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The extract of the flowers of *Prunus persica*, a new cosmetic ingredient, protects against solar ultraviolet-induced skin damage *in vivo*

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Synopsis

The flowers of *Prunus persica* Batsch have been used for skin disorders in East Asia from ancient times. In this investigation, the ethanol extract from this plant material was prepared and several major constituents were isolated. In addition, the protective effects of the extract were evaluated against solar ultraviolet (UV)-induced skin damage using *in vivo* animal models of UVB-induced erythema in guinea pigs and ear edema in ICR mice. From the extract, four kaempferol glycoside derivatives were successfully isolated and their contents were measured with HPLC. Among the derivatives isolated, the content of multiflorin B was highest (3.3%, w/w). The *P. persica* extract clearly inhibited UVB-induced erythema formation dose dependently when topically applied (IC_{50} = 0.5 mg/cm^2). It also inhibited UVB-induced ear edema (49% inhibition at 3.0 mg/ear). Moreover, multiflorin B inhibited UVB-induced erythema formation (80% inhibition at 0.3 mg/cm^2), indicating that this compound is one of the active principles of the extract. All these results suggest that *P. persica* extract may be useful for protection against UVB-induced skin damage when topically applied.

INTRODUCTION

In order to find plant materials having protective effects on solar ultraviolet (UV)-induced skin damage, various plant extracts were initially screened (1), and the extract of Prunus flowers was selected for further study. Topical application of the flowers of *Prunus persica* have long been used in Chinese medicine for treating skin disorders (2). Previously, the ethanol extract from the flowers of *P. persica* (Ku-35) was tested for protective effects against UV-induced damage in keratinocytes and fibroblasts *in vitro*. Ku-35 significantly inhibited the release of UVB-induced arachidonate acid metabolites

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from normal human keratinocytes (3). Ku-35 also potently inhibited UV-induced DNA breakage from skin fibroblasts, measured by the single cell gel electrophoresis assay (4). These previous findings strongly suggest that Ku-35 may give protection from UV-induced skin damage such as erythema, premature aging, wrinkle formation, and ultimately skin cancer, when topically applied. To check this possibility, Ku-35 was evaluated in this investigation for its inhibitory activity against *in vivo* animal models of UVB-induced erythema and edema formation. From the results, it was found, for the first time, that topical application of Ku-35 significantly inhibits the formation of UVB-induced erythema as well as edema. In addition, four flavonoid constituents (compounds I–IV) were isolated from this plant material and structurally identified as kaempferol 3-O-[β-D-glucopyranosyl(1→4)-α-L-rhamnopyranoside], kaempferol 3-O-β-D-galactopyranoside, kaempferol 3-O-α-L-rhamnopyranoside, and kaempferol 3-O-β-D-glucopyranoside, and their contents were measured using HPLC separation techniques.

MATERIALS AND METHODS

MATERIALS AND APPARATUS

^{1}H- and ^{13}C-NMR were measured in JEOL 200 MHz NMR using an internal standard. Melting points were measured with the Fisher-Johns melting point apparatus and were uncorrected. Silica gel (70–230 mesh), TLC plates (F_{254}), and a lobar column (RP-18) were from Merck (Germany).

PLANT MATERIAL

The flowers of *P. persica* (Rosaceae) were collected in several orchards located in Kangwon province (Korea) in April 1997 and 1998, and dried in the dark. A brochure specimen was deposited at the College of Pharmacy, Kangwon National University.

ANIMALS

Male Hartly guinea pigs and male ICR mice were purchased from Charles River Laboratories (Japan). The animals were maintained in the SPF animal facility at KNU under conditions of 20°–22°C, 40–60% relative humidity, and a 12 hr/12 hr (L/D) cycle.

UVB SOURCE

For irradiation on animals, the strength of a UVB lamp (Model XX-15B, medium wavelength [312 nm], Spectroline, Westbury, NY) was adjusted using a DRC-100X digital radiometer (Spectroline) as described previously (3).

PREPARATION OF THE EXTRACT AND ISOLATION OF KAEMPFEROL GLYCOSIDES

The dried flowers of *P. persica* were extracted with 80% aqueous ethanol according to the previous report (3). The extract (Ku-35) was filtered and dried *in vacuo*. For *in vivo* study,

the dried residue was mixed with an oil-based vehicle and applied to the animal skin. For isolating the constituents, the residue was dissolved in a small amount of methanol. The dissolved residue was poured into a silica gel column and eluted with chloroform: methanol:water (10:3:0.5) as a mobile phase, giving ten subfractions. Subfraction 7 was dried and further separated in the RP-18 column using methanol:water (1:1), giving compound I. From subfraction 5, the repeated silica gel column chromatography yielded compound II. From subfraction 4, the repeated silica gel column chromatography and subsequent separation with the RP-18 column using methanol:water (55:45) gave compounds III and IV.

