Journal of the Korea Convergence Society
Vol. 10. No. 7, pp. 267-279, 2019

ISSN 2233-4890
https://doi.org/10.15207/JKCS.2019.10.7.267

Effect of Photoprotective activities of Poncirustrifoliata immature Fruit extract and Naringin compound

Sang-Hee Park[1], In- Sil Kwak[2*]
[1]Professor, Division of Skin health, Daejeon institute Science and Technology
[2]Professor, Dept. of Beauty Art, Howon University

지실 추출물과 Naringin의 광방어 효과

박상희[1], 곽인실[2*]
[1]대전과학기술대학교 피부보건과 교수, [2]호원대학교 미용예술학과 교수

Abstract The study studied dermal protection and crease improvement from the sunlight of the lipid extract and narinjin. Sunlight was investigated in HR-1 (motherless mice) to identify changes in epithelial thickness and changes in collagen fibers, which account for around 90% of dermis, as the inhibitory efficacy of collagenase dissolving collagen also plays an important role in wrinkles. The experiment was validated using narinjin and jisil extract. First: The components of jisil and narinjin were analyzed. Second, antioxidant capabilities were confirmed with DPPH. Third: The inhibitory activity of collagen was measured. Studies have shown that the skin's upper skin thickness suppression of dermal extracts and narinzine has increased and that collagen thicknesses and wrinkles have decreased significantly compared to controls.

Key Words : Poncirustrifoliata immature Fruit, Naringin, Collagenase, Wrinkles, Anti-aging

요 약 본 연구는 지실추출물과 나린진의 햇빛으로부터 피부 보호 및 주름개선에 대한 연구를 하였다. HR-1(무모쥐)에 햇빛을 조사하여 상피 두께의 변화와 진피의 90% 정도를 차지하는 콜라겐 섬유의 변화를 확인하였다, 콜라게나아제의 억제 효능도 주름에 중요하게 작용하므로 같이 확인하였다. 실험은 나린진과 지실추출물을 사용하여 효능을 검증하였다. 첫째: 지실과 나린진의 성분을 분석하였다. 둘째: DPPH로 항산화 능력을 확인하였다. 셋째: 콜라겐의 변화를 측정하였다. 연구 결과로 지실추출물 및 나린진이 피부 상피두께 억제와 피부의 콜라겐 두께가 상승하였고 콜라겐 분해 효소 억제 및 주름이 대조군에 비하여 감소되었으며 유의한 차이가 나타났다.

주제어 : 지실 추출물, 나린진, 콜라게나아제, 주름, 광노화

1. Introduction

Nowadays, people are easily exposed to light aging and heat aging in their daily lives. Repeated exposure to sunlight causes photo-aging and UVB, the main culprit of skin aging, plays the biggest role in causing wrinkles. UVB irradiation destroys the function of various skin cells through DNA damage, by generating reactive oxygen species (ROS). In particular, ROS not only

This paper is written based on the doctoral dissertation of the first author.
*Corresponding Author : In-Sil Kwak(kwak3144@naver.com)
Received April 23 2019 Revised June 5 2019
Accepted July 20, 2019 Published July 28, 2019

destroys collagen directly or indirectly, but also plays an important role in the synthesis and collagen metabolism of MMPs[1]. In the case of photo-aging, it is known that skin be thickening, wrinkle increase, elasticity reduction, skin dryness increase will occur, plus it also has possibilities to produce freckles, and black mushrooms at the same time[2].

Collagen, the main constituent of skin cells, accounts for 70-80 percent of the dermis dry weight. Type I collagen accounts for 80% of the total collagen and type III collagen follows as 15%[3]. In general, the balance of synthesis of type I collagen and the activity of its decomposing enzyme, MMP-1, maintain the skin's elasticity[4], but if this balance breaks by aging and extraterrestrial factors, it is considered as the main element of wrinkles[5]. Various factors are involved in the active regulation of MMP. Among these factors, tissue inhibitors of metalloproteinase(TIMP) is the most representative natural inhibitor of MMP and are found in most tissues and body fluids with MMPs. Overproduction of TIMP inhibits its activity by binding it to the zinc ion part of the active MMP. This forms a stoichiometric complex through making noncovalent bond with MMP. The active-restraint reactions resulting from the balancing of molecular levels between MMP and TIMP works as a crucial factor to the physiological processes such as wound healing, tissue modification, and vessel production[6].

Poncirustrifoliata immature Fruit(unripe trifoliate orange) is an unripe fruit of trifoliate orange tree- which is the an evergreen small tree belonging to Rutaceae -, and is 1~2cm small and is harvested in May and June. It is grown in various southern regions in Korea[7]. There were various flavonoid glycosides contained in poncirustrifoliata immature fruit[8]. People living in this society spends a myriad of time and money pursuing beauty and health. As a measure of health and beauty, the skin acts as an important reference element.

In this study, in vivo experiment was conducted to measure the effects of naringin and Poncirustrifoliata immature Fruit extracts on animal models such as HR-1 mice which underwent photo-aging. And the effects of them - including suppressing epithelium thickness and increasing collagen fiber contents - were well verified. Plus, the photo-defense effect and wrinkle improvement effect of Poncirustrifoliata immature Fruit(PT-iF) and naringin were tested as well.

2. Methods

2.1 Research Method

2.1.1 Preparation of poncirustrifoliata immature fruits Extracts(PT-iF) and Naringin

Poncirustrifoliata immature fruits used in the experiment were purchased from oriental medicine supplier-Omniherb(www.omniherb.com, Korea). After completely drying up the fruits, 100g were grinded as powder form. By adding 1.0L water with 70% ratio, we extracted it again for 24 hours using Shaker(Dongwon Science Co., Busan, Korea).

2.1.2 Analysis of poncirustrifoliata immature fruits

HPLC analysis was conducted for the poncirustrifoliata immature fruits extracts. A solvent was 100% tertiary distilled water and B solvent was 100% acetonitrile, and each of them included 0.1% formic acid. Respectively, the solvent system was shown in Table 1 as 'solvent slope elution'. As a standard specimen, naringin was used as an indicator, and the components of PT_iF extract were analyzed.

2.1.3 Antioxidant activity by DPPH dissipation

For antioxidant activity testing by free radial dissipation of DPPH (2.2-diphenyl-1-picrylhydryzyl),

16 mg of DPPH was dissolved in a 100ml ethanol solution, heated for 20 seconds, and ethanol was added to reach the absorption level of 0.93~0.97 and ultimately became the DPP solution.

