Regular Article

The Standardized Extract of *Juniperus communis* Alleviates Hyperpigmentation *in Vivo* HRM-2 Hairless Mice and *in Vitro* Murine B16 Melanoma Cells

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In European folk medicine, the fruits of *Juniperus communis* are used in the treatment of skin-related disorders such as skin infection, itching, and psoriasis. Previously, we reported that the EtOAc fraction of *J. communis* (EAJC) contained tyrosinase inhibition properties *in vitro* non-cellular experiment. The aim of this study was to evaluate anti-melanogenic effect of standardized EAJC on a hyperpigmentation animal model. Therapeutic effects of EAJC toward skin hyperpigmentation were confirmed by both *in vivo* experiment and *in vitro* cell-based assay. Skin depigmenting effect was detected by topical treatment of EAJC for 11d to HRM-2 melanin-possessing hairless mice. Histologic findings including significantly decreased melanin depositions could be observed in dorsal skin samples of EAJC-treated group. In addition, the EAJC (50 µg/mL) attenuated melanin production through down-regulation of tyrosinase activity and protein expression in B16 murine melanoma cells. According to the phytochemical analysis, EAJC was found to contain hypolaetin-7-*O*-β-D-xylopyranoside and isoscutellarein-7-*O*-β-D-xylopyranoside as main components. Hypolaetin-7-*O*-β-D-xylopyranoside was responsible for the skin-lightening effect of EAJC by reducing the number of melanocytes in dorsal skins of HRM-2 mice. The present study provided direct experimental evidence for skin-lightening effect of EAJC in UV-irradiated hairless mouse model. Therapeutic attempts with the *J. communis* might be useful in the management of skin pigmentation-related diseases.

Key words *Juniperus communis*; anti-melanogenesis; HRM-2 melanin-possessing hairless mouse; melanin content; tyrosinase inhibition; hypolaetin-7-*O*-β-D-xylopyranoside

Melanogenesis is a process leading to the formation of melanin pigments which is responsible for skin color under the normal physiology.¹⁾ Synthesized melanin in the melanocyte of skin distributes to the keratinocytes where it primarily plays role for the protection against harmful UV irradiation.¹⁾ Excellent photochemical properties of melanin is well suited to absorbing UV thus protecting skin from further damage.²⁾ However, excessive melanin synthesis and accumulation can cause serious pathologic and esthetic problems including freckles, age spots, and melasma, which may result in skin problems.³⁾ Thus, the strategy to modulate melanin synthesis has been interesting target for managing problem associated with excessive skin pigmentation.

Melanin is synthesized by a series of enzymatic reactions.¹⁾ A number of specific enzymes are needed to produce melanin in the melanocytes. Among them, tyrosinase and tyrosinase related proteins are critical for the production of melanin.⁴⁾ Tyrosinase, which initiates the melanogenesis by catalyzing oxidation of tyrosine to dopaquinone, has been interesting target because it is the rate-limiting enzyme for the synthesis of melanin. Due to its pivotal role in melanogenesis, the identification of tyrosinase inhibitors has been of considerable interest.⁴⁾ As a result of research efforts, naturally occurring and synthesized tyrosinase inhibitors have been identified.⁵⁾ In addition, these tyrosinase inhibitor has been applied in the medication, cosmetic industry, and even in the food industry as an anti-browning agent.^{6,7)} Although several whitening agents

such as kojic acid and hydroquinone has been developed commercially, their cytotoxic and harmful problems became an object of concern.⁸⁾ Therefore, effective whitening agents with more safety are needed.

Common juniper, *Juniperus communis* L. (Cupressaceae), is one of the gymnosperms which shows a continuous distribution in the Holarctic.⁹⁾ Diverse biological effects of *J. communis* including antiseptic, diuretic, and anti-inflammatory properties have been reported.^{10,11)} The fruits of *J. communis* are specifically used in traditional folk medicine for skin injuries and problems such as boils, skin infection, and itching.^{11–14)} However, little scientific research has been performed to reveal the anti-pigmentation effect of Juniper berries. According to our current pharmacological studies, the EtOAc fraction of *J. communis* significantly inhibited mushroom tyrosinase *in vitro*.¹⁵⁾ On the basis of previous findings, we investigated direct effect of the *J. communis* EtOAc extract on hyperpigmentation in animal skins exposed to UVB. To elucidate the anti-melanogenic mechanism of *J. communis*, melanin contents and tyrosinase inhibitions were evaluated using B16F10 melanoma cells.