Compound I: Recrystallized from methanol (yellowish amorphous powder). ^{1}H-NMR (DMSO-d_6) δ 12.61 (s, 1H, 5-OH), 7.76 (d, 2H, J = 8.6 Hz, H-2′ and 6′), 6.93 (d, 2H, J = 8.6 Hz, H-3′ and 5′), 6.41 (s, 1H, H-8), 6.21 (s, 1H, H-6), 5.18 (s, 1H, rhamnosyl anomeric H), 4.30 (d, 1H, J = 8.0 Hz, glucosyl anomeric H), 0.90 (d, 3H, J = 5.6 Hz, rhamnosyl-CH_3). For ^{13}C-NMR, see Table I. Acid hydrolysis products: kaempferol, glucose, rhamnose.

Compound II: Recrystallized from methanol (yellowish platelets), m.p. = 233°–235°C, ^{1}H-NMR (DMSO-d_6) δ 12.70 (brs, 1H, 5-OH), 8.06 (d, 2H, J = 8.0 Hz, H-2′ and 6′), 6.86 (d, 2H, J = 8.0 Hz, H-3′ and 5′), 6.42 (d, 1H, J = 2.0 Hz, H-8), 6.19 (d, 1H, J = 2.0 Hz, H-6), 5.40 (d, 1H, J = 7.6 Hz, galactosyl anomeric H). Acid hydrolysis products: kaempferol, galactose.

Compound III: Recrystallized from acetone (yellowish needles), m.p. = 174°–177°C, ^{1}H-NMR (DMSO-d_6) δ 12.63 (s, 1H, 5-OH), 7.75 (d, 2H, J = 8.6 Hz, H-2′ and 6′), 6.91 (d, 2H, J = 8.6 Hz, H-3′ and 5′), 6.41 (d, 1H, J = 2.0 Hz, H-8), 6.20 (d, 1H, J = 2.0 Hz, H-6), 5.29 (s, 1H, rhamnosyl anomeric H), 0.78 (d, 3H, J = 5.5 Hz, rhamnosyl-CH_3). Acid hydrolysis products: kaempferol, rhamnose.

Compound IV: Recrystallized from acetone (yellowish prisms), m.p. > 250°C, ^{1}H-NMR (DMSO-d_6) δ 12.61 (brs, 1H, 5-OH), 8.04 (d, 2H, J = 8.8 Hz, H-2′ and 6′), 6.88 (d, 2H, J = 8.8 Hz, H-3′ and 5′), 6.42 (s, 1H, H-8), 6.20 (s, 1H, H-6), 5.45 (d, 1H, J = 7.0 Hz, glucosyl anomeric H). Acid hydrolysis products: kaempferol, glucose.

HPLC ANALYSIS

The contents of the isolated flavonoids in crude extract (Ku-35) were measured using HPLC (Shimadzu, Japan) equipped with a reverse-phase ODS-II column (4.6 × 150 mm, Shinwa Chem.) and UV_{360} nm. The solvent gradient [3%→90%, 1% acetic acid in water: 1% acetic acid in acetonitrile] was used as a mobile phase at 1 ml/min for 75 min. The retention times for compounds I–IV were found to be 34.6, 38.3, 33.2, and 36.3 min, respectively.

IN VIVO ERYTHEMA TEST

Dorsal hairs of guinea pigs were shaved and depilated by application of Nair®. After 4 hr, the plastic film having six circular holes was wrapped with rubber bands around each animal. Test compounds, including Ku-35 pre-mixed with an oil-based vehicle, were applied (20 mg of compound plus vehicle/1.1 cm^2/site). Control sites received only 20 mg of vehicle/site. Five hours later, the sites were irradiated with UVB (1 J/cm^2,