2.1.4 Collagenase Inhibitory Activity measurement

The collagenase inhibitory activity was measured to check the wrinkle improvement activity of the PT_iF in a test tube. Collagenase inhibitory activity is shown as the rate of reduction of absorbency of additives and additives to the specimen solution.

$$\text{Collagenase Inhibition rate of activity} = \frac{1 - Absorbance\ of\ the\ sample}{Absorbance\ of\ control} \times 100$$

2.2 In vivo: effect assessment on wrinkles caused by UVB on animal models(mice)

2.2.1 UVB Investigation and Induction of skin wrinkles

Table 1. UVB irradiation and medication schedule

	1 wk (times)	2 wk (times)	3 wk (times)	4 wk (times)	5 wk (times)	6 wk (times)	7 wk (times)	8 wk (times)	9 wk (times)	10 wk
No of UVB irradiation	7	3	3	3	3	3	3	3		
No. of drug treatment	7	7	7	7	7	7	7	7		
Wrinkle photographed						measurement			measurement sacrifice	

Table 2. Poncirus trifoliata Naringin in immature fruit and ointment cream formulations.

	2 % PT_iF cream	0.5% PT_iF cream	1%/0.5% Naringin cream
Water phase(%)			
Glycerine	10.00	10.00	10.00
Silicon derivatives	0.20	0.20	0.20
Microcide-C	0.20	0.20	0.20
EDTA-2Na	0.01	0.01	0.01
Keltrol F(1 %)	5.00	5.00	5.00
Sepiplus 400	0.30	0.30	0.30
Oil phase(%)			
Lanette O	2.00	2.00	2.00
Olive M 1000	1.00	1.00	1.00
Tego care 450	2.00	2.00	2.00
Puresyn 4	2.00	2.00	2.00
TCG-M	3.00	8.00	8.00
DC 200/6cs	2.50	2.50	2.50
Lipex Shea	1.00	1.00	1.00
Vitamin E	0.50	0.50	0.50
D-P	0.10	0.10	0.10
BHT	0.02	0.02	0.02
Stabilizer(%)			
TEA	0.10	0.10	0.10
DW	2.00	2.00	2.00
Additives(%)			
2 % PT_iF extract	2.00		
0.5 % PT_iF extract		0.5	
Naringin compound			1% or 0.25 %

Table 3. Primer sequences (wrinkle-related genes) for real-time PCR analysis.

Target gene	Primer/probe	Sequences
MMP-2	Forward	5'-CAG GGA ATG AGT ACT GGG TCT ATT-3
	Reverse	5'-ACT CCA GTT AAA GGC AGC ATC TAC-3'
MMP-9	Forward	5'-AAT CTC TTC TAG AGA CTG GGA AGG AG-3
	Reverse	5'-AGC TGA TTG ACT AAA GTA GCT GGA-3'
COX-2	Forward	5'-ATG GAT CGA AGA CTA CGT GCA A-3'
	Reverse	5'-GGG ATT TCC CAT AAG TCC TTT C-3'

Table 4. Primer sequences (wrinkle-related genes) for real-time PCR analysis.

Target gene	Primer/probe	Sequences
MMP-2	Forward	5'-CAG GGA ATG AGT ACT GGG TCT ATT-3
	Reverse	5'-ACT CCA GTT AAA GGC AGC ATC TAC-3'
MMP-9	Forward	5'-AAT CTC TTC TAG AGA CTG GGA AGG AG-3
	Reverse	5'-AGC TGA TTG ACT AAA GTA GCT GGA-3'
COX-2	Forward	5'-ATG GAT CGA AGA CTA CGT GCA A-3'
	Reverse	5'-GGG ATT TCC CAT AAG TCC TTT C-3'

2% and 0.5% of PT_iF, and naringin (1%, 0.25%) - which were made based on vehicle(30% ethanol, 70% polyethylene glycol) - were applied to the skin of the HR-1 mice and the mice were investigated under UVB at a distance of 15 cm for five to ten minutes.

2.2.2 Measurement of skin creases

Attach Double-Stick Disc to the skin of the mouse and spray the mixture using DETAX System II to see the degree of wrinkles by removing the fully hardened disc after 2-3 minutes. In the evaluation, grade 0 is defined as the case with no wrinkles, grade 1 is the case with a few shallow wrinkles, grade 2 is the case with some wrinkles, and grade 3 is defined as the case with several deep wrinkles[9].

2.2.3 Observation of the improvement of wrinkles on the skin

The skin tissue of anesthetized mice was filmed 400 times bigger to examine the changes in wrinkles in detail, by using USB Digital Microscope (x400, CE FOROHS, China). To compare wrinkles among the entire back area, left back and right back were separately photographed, and the left back pigmented areas were observed for crease formation and degree of improvement.

2.2.4 Analysis of the expression of the gene in the dorsal tissue related to wrinkles

Real-time PCR was performed to determine how much effect the UVB would have on the wrinkle related genes - matrix metalloproteinase MMP-2, MMP-9 mRNA - and skin damage related genes, IL-1β, IL-6, TNF-α, NOS-II and COX-2 mRNA.

2.2.4.1 RNA extraction

The skin of the mice which were pigmented were extracted(removed), and the cells were broke down with homogenizer with a trizol 500μl. Then 100μl of chloroform ($CHCl_3$) were added to it, and it was mixed again for 15 seconds. After leaving it on ice for 15 minutes, centrifugation was performed at 13,000 rpm, and about 200μl of the supernatant was recovered, mixed with 200μl of 2-propanol and left for 15 minutes. Again, RNA was extracted by centrifuging it at 13,000 rpm and then rehydrating it at 80% EtOH and drying it at the

vacuum pump for 3 minutes. The extracted RNA was dissolved in 20μl of distilled water treated with DEPC to activate the fire at 75°C of heating block and used for first strand cDNA synthesis.

2.2.4.2 reverse transcription

The total RNA 3 μg from the skin of the wrinkled mouse was denatured at 75°C for 10 minutes, followed by 2.5 μl of 10 mM dNTPs mix, 1 μl radium sequence hexanuculeides (25 mole/25 μl). 1 μl RNase inhibitor (20 U/μl), 1 μl 100 mM DTT, 4.5 μl 5 × RT buffer (250 mM Tris-HCl, pH 8.3, 375 mM KCl, 15 mM MgCl2)RNA inhibitor 1 μBi were additionally performed, M-MLV RT (200 U/μl) of 1 μl was finally applied and the final product volume had to reach 20μl. The reaction mixture of 20μl was mixed well, then the reaction mixture was then centrifuged for 5 seconds at 2,000 rpm to synthesize the first-strand cDNA at 37°C heating block for 60 minutes, and then left at 95°C for 5 minutes to deactivate the M-MLV RT before using the synthesized cDR PC.