MATERIALS AND METHODS

Chemicals Mushroom tyrosinase, L-tyrosine, L-3,4-dihydroxyphenylalanine (dopa), α-melanocyte stimulating hormone (α-MSH), and other reagents were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Antibodies against tyrosinase and β-actin were purchased from Santa Cruz Biotechnology

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(Santa Cruz, CA, U.S.A.).

Plant Material and Sample Preparation The fruits of *J. communis* were purchased from herbmoul and the plant material was identified by Prof. Eun Ju Jeong of Department of Agronomy and Medicinal Plant Resources, Gyeongnam National University of Science and Technology. A voucher specimen (PNU-0020) has been deposited in the Medicinal Herb Garden, Pusan National University. The EtOAc fraction of *J. communis* extract (EAJC) were prepared as previously described.¹⁵⁾

In Vivo Depigmenting Activity Test The *in vivo* depigmenting efficacies of EAJC and hypolaetin-7-*O*- β -D-xylopyranoside were assessed in animal experiments that were performed in accordance with the guidelines for animal experimentation issued by Pusan National University (PNU-2016-1229). Six-week-old male HRM-2 melanin-possessing hairless mice were obtained from Hoshino Laboratory Animals (Yashino, Saitama, Japan) and housed in a controlled room (23 $\pm$ 1°C, 55 $\pm$ 5% humidity, 12-h light/dark cycle) with *ad libitum* access to water and standard laboratory diet. After an acclimation period (1 weeks), mice were randomly divided into 6 groups of 5 animals. EAJC (5, 10, 50 μ g/mL) and hypolaetin-7-*O*- β -D-xylopyranoside (50 μ M) were prepared in a solution of propylene glycol and ethanol (3:7). Kojic acid solution (50 μ M) was also prepared in same solution, and used as a positive control. Each solution (200 μ L) was topically applied to a designated site (3 $\times$ 3 cm) on the dorsal skin once daily. Animals were exposed to UVB in a CROSSLINKER (BEX-800, Ultra-Lum, Inc., Claremont, CA, U.S.A.) at 50 mJ/cm² according to experimental schedule. Colors of skin sites were measured using a CR-10 spectrophotometer (Konica Minolta Sensing, Inc., Sakai, Osaka, Japan), which describes colors using *L** values as described by the Commission Internationale de l'Eclairage color system.

Cell Culture System The anti-melanogenic effects of EAJC were further tested using B16F10 murine melanoma cells. Murine melanoma B16F10 cells were obtained from the American Type Culture Collection (Manassas, VA, U.S.A.). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS; Gibco, NY, U.S.A.) and penicillin/streptomycin (100 IU/50 μ g/mL) in a CO₂ incubator (Panasonic, Japan) with 5% CO₂ at 37°C.

Cell Viability Assay Cell viability assays were carried out using Ez-cytox cell viability test kit (Dogen Bio, Korea). Briefly, B16F10 cells (1 $\times$ 10⁴) were plated in each well of a 24-well plate, treated with EAJC at different concentrations for 24 h, and water soluble tetrazolium salt-1 was added. The derivative formed by cellular dehydrogenase was solubilized in a medium of the cells, and absorbance was measured at 450 nm using a microplate reader.

Determination of Melanin Contents Amounts of total melanin present in B16 cells were used as an index of melanogenesis. Briefly, B16F10 cells were plated on a 6-well dish at a density of 5 $\times$ 10⁴ cells/mL and incubated in the presence or absence of 10 μ M α -MSH. Cells were then incubated for 24 h with or without EAJC at different concentrations. After washing twice with phosphate buffered saline (PBS), cells were dissolved in 500 μ L of 1 N NaOH, incubated at 60°C for 1 h, and mixed to solubilize the melanin. Total melanin contents were determined by measurement of absorbance at 405 nm using a microplate reader.