Table I
^{13}C-NMR Assignments of the Isolated Flavonoid Derivatives

Compound	I	II	III	IV
C-2	157.3[a]	156.4[d]	157.3	156.5
3	134.5	133.2	134.2	133.2
4	177.8	177.5	177.8	177.5
5	161.4	161.2	161.4	161.2
6	98.9	98.8	98.8	98.8
7	164.6	164.2	164.5	164.5
8	93.9	93.7	93.8	93.7
9	156.6[a]	156.3[d]	156.6	156.2
10	104.1	103.8	104.1	103.9
1′	120.6	120.9	120.6	121.0
2′	130.7	131.0	130.7	130.9
3′	115.5	115.0	115.4	115.1
4′	160.1	159.9	160.0	160.0
5′	115.5	115.0	115.4	115.1
6′	130.7	131.0	130.7	130.9
Rham-1	102.0		101.8	
2	70.3[b]		70.1	
3	69.8[b]		70.3	
4	82.0		71.1	
5	69.0[b]		70.7	
6	17.3		17.5	
Glu-1	104.8			100.9
2	74.5			74.2
3	76.7[c]			77.5[e]
4	69.8[b]			69.9
5	77.0[c]			76.4[e]
6	61.0			60.8
Gal-1		101.7		
2		71.2		
3		73.1		
4		67.9		
5		75.8		
6		60.2		

All NMR spectrums were measured in DMSO-d_6.
[a,b,c,d,e] Interchangeable values in each column.

irradiation time: 3 min) and the formation of erythema was observed regularly up to 72 hr according to the criteria previously described (5).

IN VIVO EAR EDEMA TEST

Ku-35 pre-mixed with an oil-based vehicle was applied to the ears of mice. After 1 hr, mice lightly anesthetized with ether were irradiated with UVB (4 J/cm^2, irradiation time: 12 min). Twenty hours later, the ear thickness of each mouse was measured with a dial thickness gauge (Lux Scientific Instrument, USA) after washing out the applied vehicle. The increase in ear thickness after UV irradiation was regarded as edema formation.

STATISTICS

All data were represented as arithmetic mean ± S.D. The statistical significance was

evaluated with one-way ANOVA. P values less than 0.05 were regarded as significantly different.

RESULTS AND DISCUSSION

In order to develop new plant materials providing protection from UV-induced skin damage for safer and long-term use, *P. persica* extract (Ku-35) was evaluated in *in vivo* animal models that included UVB-induced erythema and edema formation.

From the flowers of *P. persica*, several constituents such as afzelin, quercitrin, multiflorin A and B, multinoside A, and chromogenic acid were previously isolated (6). In the present investigation, four flavonol glycosides (compounds I–IV) were successfully isolated from the extract. Compound I was structurally confirmed based on ^{1}H-, ^{13}C-, and COZY NMR analysis as kaempferol 3-O-[β-D-glucopyranosyl(1→4)-α-L-rhamnopyranoside] or multiflorin B. The ^{13}C-NMR spectrum was well matched with the results of that previously described (7,8). Multiflorin B was initially isolated from *Rosa multiflora* (9). Since a glucopyranosyl(1→4)rhamnopyranosidic bond of flavonoid glycoside is very rate in plants, compound I may be used as a standard compound for the *P. persica* extract. Compounds II and III were structurally identified as kaempferol-3-O-β-D-galactopyranoside (trifolin) and kaempferol-3-O-α-L-rhamnopyranoside (afzelin), respectively, by comparison of the spectral results of the published data (9,10). Compound IV was directly compared with an authentic standard isolated previously from the flowers of *Carthamus tinctorius* (11) and identified as kaempferol-3-O-β-D-glucopyranoside (astragalin). Figure 1 demonstrates the elution profiles of the flavonoid