2.2.4.3 Real-time RT-PCR

Real time quantitative PCR was performed using the Applied Biosystems 7500 Real-Time PCR system (Applied Biosystems, USA) and the primer sequence used in the experiment is as shown in Table 3 and 4. The manifest of mRNA gene in Table 3 and 4 used TaqMan probe (FAM di-labeled, ABi, USA) and GAPDH probe set; Endogenous Control (VIC® / MGB Probe, Probe limited) from Applied Biosystems (4352339E) was used as the internal standard. The concentration of SYBR primer was 200 nM. The conditions of real time PCR were pre-denaturation for 2 minutes at 50 °C, 10 minutes at 94 °C, and for 40 cycles for 1 minute at 95 °C. The experimental and control groups used GAPDH as their internal standard.

Table 5. Taqman probes and primer sequences (pro-inflammatory gene) for real-time PCR analysis.

Target gene	Primer/probe	Sequences
IL-1β	FAM	5'-CTGTGTAATGAAAGACGGCACACCCACC-3'
IL-6	Forward	5'-TCCAGTTGCCTTCTTGGGAC-3'
	Reverse	5'-GTACTCCAGAAGACCAGAGG-3'
TNF-α	FAM	5'-CACGTCGTAGCAAACCACCAAGTGGA-3'
NOS-II	Forward	5'-CAG CTG GGC TGT ACA AAC CTT-3'
	Reverse	5'-ATG TGA TGT TTG CTT CGG ACA-3'

2.2.5 Measurement of MMP-2 ELISA

Using the MMP-2 ELISA (R&D System, USA) kit for mice, the MMP-2 coating antibody was distributed 100μl to each of the microwell, left at 4°C for 16 hours, washed with a wash buffer, added 200μl of assay diluent water, left for 1 hour, and was cultivated in the normal temperature. After diluting the standard product, and diluting the culture supernatant for 20 times, we cleaned the microplate, added 100μl of each of the liquid, covered the well for two hours, and incubated them at room temperature. The microplate was cleaned, the working detector was made, 100μl was put into each well, blocked the well for one hour, and then cultivated at room temperature. The microplate was cleaned, the working detector was made, and 100μl was added to each well for an hour and were cultivated in a dark room at room temperature. Again, microplate was cleaned, suvstrate solution was made, and 100μl was added to each well for an hour and were cultivated in a dark room at room temperature. Finally, stop solution was made, and 50μl was added to each well and the absorbance was measured at the microplate spectrophotometer at 450nm.

2.3 Statistical processing

The result values of each group were statistically processed using T-test statistics program and a significance test was performed at a level of $p<0.05$.

3. Research Results and Reviews

3.1 In vivo: Effect of skin damage through UVB irradiation of PT-iF and naringin

3.1.1 Effects of PT-iF and naringin on the epithelial tissue thickness of HR-1 mice

In order to measure the effect of Naringin and PT_iF extract on skin wrinkle improvement, UVB was irradiated to the skins of HR-1 mice to make wrinkles, and the tissues of the skin were dyed with H&E after 8 weeks to easily find out the test result. When measuring the skin tissue's thickness with a microscope, UVB group's skin was 4 times thicker compared to control group (Fig. 3A~D). However, the application of 2% PT_iF extract (Fig. 5A-D), 0.5% PT_iF extract (Fig. 4A-D), 1% naringin(Fig. 6A-D) and 0.25% naringin (Fig. 7A-D) has brought a statistically meaningful decrease to epithelial tissue thickness when compared with UV_control, and especially the 1% naringin(Fig .6A-D) has brought a significant decrease, which decreased more than 64.3%.

3.1.2 Skin Collagen Fiber Changes in HR-1 mice due to PT-iF and Naringin

As a result of measuring the changes in collagen fiber, it is thought that the breakdown of collagen fibers and wrinkle formation has accelerated, since the strength of M-T dying was significantly decreased in the UVB_control group(Fig. 9A~B), when compared to normal group (Fig. 8A~B), as shown in Fig. 8-13. However, it showed that the amount of collagen fiber was increased by the application of 2% PT_iF extract(Fig. 11A-B), 1% naringin (Fig. 8A~B), and 0.25% naringin(Fig. 12A~B) when compared to the UVB_control group, and therefore it resulted in the improved in wrinkles.

3.1.3 Effects of PT-iF and Naringin on the Formation of Skin Wrinkle in HR-1 mice

8 weeks after inducing photo-aging on the HR-1 mice by irradiating UVB on them, the back skin of the mice were observed with digital camera (Fig. 13 to 18), and the back skin folds with digital microscope were observed 400x (Fig. 19 to 24). As a result, UVB_controls (Fig. 14A-B, Fig. 20A-D) had significantly bigger number of wrinkle formation compared to normal controls (Fig. 13A-B, Fig. 19A-D), as shown in Fig. 13-22. However, 2% PT_iF extract (Fig. 16A~B, Fig. 22A~D) and 1% naringin (Fig. 18A~B, Fig. The application of 24A-D) and 0.25% naringin (Fig. 17A-B, Fig. 21A-D) showed a significant decrease in dermal creases compared to controls

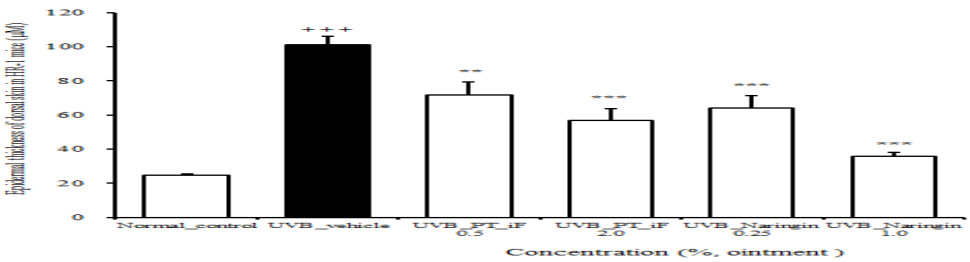

Fig. 1. Inhibition of skin thickening by PT_iF and naringin on epidermis induced by UVB irradiation.

