

한방약용식물의 항산화효과 및 기초화장품소재 응용

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Antioxidizing Effects of Herbal Medicine Plants and Their Applications to the Materials of Skin Care Cosmetic Base

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요약. 우리나라 서남단의 지리산에는 천연야생에서 자생하는 한방약초의 보고라고 할 수 있다. 현재 약초로 분류되는 식물로는 1200여종이 자생 혹은 재배되고 있다. 최근들어 이들 중 현대인의 피부노화억제와 관련하여 관심을 끌고 있는 약초로 홍화에 관한 연구가 활발히 연구되고 있는데 금번연구에서는 홍화의 주성분인 폴리페놀 화합물(Polyphenol cocktail)이 30 - 40대 중년기의 여성에게 주름생성을 억제시켜 피부노화에 대한 지연효과를 줄 수 있는 천연물질임을 확인하고자 한다. 본 연구에서는 1차적으로 *in vitro*법으로 DPPH assay로 항산화효과를 확인하고 더불어 인체에 대한 임상시험을 통해 얼굴피부의 주름발생에 대한 억제 혹은 개선효과가 있음을 입증하고자 한다. 또한 홍화추출물을 주성분으로 하는 항노화 마스크팩 시제품의 개발로 진일보하여 기능성화장품 산업의 발전에 기여할 것임을 확신한다.

주제어: 홍화, 폴리페놀, 항노화, 항산화, 주름

ABSTRACT. Mt. Jiri, where is located in southwestern area of Korea, can be a treasury of herbal medicine plants grown wild. More than 1200 species currently classified as herbs are grown or cultivated. Recently, the studies on the safflower which attracts the interests in the inhibition of skin aging in the modern people have been actively investigated, and this study aims to confirm that polyphenol compound, the main ingredient of safflower, can be a natural material to cause the delayed effect on the skin aging, inhibiting the generation of wrinkle in the 30 to 40s middle aged females. Primarily, this study is to confirm the anti-oxidizing effect by DPPH assay *in vitro*, and to evaluate the efficacy of inhibition or improvement on the wrinkle generation in the face skin by clinical trials, additionally. In addition, we are confident to contribute on the improvement of functional cosmetic industry, developing the sample product of anti-aging mask pack which has the main ingredient with the extract of safflower.

Keywords: Safflower, Polyphenol, Anti-aging, Anti-oxidizing, Wrinkle

INTRODUCTION

In January 2009, Ministry of Health and Welfare selected the cosmetic industry among high value added healthcare industries with pro-environmental green technology as a promising sector (as of Jan. 15, 2009), and she supports with the regulatory enhancement of Cosmetic Law and export promising industry activation to this industry where shows the lipstick effect that enjoys the prosperous condition even in the slack time. In fact, Sulhwassoo, which is the sole brand of herbs, has recorded the sales over KRW500 billion every year achieving the amazing performance as a single brand.

The domestic herb cosmetic market has been expanding rapidly with the size of KRW1.2 trillion. Recently, the local cosmetics show the increasing trend every year and are anticipated to export more actively penetrating the worldwide market with Korean wave fad by the export enhancing policy of MOHWF since 2000. Hence, the technical development of skin care cosmetics has been enhanced to meet the needs of the consumers together with the increase of interests in Korean female skin aging and skin care, where the rate becoming aging society is the fastest. Therefore, Sanchong-gun, Hadong-gun, and Hamyang-gun near Mt. Jiri in South Kyung-sang Province are fundamental locations for tradi-

tional herbs cultivation, traditional agricultural products and herbs producing are as, and the beneficial geographical location to industrialize the herb materials and cosmetics.

THEORETICAL BACKGROUND

Safflower (*Carthamus tinctorius L.*) which has been applied to this study as the major herbal medicine plant is growing in Mt. Jiri. It is a yearly herbaceous plant in Asteraceae originated from Egypt or Mesopotamia region and being cultivated in Korea, China, Japan, and so on. Both its flower and seed are used for food or medicinal purpose. The flower of safflower consists of 0.3 - 0.6% carthamin and 20 - 30% safflower yellow, and especially carthamin, which is the main ingredient of safflower, has been called as red pigment and utilized as the natural coloring matter since long time before. There were reports as the studies on the physiological activity of safflower that carthamin, a red pigment of safflower, had lots of polyphenol compound and effects on the anti-oxidizing activation and anti-aging.¹⁻³ This study aims to validate the effectiveness by DPPH assay *in vitro* on the anti-oxidizing effect of safflower extract from safflower, and to conduct the clinical trial with mask pack sample product absorbed the mixture of safflower extract and aroma oil, which may have the efficacy to improve the wrinkle.