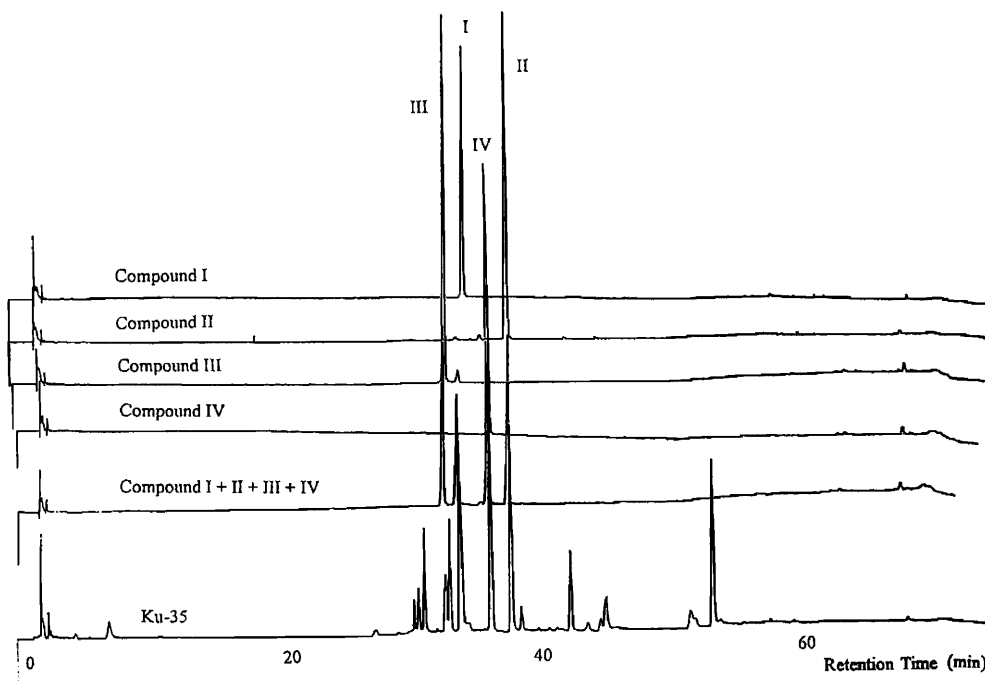

Figure 1. HPLC elution profiles of the isolated flavonoids and *P. persica* extract.

Table II
The Contents of Kaempferol Glycosides in the Extract (Ku-35) of the Flowers of *P. persica*

Compound	I	II	III	IV
Contents (%)	3.3 ± 0.5	0.6 ± 0.2	1.8 ± 0.2	2.0 ± 0.3

From 1 g of the dried flowers of *P. persica,* 120 ± 25 mg of dried Ku-35 were obtained (n = 3). The contents represented here are percentages of the isolated compounds based on the weight of Ku-35 (w/w, n = 3).

glycosides isolated and the extract (Ku-35) in HPLC. The contents of compounds I–IV in Ku-35 are represented in Table II. Among the four kaempferol glycosides, the multiflorin B content was highest. This result also supports our suggestion that multiflorin B may be used as a standard compound of Ku-35.

Table III demonstrates the protective effects of Ku-35 on UVB-induced erythema formation in guinea pig skin. In this experiment, the erythema formation gradually increased up to 6 hr. After this point, the intensity did not drastically change until 72 hr. Ku-35 dose dependently inhibited the erythema formation up to 72 hr. The IC_{50} value of Ku-35 was determined to be 0.5 mg/cm^2 at 6 hr after UVB irradiation by linear regression analysis, while indomethacin (0.1 mg/cm^2) showed significant inhibition of the erythema formation at early time points. At 5.0 mg/cm^2, Ku-35 completely blocked the erythema formation (Figure 2). It is noteworthy that the inhibitory effect of Ku-35 at concentrations $\geq$ 2 mg/cm^2 was well maintained during the experiment, whereas the inhibitory activity by indomethacin gradually decreased from 24 hr after UVB irradiation up to 72 hr (data not shown). Multiflorin B also inhibited the erythema formation (80%) at the dose of 0.3 mg/cm^2, suggesting that this compound is one of the active principles of Ku-35. When the inhibitory effects of Ku-35 on UVB-induced ear edema were evaluated, they also showed reduction of ear edema at doses of 0.3–3.0 mg/ear (49% inhibition at 3.0 mg/ear), as shown in Figure 3. All results obtained above clearly demonstrated for the first time, that Ku-35 protected the formations of UVB-induced erythema and edema *in vivo* by topical application.

Previously, Ku-35 inhibited the release of arachidonic acid (AA) metabolites from UVB-induced human keratinocytes in culture (3). The inhibitory activities of Ku-35 against *in vivo* UVB-induced erythema and edema formation may be partly due to the inhibitory activity of the release of AA metabolites, since AA and its major metabolite,

Table III
Effect of *P. persica* Extract (Ku-35) on UVB-Induced Erythema in Guinea Pigs

	mg/cm^2	1 hr	4 hr	6 hr	72 hr
UVB	—	1.0 ± 0.0	2.0 ± 0.0	3.0 ± 0.0	2.5 ± 0.5
Indomethacin	0.1	0.0 ± 0.0*	0.5 ± 0.5*	1.0 ± 0.7*	2.0 ± 0.5
Ku-35	0.2	0.8 ± 0.4	1.3 ± 0.4	2.0 ± 0.7	2.5 ± 0.7
	1.0	0.3 ± 0.4	0.5 ± 0.5*	0.8 ± 0.4*	1.0 ± 0.7
	2.0	0.0 ± 0.0*	0.3 ± 0.4*	0.5 ± 0.5*	0.5 ± 0.5*
	5.0	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*
Multiflorin B	0.3				0.5 ± 0.5*

Numericals in this table indicate the degree of erythema: 0 = none, 1 = mild, 2 = moderate, and 3 = severe (n = 4).
* $P < 0.05$, significantly different from UVB-irradiated group by one-way ANOVA test.