Measurement of Cellular Tyrosinase Activity Tyrosinase activity in B16F10 cells was examined by measuring the rate of oxidation of L-DOPA. B16F10 cells were plated in 6-well dish at a density of 5 $\times$ 10⁴ cells/mL, incubated in the presence or absence of 10 μ M α -MSH, and treated for 24 h with various concentrations of EAJC. Cells were then lysed in 500 μ L of 50 mM sodium phosphate buffer (pH 6.8) containing 25 μ L of 1% Triton X-100 and 25 μ L of 0.1 mM phenylmethylsulfonyl fluoride, and frozen at -80°C for 30 min. After thawing and mixing, cellular supernatants were obtained by centrifugation at 12000 $\times g$ for 30 min at 4°C. Supernatants (80 μ L) and 20 μ L of L-DOPA (2 mg/mL) were placed in a 96-well plate, and the absorbance at 492 nm was read every 10 min for 1 h at 37°C using a microplate reader. The changes in absorbance were calculated and used as cellular tyrosinase activity.

Western Blotting Cell lysates for Western blotting were prepared using RIPA protein extraction solution (TransLab, Korea) with addition of protease inhibitor mixture (GenDEPOT, TX, U.S.A.) just before use. Cell lysates (20 μ g of protein each) were boiled for 5 min in gel-loading buffer (0.06 M Tris-HCl, pH 6.8, 2% sodium dodecyl sulfate (SDS) 5% 2-mercaptoethanol, and 0.1% bromophenol blue). Samples were then separated by SDS-polyacrylamide gel electrophoresis in 10% acrylamide gels and transferred to polyvinylidene fluoride (PVDF) membranes, which were immediately placed in blocking buffer (5% non-fat milk) containing 10 mM Tris (pH 7.5), 100 mM NaCl, and 0.1% Tween 20 for 1 h. Membranes were then incubated with specific primary antibodies at 4°C overnight followed by horseradish peroxidase (HRP)-conjugated anti-mouse antibody (Santa Cruz, 1:10000), or an anti-goat antibody (Santa Cruz, 1:10000) at 25°C for 1 h. Resulting immunoblots were visualized using Western Bright Peroxide solution (Advansta, CA, U.S.A.) and Davinch-chemi CAS-400 (Davinch-K, Seoul, Korea), according to the manufacturers' instructions.

Chromatographic Conditions The EAJC sample was analyzed using an Agilent 6530 Accurate-Mass Q-TOF LC/MS system (Agilent Technologies) for phytochemical characterization. A Poroshell 120 EC-C18 column (3.0 $\times$ 100 mm, 2.7 μ m, Agilent) was used for analysis at a flow rate of 0.3 mL/min. The mobile phase consisted of acetonitrile (solvent A) and water (solvent B), using a linear gradient elution: 5–95% A (0–20 min); 10% A (20–30 min). All acquisitions were performed under positive ionization mode. Mass spectra were recorded across the range *m/z*=100–1500 with accurate mass measurement of all mass peaks.

Statistical Analysis Data are expressed as the mean $\pm$ standard deviation (S.D.). The values were expressed as percent changes from the mean value of the control experiment. Statistical analyses were performed by a one-way ANOVA using Statistical Package. *p* values less than 0.05 were considered statistically significant.

RESULTS

Inhibitory Effects of EAJC on UV-Induced Skin Pigmentation *in Vivo* Experiments were carried out using HRM-2 hairless mice to determine the inhibitory effects of EAJC on skin pigmentation *in vivo*. The mice treated with vehicle or EAJC/vehicle for 11 d resulted in no adverse reaction

Fig. 1. Effects of EAJC on Pigmentation in Animal Skins Exposed to UVB

HRM-2 mice were treated with vehicle or EAJC on dorsal skin for 11 d and animals were irradiated six times at the indicated doses (A); Changes in the L^* (lightness) values of exposed skins. Skin lightness was measured prior to sample or vehicle application daily. (B); Graph showing changes in L^* values [ΔL values were defined as average values after final UVB exposure (day 11) minus average baseline values before treatment (day 1)]. $^{\#}p<0.001$ for control versus UV. $^*p<0.05$ and $^{**}p<0.01$, $^{***}p<0.001$ for UV versus test groups (C). The photographs indicate pigmentation differences between the dorsal skins (D).