HR-1 hairless male mice by UVB irradiation was performed using UVM-225D Mineralight UV Display Lamps(UVP, Phoenix, AZ, U.S.A.) emitting wavelength of 302 nm. UV strength was measured using a HD2102-2 UV meter(Delta OHM, Padova, Italy). UVB irradiation for 8 weeks with vehicle cream-ointment , UVB irradiation for 8 weeks with skin apply of 2% PT_iF-cream ointment(UVB_2% PT+iF), UVB irradiation for 8 weeks with skin apply of 0.5% PT_iF-cream ointment(UVB_0.5% PT+iF), UVB irradiation for 8 weeks with skin apply of 2% naringin-cream ointment(UVB_1% naringin), and UVB irradiation for 8 weeks with skin apply of 0.25% naringin-cream ointment(UVB_0.25% naringin). Dorsal skin Left part and Light part photograph image analysis. The HR-1 skin whitening values showing brightness of skin colour were measured using a Image analysis software(*Each system ships with 1 full version of Quantity One and unlimited copies of Quantity OneBasic Mode. Bio-Rad, USA*). Hematoxylin and eosin(H&E) staining of UVB-irradiated hairless mice skin. Original magnification 200x.

(UVB_control), and that PT_if and Naringin actually had significant improvement effect on wrinkle formation which occurred due to the photo-aging.

3.1.4 Analysis of Dermal Inflammation and Wrinkle-related Genetic Expression in the Dermal Damage-causing Pathological Model through UVB irradiation

3.1.4.1 Proflammatory cytokine analysis of HR-1 mice

To measure the effect of Naringin and PT_iF extracts on skin crease improvement, UVB was irradiated to the HR-1 mice group to induce wrinkles. 8 weeks later, they separated the skin tissues of the mice. With the result which came from investigating the inflammatory mRNA genes((IL-1β (A), IL-6(B), TNF-α(C)) and NOS-II(D), and making UVB control group's IL-1β, IL-6, TNF-α, and NOS-II mRNA gene expression's RQ value as 1, we analyzed the experimental grou's value of naringin and PT_iF extract.

As a result, UVB_control (UVB_vehicles) all showed increase more than two times in IL-1β-1, IL-6, TNF-α, and NOS-II mRNA gene expression. In the experimental group, the application of 1% naringin (UVB_Naringin 1.0) and 2% UVB_PT_iF2.0 extract showed statistically significant decrease in iL-1β, IL-6, TNF-α, and NOSII mRNA expression when compared to the control group (UVB_vehicles). ($p<0.01$, $p<0.001$). However, the 0.5% lUVB_PT_iF 0.50 extract and the application of 0.25% naringin (UVB_Naringin 0.25%) reduced the expression of IL-1β, IL-6, TNF-α, and NOS-II mRNA when compared to control group (UVB_vehicles), but no statistical significance was shown.

3.1.4.2 Analysis of MMP and related gene expression in HR-1 mice by PT-iF and naringin

To investigate the wrinkle improvement effect of Naringin and PT_iF extract, UVB was irradiated to the HR-1 mice. After 8 weeks, the tissues were separated from the body, and to figure out the wrinkle formation process due to the collagen decomposition, the expression of COX-2 (A), MMP-2 (B), and M-9PC-2 (MMPC) was examined. And, With the result which came from investigating the the expression of COX-2 (A), MMP-2 (B), and M-9PC-2 (MMPC), and making UVB control group's COX-2, MMP-2, and MMP-9 gene expression's RQ value as 1, we analyzed the experimental grou's value of naringin and PT_iF extract.

As a result, more likely as we can see from the expression of MMP-9 mRNA gene, the UVB_control group all showed a significant increase in the expression of COX-2, MMP-2, and MMP-9 mRNA when compared to the Normal control group, all more than two from four times. In the experimental group, COX-2, MMP-2, and MMP-9 mRNA expressions showed statistically significant decrease ($p<0.05$, $p<0.01$, $p<0.001$) due to the appliance of UVB_PT_iF2.0 and UVB_Naringin 1.0. However, UVB_PT_iF 0.50 extract and the application of 0.25% naringin (UVB_Naringin 0.25%) slightly decreased the expression of COX-2, MMP-2, and MMP-9 mRNA when compared to control group (UVB_vehicles), but no statistical significance was shown.

3.1.5 MMP-2 protein expression analysis in the skin-impaired pathological model through UVB irradiation

As a result of measuring MMP-2 protein production in order to observe the change in skin crease formation which is formed by dermal collagen decomposition due to UVB light aging, the production amount of PT_iF extract and naringin from the skin tissues were analyzed in UVB control group by studying MMP-2 protein expression. As a result, the amount of MMP-2 protein expression.

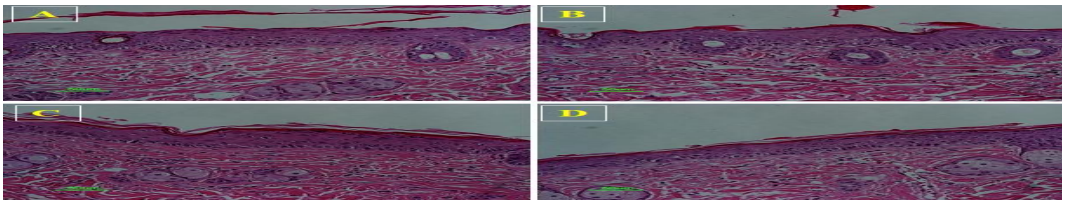

Fig. 2. Inhibition of skin thickening by vehicle control on epidermis induced by UVB irradiation.
HR-1 hairless male mice dorsal skin (A)~(D) Hematoxylin and eosin(H&E) staining of UVB-irradiated hairless mice skin. Original magnification 200x

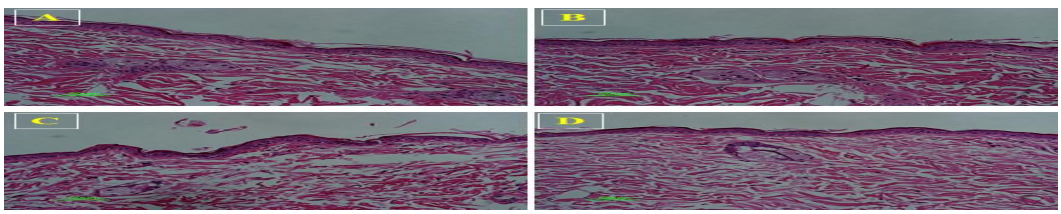

Fig. 3. HR-1 hairless male mice dorsal skin
HR-1 hairless male mice dorsal skin (A)~(D) Hematoxylin and eosin(H&E) staining of UVB-irradiated hairless mice skin. Original magnification 200x.