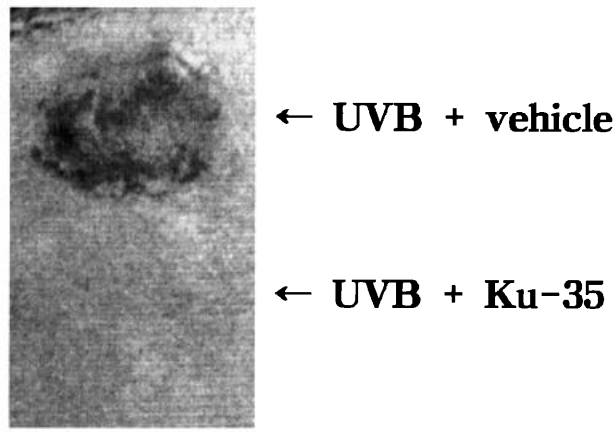

Figure 2. Photograph of UVB-induced erythema in guinea-pig. Ku-35 (5.0 mg/cm^2) was applied. This figure represents the complete protection of Ku-35 at 6 hr after UVB irradiation.

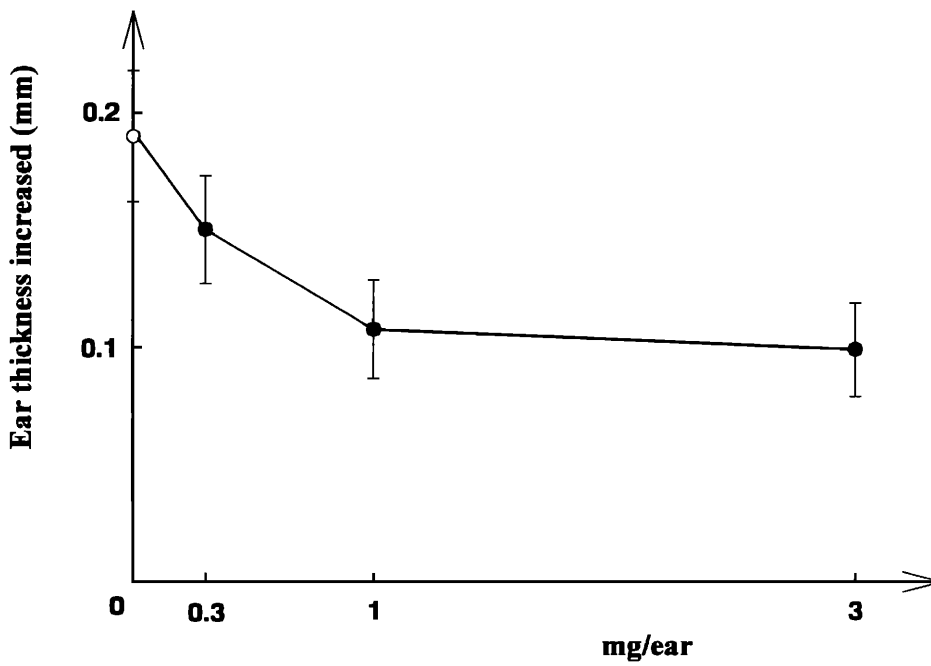

Figure 3. Effects of *P. persica* extract (Ku-35) on UVB-induced ear edema in mice. UVB-irradiated control (○). Ku-35 (●). No statistically significant difference among Ku-35 treated groups was observed (n = 5).

prostaglandin E_2 (PGE_2), are well related with inflammatory responses including erythema and edema. We also described that Ku-35 inhibited UVB- and C-induced DNA breakage in fibroblasts and UVB-induced skin carcinogenesis in SKH-1 mice by topical treatment (4). The major constituents of Ku-35 were found to be kaempferol glycosides (flavonoid). Therefore, the well-known anti-oxidative and UV-filtering activity of flavonoids may contribute to the inhibition of UV-induced skin damage by Ku-35.

CONCLUSIONS

The extract (Ku-35) of flowers of *P. persica* were prepared, and four flavonoid glycosides (multiflorin B, trifolin, afzelin, and astragalin) were isolated. Ku-35 protected the formation of UVB-induced erythema and edema in animal models. Ku-35 may be a new cosmetic ingredient for protection from UVB-induced skin damage by topical application.

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