2′, 6′), 12.0 (1H, 5-OH), ^{13}C-NMR ($CDCl_3$, 125 MHz); δ 43.2 (C-3), 55.7 (7-OMe), 77.3 (C-2), 94.3 (C-8), 95.2 (C-6), 103.2 (C-10), 115.7 (C-3′, 5′), 128.0 (C-2′, 6′), 130.7 (C-1′), 156.0 (C-4′), 162.9 (C-9), 164.2 (C-5), 168.1 (C-7), 196.0 (C-4); EI-MS; m/z 286 $[M]^+$ (100), 167 $[C_6H_2(OCH_3)(OH)(OH)CO]^+$ (94), 119 $[C_6H_4(OH)CH{=}CH_2]$ (92); $[\alpha]_D$ −31.2° (c=0.32, MeOH).

Animals Male ddY mice (SPF grade), 5 weeks old, were obtained from Japan SLC (Shizuoka, Japan) and housed at 24 °C. Food and water were available *ad libitum*.

All animal experiments were performed in accordance with the Guidelines for Animal Experiments of Mukogawa Women's University.

HEL Sensitization Immunization with hen egg-white lysozyme (HEL) was performed as previously described[16] with slight modification. Male ddY mice of 5 weeks of age were sensitized interperitonealy with 50 μg of HEL in complete Freund's adjuvant (DIFCO) on day 0.

***In Vivo* Assay Method for Allergy Preventive Substances** Subcutaneous blood flow in the mouse tail was monitored using a laser doppler blood flow meter of the contact type (OMEGA FLO-C1 Neuroscience Inc., Tokyo, Japan) as previously described.[15] Each mouse was pre-warmed for 10 min at 36 °C prior to the experiment and placed on a holder in a measuring chamber kept at 36 °C throughout the measurement. The normal blood flow was measured for 10 min at 20 min before the experiment. The blood flow of the sensitized mouse was measured for 10 min without anesthesia. The results were expressed as mean±S.E. of the percent of the normal blood flow of each mouse.

Allergy-Preventive Effects Each of the extracts (100 mg/kg) and compounds (10 mg/kg) was administered orally to HEL-sensitized mice at 0 (the start day), 3, 6 and 9 d. None of the reagents affected the blood flow. The measurements of blood flow were carried out every day for 9 d. The statistical calculations were determined in comparison with the HEL-sensitized mice (control group).

Platelet Aggregation The platelet aggregation of whole blood induced by ADP (8 μM) was measured with a whole blood aggregometer (WBA) analyzer (MC Medical Co., Ltd., Tokyo, Japan) using a screen filtration pressure (SFP) method. The whole blood was obtained from each anesthetized mouse with diethyl ether on day 9. The samples were anticoagulated with 3.8% sodium citrate and then left to stand for 30 min at room temperature for stable aggregation. The results are expressed as the mean±S.E. of the cohesion rate for control and pretreatment with test compounds (n=5).

Statistical Analysis Two-way analysis of variance (ANOVA) was used to test for statistical differences. When significant differences (p<0.05) were identified, the data were further analyzed by Dunnett's multiple range test with Bonferroni inequality for significant differences between each test group and the control group.

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