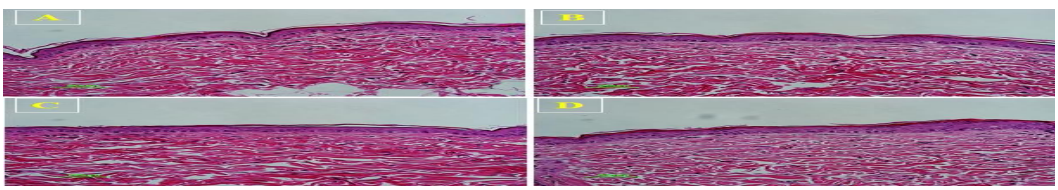

Fig. 4. Inhibition of skin thickening by 2.0% PT_iF on epidermis induced by UVB irradiation.
HR-1 hairless male mice dorsal skin (A)~(D) Hematoxylin and eosin(H&E) staining of UVB-irradiated hairless mice skin. Original magnification 200x.

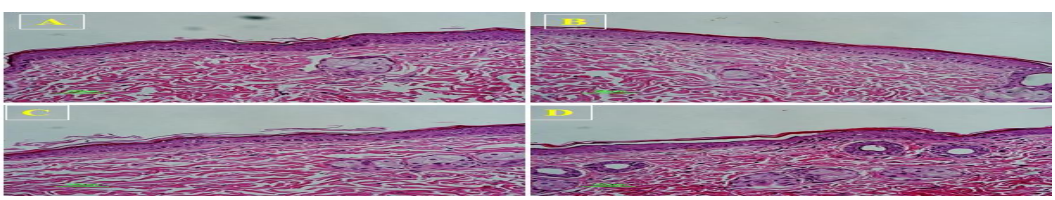

Fig. 5. Inhibition of skin thickening by 0.25% naringin on epidermis induced by UVB irradiation.
HR-1 hairless male mice dorsal skin (A)~(D) Hematoxylin and eosin(H&E) staining of UVB-irradiated hairless mice skin. Original magnification 200x.

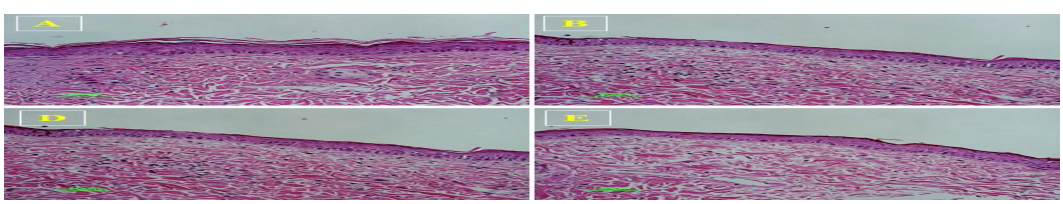

Fig. 6. Inhibition of skin thickening by 1.0% naringin on epidermis induced by UVB irradiation.
HR-1 hairless male mice dorsal skin (A)~(E) Hematoxylin and eosin(H&E) staining of UVB-irradiated hairless mice skin. Original magnification 200x.

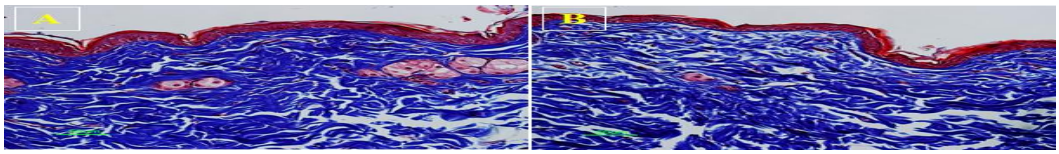

Fig. 7. HR-1 hairless male mice dorsal skin
HR-1 hairless male mice dorsal skin (A)~(B) Masson's Trichrome(M-T) staining of not UVB-irradiated hairless mice skin. Original magnification 200x.

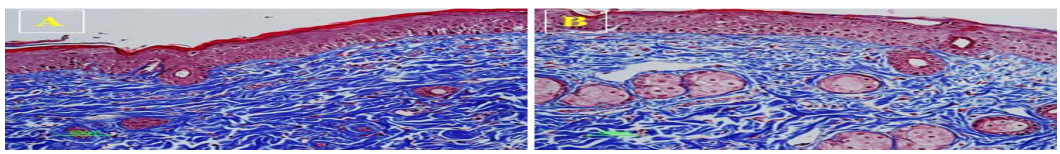

Fig. 8. Collagen deposition by vehicle control on epidermis induced by UVB irradiation.
HR-1 hairless male mice dorsal skin (A)~(B) Masson's Trichrome(M-T) staining of UVB-irradiated hairless mice skin. Original magnification 200x.

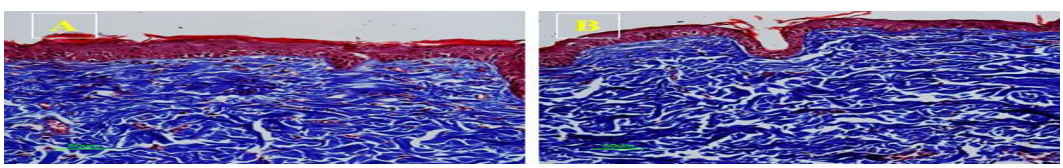

Fig. 9. Collagen deposition by 0.5% PT_iF extract cream-ointment on epidermis induced by UVB irradiation.
HR-1 hairless male mice dorsal skin on skin aging in terms of collagen fiber changes evaluated by (A)~(B) Masson's Trichrome(M-T) staining of UVB-irradiated hairless mice skin. Original magnification 200x.

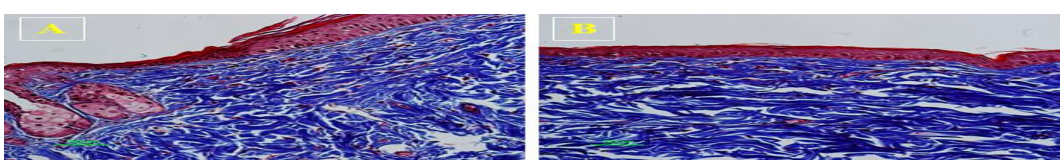

Fig. 10. Collagen deposition by 2% PT_iF extract cream-ointment on epidermis induced by UVB irradiation.
HR-1 hairless male mice dorsal skin on skin aging in terms of collagen fiber changes evaluated by (A)~(B) Masson's Trichrome(M-T) staining of UVB-irradiated hairless mice skin. Original magnification 200x.