Fig. 2. Effects of EAJC on the Histological Changes of Skin Pigmentation in HRM-2 Melanin-Possessing Hairless Mice

Fontana–Masson staining was carried out with the dorsal skin sections. After skins were excised from each mouse, they were fixed in 4% paraformaldehyde overnight at room temperature and stained for melanin using a Fontana–Masson staining kit.

or skin irritation. Obvious skin pigmentation was induced by irradiation of UVB light (50 mJ/cm², total 6 times in accordance with experimental schedule) (Fig. 1A). Repeated UVB exposure gradually decreased in L^* values, which represents a significant skin pigmentation (Fig. 1B). EAJC treatment dose-dependently inhibited the development of skin pigmentation in the concentration range of 5–50 µg/mL (Fig. 1B). Changes in L^* value, ΔL , was calculated by subtracting the L^* value of the first day from that of the last day and shown in Fig. 1C. The ΔL of UVB group was distinctly regressed after treatment of 50 µg/mL of EAJC (Fig. 1C). Additional Fontana–Masson staining was performed to confirm the inhibitory effect of EAJC on skin pigmentation of hairless mice. As a result, EAJC (50 µg/mL)+UVB treated mice showed less pigment spot of melanin than that of control mice (Figs. 1D, 2).

Effect of EAJC on Melanin Contents in α -MSH-Treated B16F10 Melanoma Cell As shown in Fig. 3A, EAJC did not cause any cell cytotoxicity at the highest concentration of 50 µg/mL in murine B16F10 melanoma cells. Upon exposure to 10 µM α -MSH alone, the melanin contents of B16F10 cells was elevated to 2.7-fold of vehicle control (Fig. 3B). The amount of melanin produced by α -MSH was dose-dependently reduced by treatment of EAJC (5, 10, 50 µg/mL). At a concentration of 50 µg/mL, EAJC significantly attenuated the melanin formation by 37.6% compared to α -MSH-stimulated control cells ($p<0.01$). The highest concentration of EAJC (50 µg/mL) was more efficient than the positive control, 50 µM kojic acid (22.3% decrease, $p<0.05$).

Tyrosinase Inhibitory Activity of EAJC in α -MSH-Treated B16F10 Melanoma Cells In order to investigate the tyrosi-

Fig. 3. Effects of EAJC on Cell Cytotoxicity (A), Melanin Contents (B), and Cellular Tyrosinase Activity (C) in B16F10 Murine Melanoma Cells

Cells were exposed to 10 µM α -MSH in the presence or absence of samples at different concentrations (5, 10, 50 µg/mL). Kojic acid (50 µM) was used as positive control. Values are means $\pm$ S.D. of triplicate experiments; [#] $p < 0.001$ compared to the control and ^{*} $p < 0.05$ and ^{**} $p < 0.01$ compared to the α -MSH-treated cells.

nase inhibitory effects on *J. communis*, various concentrations of EAJC (5, 10, 50 µg/mL) were applied to B16F10 cells in the presence of α -MSH. Cellular tyrosinase activity was increased

upto 1.87-fold of control by 24h treatment of α -MSH (10 µM) (Fig. 3C). When the α -MSH and EAJC sample were treated together, the activity of tyrosinase was obviously decreased in comparison to the α -MSH-treated control group. At the highest concentration of 50 µg/mL of EAJC, the percent inhibition of tyrosinase activity was decreased to 48% ($p < 0.05$) compared to the control (100%). Kojic acid at a dose of 50 µM also reduced tyrosinase activity, which was similar to the result of 50 µg/mL of EAJC.

Effect of EAJC on Tyrosinase Protein Expression in α -MSH-Treated B16F10 Melanoma Cells Additional experiment was carried out to identify whether EAJC affects tyrosinase protein expression in B16F10 murine melanoma cells. Tyrosinase expression induced by 10 µM of α -MSH was 1.5-fold greater than that of control cells. The α -MSH-stimulated increase in tyrosinase expression was reversed by treatment of EAJC (Fig. 4A). A dose-dependent decrease in tyrosinase protein level was observed in the EAJC-treated cells (Fig. 4B). EAJC (50 µg/mL) statistically inhibited tyrosinase expression in B16F10 melanoma cells by 52% ($p < 0.05$). Under similar conditions, kojic acid showed no significant inhibitory effect.

The Standardization of EAJC Using HPLC/MS For the simultaneous determination of major constituents of *J. communis*, the optimized chromatographic condition has been investigated. The optimal mobile phase consisting of acetonitrile/water was subsequently employed for the analysis of EAJC led to a good resolution and satisfactory peak shape. The presence of five compounds 1: hypolaetin-7-*O*- β -D-xylopyranoside (m/z 433.08 at t_R 7.4 min), 2: isoscutellarein-7-*O*- β -xylopyranoside (m/z 417.08 at t_R 8.1 min), 3: apigenin (m/z 269.04 at t_R 10.3 min), 4: cupressuflavone (m/z 537.08 at t_R 10.5 min), and 5: podocarpusflavone A (m/z 551.09 at t_R 14.1 min) in EAJC was verified by comparing each retention time and UV spectrum with those of each standard compound and spiking with authentic standards (Fig. 5).