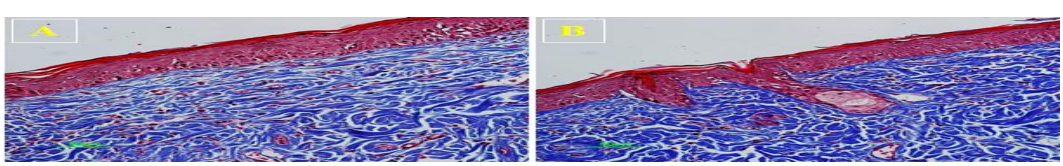

Fig. 11. Collagen deposition by 0.25% naringin cream-ointment on epidermis induced by UVB irradiation.
HR-1 hairless male mice dorsal skin on skin aging in terms of collagen fiber changes evaluated by (A)~(B) Masson's Trichrome(M-T) staining of UVB-irradiated hairless mice skin. Original magnification 200x.

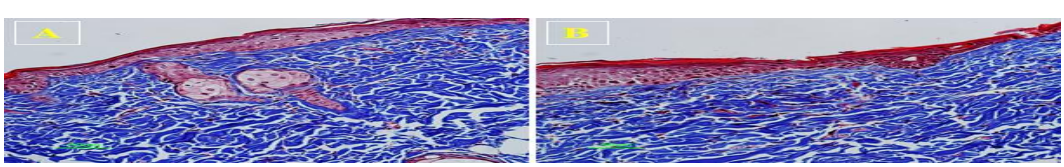

Fig. 12. Collagen deposition by 1.0% naringin cream-ointment on epidermis induced by UVB irradiation.
HR-1 hairless male mice dorsal skin on skin aging in terms of collagen fiber changes evaluated by (A)~(B) Masson's Trichrome(M-T) staining of UVB-irradiated hairless mice skin. Original magnification 200x.

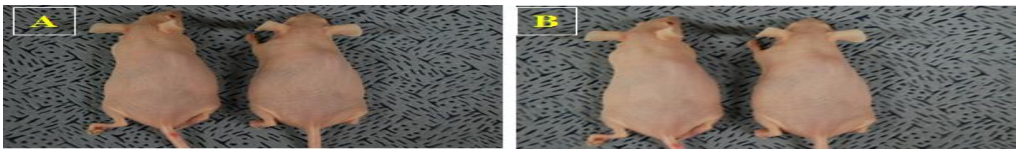

Fig. 13. HR-1 hairless male mice dorsal skin on wrinkle formation
HR-1 hairless male mice dorsal skin (A)~(B). Features of hairless mouse dorsal skin and images of mouse backs were recorded using a digital camera(Nikon, Japan). Original magnification 200x.

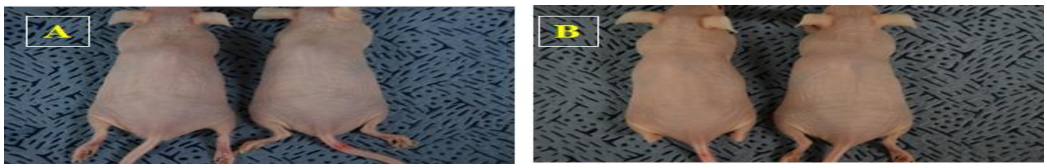

Fig. 14. Effects of vihicle control on UVB-induced wrinkle formation in hairless mice
HR-1 hairless male mice dorsal skin (A)~(B), Features of hairless mouse dorsal skin and images of mouse backs were recorded using a digital camera(Nikon, Japan). Original magnification 200x.

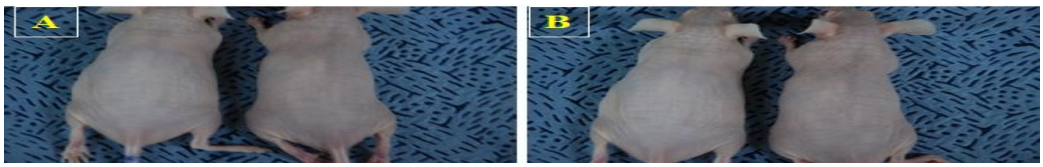

Fig. 15. Effects of 2% PT_iF extract cream-ointment on UVB-induced wrinkle formation in hairless mice
HR-1 hairless male mice dorsal skin (A)~(B), Features of hairless mouse dorsal skin and images of mouse backs were recorded using a digital camera(Nikon, Japan). Original magnification 200x.

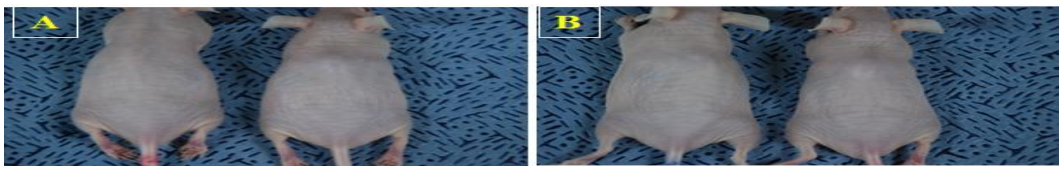

Fig. 16. Effects of 0.5% PT_iF extract cream-ointment on UVB-induced wrinkle formation in hairless mice
HR-1 hairless male mice dorsal skin (A)~(B), Features of hairless mouse dorsal skin and images of mouse backs were recorded using a digital camera(Nikon, Japan). Original magnification 200x.

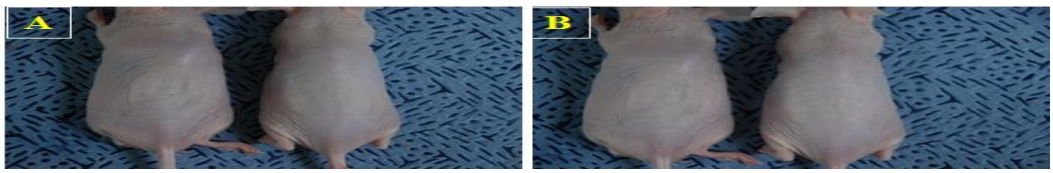

Fig. 17. Effects of 1.0% naringin cream-ointment on UVB-induced wrinkle formation in hairless mice
HR-1 hairless male mice dorsal skin (A)~(B), Features of hairless mouse dorsal skin and images of mouse backs were recorded using a digital camera(Nikon, Japan). Original magnification 200x.

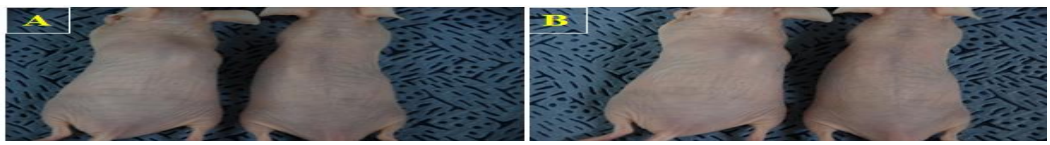

Fig. 18. Effects of 0.25% naringin cream-ointment on UVB-induced wrinkle formation in hairless mice
HR-1 hairless male mice dorsal skin (A)~(B), Features of hairless mouse dorsal skin and images of mouse backs were recorded using a digital camera(Nikon, Japan). Original magnification 200x.

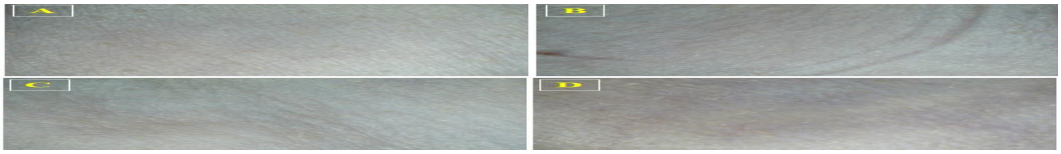

Fig. 19. HR-1 hairless male mice dorsal skin on wrinkle photograph.
HR-1 hairless male mice dorsal skin (A)~(D). Photographs of USB Disital Microscope were taken from dorsal skin of hairless mice after UVB-irradiation. Original magnification 200x.

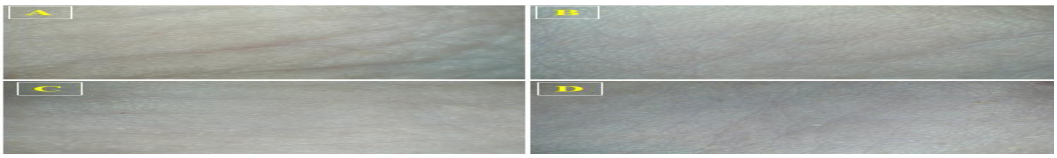

Fig. 20. Effects of vihicle control on UVB-induced wrinkle photograph in hairless mice
HR-1 hairless male mice dorsal skin (A)~(D), Photographs of USB Disital Microscope were taken from dorsal skin of hairless mice after UVB-irradiation. Original magnification 200x.

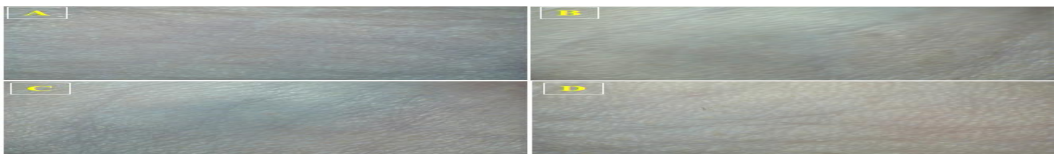

Fig. 21. Effect of 2% PT_iF Extracted Cream Ointment on UVB-induced Wrinkle Photographs in Unhulled Mice
After UV microphotography in dorsal skin (A) to (D) of male HR-1 hairless rats, I took a USB microscope photo. Research. Original magnification 200x.

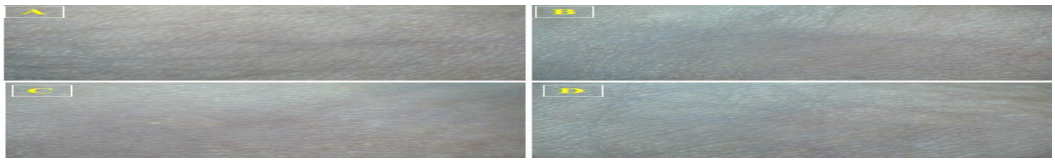

Fig. 22. Effect of 0.5% PT_iF Extract Cream Ointment on UVB-Induced Wrinkle Photographs of Rehmannia Rat
HR-1 From the dorsal skin (A) to (D) of the hairless male rats, pictures of the USB digital microscope were taken from mice without hair after UVB irradiation. Original magnification 200x.

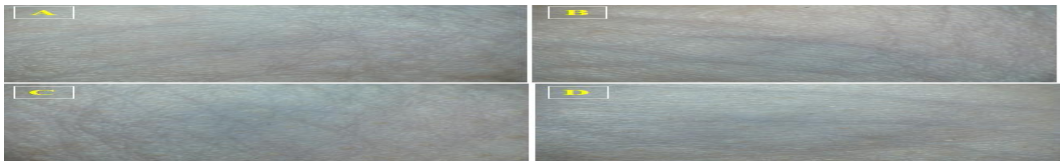

Fig. 23. Effect of 1.0% Naringin cream ointment on UVB-induced wrinkles in hairless mice HR-1 Dorsal skin (A) - (D) of a male rats without hair, Tphotographs of the USB digital microscope were taken from the dorsal skin of the hairless mouse after UVB irradiation.. Original magnification 200x.

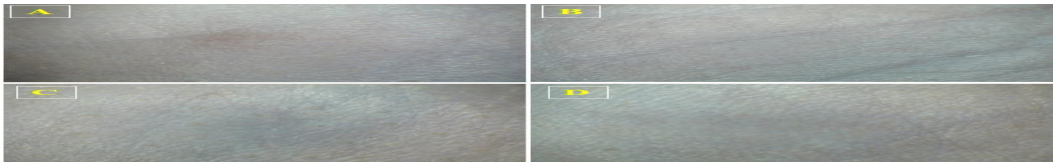

Fig. 24. Effect of 0.25% Naringin Cream Ointment on UVB-Induced Wrinkle Photographs of Hairless Rats HR-1
Dorsal skin (A) to (D) of male rats without hair, USB microscope photography from the skin of the hairless mouse back after UVB irradiation. Original magnification 200x.

As a result, the amount of MMP-2 protein increased more than 50 times in the UVB_control group when compared to the Normal control group. In the experimental group, UVB_PT_iF2.0 Extract ($p<0.001$), 0UVB_PT_iF 0.5 Extract ($p<0.01$), 1% naringin (UVB_Narlingin 1.0) ($p<0.001$), and 0.25% naringin (UVB_Naringin 0.25)($p<0.01$) were applied, and the MMP-2 protein expression showed a statistically significant decrease.