Inhibitory Effects of Hypolaetin-7-*O*- β -D-xylopyranoside from EAJC on UV-Induced Skin Pigmentation *in Vivo* Skin hyperpigmentation was observed on the dorsal skin in HRM-2 mice after UVB irradiation, whereas treatment with 50 µM hypolaetin-7-*O*- β -D-xylopyranoside from EAJC tended to reduce the visible pigmentation (Fig. 6). The L^* value was markedly decreased in the UVB-treated group compared with the control group. After 10d of treatment, hypolaetin-7-*O*- β -D-xylopyranoside (50 µM) remarkably elevated the L^* to 40% of UVB control (Figs. 6A, B). Histological analysis further confirmed the depigmenting effect of hypolaetin-7-*O*- β -D-xylopyranoside. The number of melanocytes in dorsal skin regions was significantly decreased by treatment of hypolaetin-7-*O*- β -D-xylopyranoside, a major component of EAJC (Fig. 7).

DISCUSSION

Human skin hyperpigmentation is induced by diverse factors such as long-term UV irradiation, chronic inflammation, and α -MSH overexpression.^{16–18} Dermal and epidermal hyperpigmentation is associated with elevated number of melanocytes and activity of melanogenic enzymes.^{18,19} Three enzymes of melanogenesis, tyrosinase, tyrosine hydroxylase isoform I (THI), and phenylalanine hydroxylase (PAH), are

Fig. 4. Effects of EAJC on the Protein Expression of Tyrosinase in B16F10 Murine Melanoma Cells

Cells were exposed to 10 μ M α -MSH in the presence or absence of samples at different concentrations (5, 10, 50 μ g/mL). Kojic acid (50 μ M) was used as positive control. Values are means $\pm$ S.D. of triplicate experiments; [#] p < 0.001 compared to the control and ^{*} p < 0.05 compared to the α -MSH-treated cells.

Fig. 5. HPLC Chromatogram of EAJC (A) and Chemical Structures of Components (B)

Fingerprint analysis of EAJC was performed in positive ion mode using LC-TOF-MS. 1: hypolaetin-7-*O*- β -xylopyranoside, 2: isoscuteallarein-7-*O*- β -xylopyranoside, 3: apigenin, 4: cupressuflavone, and 5: podocarpusflavone A.

Fig. 6. Effects of Hypolaetin-7-O-β-D-xylopyranoside from EAJC on Pigmentation in Animal Skins Exposed to UVB

HRM-2 mice were treated with vehicle or hypolaetin-7-O-β-D-xylopyranoside (50 μM) on dorsal skin for 10 d and animals were irradiated six times at the indicated doses; Changes in the L^* (lightness) values of exposed skins. Skin lightness was measured prior to sample or vehicle application daily. (A); Graph showing changes in L^* values [ΔL values were defined as average values after final UVB exposure (day 11) minus average baseline values before treatment (day 1)]. # $p < 0.001$ for control versus UV. * $p < 0.05$ for UV versus test groups (B). The photographs indicate pigmentation differences between the dorsal skins (C).

Fig. 7. Effects of Hypolaetin-7-O-β-D-xylopyranoside from EAJC on the Histological Changes of Skin Pigmentation in HRM-2 Melanin-Possessing Hairless Mice

Fontana–Masson staining was carried out with the dorsal skin sections. After skins were excised from each mouse, they were fixed in 4% paraformaldehyde overnight at room temperature and stained for melanin using a Fontana–Masson staining kit.

considered to be essential for the beginning of melanin synthesis.^{19,20} In particular, tyrosinase is a key messenger in the regulation of skin pigmentation by mediating the conversion of L-tyrosine into L-DOPA.²¹ Thus, searching for new depigmenting agents from natural materials targeting tyrosinase might be a promising strategy for treating skin pigmentation-related disorders like melasma and solar lentigo.²²