4. Discussion

Photo-aging refers to skin damage caused by continuous exposure to sunlight, and is also known as sun aging. The main symptoms of photo-aging are pigmentation such as fine wrinkles, rough skin and freckles, which accumulate in proportion to the time of exposure to the sun[10]. Research on dermal aging has been made to protect skin cells by inhibiting the biosynthesis of collagen and enzymes that break down collagen (MMPS) or by using antioxidants and anti-aging materials[11]. Recent studies have shown that PT_IF extracts have anti-alergic action[12], antibacterial action[13], anti-cancer action[14] and melanin-producing inhibiting effects[15]. The results of this experiment show that PT_iF extracts and naringin are proven to be high in electronic contribution capabilities, which results in the fact that they show excellent antioxidant action, and ultimately are thought to be available as an antioxidant. Furthermore, the test measured collagenase inhibitory activity for PT_iF extract showed low activity of IC50 at about 150 μg/ml and ascorbic acid, the positive control showed low activity of IC50 at 400 μg/ml or above 400 μg/ml.

5. Conclusion and Proposal

As a result of In vivo experiments, it is proven that on PT_iF extracts and naringin do have in a statistically significant decrease on skin creases improvement. Group which were applied 2% PT_iF extract and 1% naringin had significant decrease on epithelial tissue thickness and increase in collagen fiber when compared to UVB control (UVB_vehicles). They also had better skin crease improvement as well. Based on the above results, it is believed that PT_iF extracts and naringin actually did show positive effects on the anti- aging and anti-inflammatory effects, and they can be used as useful raw materials for developing skin protection products from plant origin.

REFERENCES

[1] Y. W. Ryoo, S. I. Suh, K. C. Mun, B. C. Kim & K. S. Lee. (2001). The effects of the melatonin on ultraviolet-B irradiated cultured dermal fibroblasts. *Journal of dermatological science, 27(3)*, 162-169.

[2] E. F. Bernstein et al. (1994). Uitto J Enhanced elastin and fibrillin gene expression in chronically photodamaged skin. *The Journal of investigative dermatology, 103(2)*, 182-186.

[3] B. A. Gilchrest. (1989). Skin aging and photoaging: an overview. *Journal of the American Academy of Dermatology, 21(3PT2)*, 610-613, 1989.

[4] J. S. Talwar, C. E. Griffiths, G. J. Fisher, T. A. Hamilton & J. J. Vorhees. (1995). Reduced type Ⅰ and type Ⅲ procollagens in photodamaged adult human skin. *The Journal of investigative dermatology, 105(2)*, 285-290.

[5] T. B. Lee. (2003). Coloured flora of Korea. Hyang-munsa, Seoul, Korea, 515-516.

[6] E. Lambert, EDasse, B. Haye B & E. Petitfrere. (2004). TIMPsas multifacial proteins. *Critical reviews in oncology/hematology, 49(3),* 187-198.

[7] J. H. Park & S. H. Sung. (2007). *The Key Medicinal Plants*. Seoul: Shinilbooks.

[8] N. Shoji, A. Umeyama, Y. Asakawa, T. Takemoto, K. Nomoto & Y. Ohizumi. (1984). Structural Determination of Nootkatol, a NewSesquiterpene Isolated from Alpinia oxyphylla MiquelPossessing Calcium- Antagonistic Activity. *Journal of pharmaceutical Sciences, 73(6)*, 843-844.

[9] K. Tsukahara, S. Moriwaki, T. Fujimura & Y. Takema. (2001). Inhibitory effect of an extract of Sanguisorba officinalis L. on ultraviolet-B-induced photodamage

of rat skin. *Biological and Pharmaceutical Bulletin, 24(9)*, 998-1003.

[10] L. Rittie & G. J. Fisher. (2002). UV-light-induced signal cascades and skin aging. *Ageing research reviews, 1(4)*, 705-720.

[11] H. S. Talwar, C. E. Griffiths, G. J. Fisher, T. A. Hamilton & J. J. Voorhees. (1995). Reduced type I and type III procollagens in photodamaged adult human skin. *The Journal of investigative dermatology, 105(2)*, 285-290.

[12] H. M. Kim, H. J. Kim & S. T. Park. (1999). Inhibition of immunoglobulin E production by Poncirus trifoliata fruit extract. *Journal of ethnopharmacology, 66(3)*, 283-288.

[13] R. H. Jeon, H. J. Choi, S. J. Moon & M. S. Na. (2010). The effects of antioxidant and antimicrobial activity with Poncirus trifoliata ethanol extracts during growth. *Journal of the Korean Society of Cosmetology, 16(4)*, 1225-1230.

[14] M. R. Cha, M. Y. Yoon, E. S. Son & H. R. Park. (2009). Selective cytotoxicity of Ponciri fructus against glucose-deprived PANC-1 human pancreatic cancer cells via blocking activation of GRP78. *Bioscience, biotechnology, and biochemistry, 73(10)*, 2167-2171.

[15] A. R. Son, J. Y. Choi, J. A. Kim, S. H. Cho, X. G. Hua, S. H. Park, S. R. Chung, T. C. Chung, Y. D. Jahng, J. K. Son & S. H. Lee. (2005). Isolation of melanogenesis inhibitors from Ponciri fructus. *Korean Journal of Pharmacognosy, 36(1),* 1-8.

박 상 희(Sang-hee Park) [정회원]

· 2012년 2월 : 대전대학교 보건 의료 대학원(보건학 석사)
· 2016년 2월 : 대전대학교 대학원 (보건학 박사)
· 2012년 2월 ~ 현재 : 대전과학기술대학교 피부보건과 겸임교수
· 2014년 7월 ~ 현재 : 상희인터내셔널 대표
· 관심분야 : 피부학, 미용경락, 발반사, 병원코디네이터, 아로마테라피, 메이크업, 두피모발관리학
· E-Mail : a586486@hanmail.net

곽 인 실(In-Sil Kwak) [정회원]

· 2008년 2월 : 인천대 대학원(체육학 석사)
· 2014년 2월 : 한양대 대학원(체육학 박사)
· 2007년 3월 ~ 2014년 2월 : 호원대학교 미용예술학과 겸임교수
· 2014년 3월 ~ 현재 : KC대학교 미용예술경영학과 겸임교수
· 2015년 1월 ~ 현재 : 웰니스테라피 연구소 대표
· 2015년 7월 ~ 현재 : 대한민국산업현장교수디자인분야(미용)
· 관심분야 : 피부학, 두피·모발관리학, 운동처방, 운동생리학 림프드레니쥐, 아로마테라피, 뱀부테라피,네일아트, 메이크업
· E-Mail : kwak3144@naver.com