Recently, EAJC was reported to inhibit mushroom tyrosinase activity in the non-cellular experiments.¹⁵ The present study was undertaken to verify its therapeutic efficacy in

the treatment of skin hyperpigmentation caused by UV irradiation. HRM-2 (melanin-possessing hairless mice) was used for the measurement of skin color in long-term experiment with EAJC. An appropriate animal model for cutaneous hyperpigmentation could be a valuable tool to evaluate and understand biological activities of depigmenting agents.^{23,24} HRM-2 mouse model developed by crossing hairless mice with C57BL/6J mice is regarded as being useful to find potential candidates for treating pigmentation and skin melanoma induced by UVB.^{24,25} Topical treatment of EAJC to HRM-2

mice for 11 d markedly suppressed skin pigmentation by 65% of UVB control at a dose of 50 $\mu\text{g/mL}$. A histological analysis of dorsal skin sections from HRM-2 mice also showed fewer melanin spots in EAJC-treated group compared with the vehicle control. Results indicated that EAJC effectively interrupted skin pigmentation in UVB-induced *in vivo* melanogenesis model, which was consistent with its *in vitro* mushroom tyrosinase inhibition.

To reveal further detail of the anti-melanogenic effect, the ability of EAJC to suppress melanogenesis was determined using α -MSH-stimulated murine melanocytes in the cell culture system. Since melanocyte stimulating hormone (MSH) is the major physiological stimulus for melanogenesis, the inhibition of α -MSH-induced melanin production is a promising target in the treatment of skin hyperpigmentation.^{26,27} A significant increase in the melanin contents was observed by treating α -MSH (10 μM) in the present study. EAJC (50 $\mu\text{g/mL}$) was efficient in decreasing α -MSH-stimulated melanin synthesis, which was mediated through down-regulation of tyrosinase activity in B16 melanoma cells. Many of the well-known skin-whitening agents such as arbutin, kojic acid, and hydroquinone are known to be strong tyrosinase inhibitors.^{28,29} In our experimental system, EAJC acted as a natural inhibitor of tyrosinase in both cell-free and cell-based assays. Whitening activity of EAJC also might be related to the inhibition of tyrosinase in α -MSH-stimulated B16 melanoma cells.

In the previous work, we isolated and identified eight flavonoids, namely apigenin, isoscutellarein-7-*O*- β -D-xylopyranoside, hypolaetin-7-*O*- β -D-xylopyranoside, amentoflavone, podocarpusflavone A, robustaflavone, cupressuflavone, and hinokiflavone, from EAJC.¹⁵ HPLC/MS analysis, using eight isolates as bioactive marker compounds, was performed to standardize the EAJC sample. The EtOAc fraction of *J. communis* was found to contain hypolaetin-7-*O*- β -D-xylopyranoside, isoscutellarein-7-*O*- β -D-xylopyranoside, apigenin, cupressuflavone, and podocarpusflavone A as major compounds. Among the eight isolates of EAJC, hypolaetin-7-*O*- β -D-xylopyranoside was responsible for tyrosinase inhibition of EAJC in the previous cell-free and cell-based assays.¹⁵ The present quantitative analysis revealed that hypolaetin-7-*O*- β -D-xylopyranoside (compound 1, 36.84%), the most potent tyrosinase inhibitory compound in EAJC, and isoscutellarein-7-*O*- β -D-xylopyranoside (compound 2, 35.39%) are main components of EAJC. Additional *in vivo* experiment was performed to observe the skin color changes in hypolaetin-7-*O*- β -D-xylopyranoside-treated mice. Hypolaetin-7-*O*- β -D-xylopyranoside from EAJC remarkably suppressed skin hyperpigmentation by achieving improved visible tone and reduction in the appearance of hyperpigmented spots. Histological analysis also showed that the compound attenuates pigment deposition by reducing the number of melanocytes in dorsal skin regions. Taken together, hypolaetin-7-*O*- β -D-xylopyranoside appears to be responsible for skin-lightening effect of EAJC.

In summary, efficient lightening effects were observed following topical application of EAJC and its active compound hypolaetin-7-*O*- β -D-xylopyranoside to UV-stimulated hyperpigmented dorsal skin of HRM-2 mice. According to microscopic histological examination, the anti-hyperpigmentation effects of EAJC and hypolaetin-7-*O*- β -D-xylopyranoside were due to depletion of melanin depositions. *Juniperus commu-*

nis reduced melanin contents by attenuating tyrosinase (key enzyme of melanogenesis) activity and expression in B16 melanoma cells. Further study is warranted to identify the therapeutic effects of EAJC against multiple exposures to UV light of human skin.

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Conflict of Interest The authors declare no conflict of interest

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