EXPERIMENTAL

Although active ingredient from safflower, IUK (safflower extract) is easily extracted by alcohols, it has the disadvantage of lower extract productivity than organic solvent if it is used as the source material of cosmetics. Adding 100 times of distilled water to 20 g sample of cultivated safflower, we extrac-

ted and filtered for 4 hours at 95 °C attaching the reflux condenser, performed decompression enrichment at 60 °C with this remaining solution, purified and extracted via liquid-liquid extraction and open column chromatography with butanol solvent, and then utilized this to anti-oxidizing test after lyophilization. The properties of the extract are shown in Table 1. Also, HPLC (Agilent 1100, USA) was used for qualitative analysis of polyphenol compound of the test material, IUK (safflower extract). This was compared and identified with the standard polyphenol cocktail (Sigma) measured by absorbance at 280 nm utilizing column (C₁₈, zorbox, 46 × 150 mm, 5 micrometer) under the isocratic condition with 10% ethanol solvent. Analysis results were shown in Figure 1.

Anti-oxidizing effect test (DPPH assay)

The anti-oxidizing test was performed *in vitro* classifying by concentration after dissolving the lyophilized powder into the solvent. The main facilities and test materials for the anti-oxidizing test of safflower extract were as follows; test equipment for the measurement was ELISA Reader system (power wave, bio-tek inc, Japan), test material were DPPH (1,1-diphenyl-2-picrylhydrazil), 96 well, pipette, Blue tip, Yellow tip, etc., and the test material was labeled as IUK (safflower extract). DPPH reactant tested by DPPH method is the colored radical, and it can directly confirm the capacity to remove the radical of the sample. First, the sample is dissolved into the solvent. 20 uL of liquid sample is put into 96 well and mixed with 180 uL of 100 uM DPPH. Incubate for 30 minutes at the normal temperature. Assess the remaining DPPH amount measured by absorbance at 540 nm. Demonstrate anti-oxidizing effect of each hydrolysate with lowered ratio compared to absorbance of control. The assessment method is to determine the existence of anti-oxidizing function through observing the elimination rate of DPPH free radical by the sample. The calculation method is (% inhibition) = [1-Absorbance

Table 1. Physicochemical properties of safflower extract

Condition	Water	2000 mL
	Material	20g
	Temperature	95 °C
	Time	4hour
Result	mL	1900 mL
	Brix	0.4
	pH	4.84
	Concentration evaporated at 60 °C	
Result	mL	80 mL
	Brix	24
	pH	4.84
	Freezing-Dry	
Final Result	g	8 g

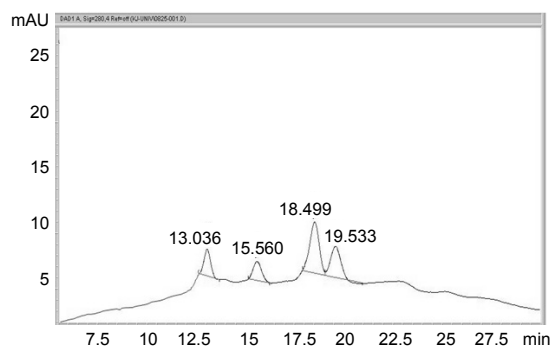


Fig. 1. HPLC analysis result of test material, IUK (mAU/min).

of test material/Absorbance of control] $\times 100$, and the analyzed standard values by statistical process were shown as Mean \pm SD.⁴⁻⁶

Clinical trial with cocktail products

The sample product was produced with mask type by mixing safflower extract (*Carthamus tinctorius* (safflower) flower extract) as a main ingredient and thunberg extract, *Portulacaoleracea* extract, aroma oil (neroli, lavender, camomile, and tea tree) as the cosmetic compound, and absorbing this into non-woven fabric. Anti-aging improvement effect of the cosmetic compound prescribed with the above method was monitored in the human-beings with the following method.

First, 19 male and female adults who complain xeroderma or consider being dry skin among the volunteers were selected and applied the mask pack on the face for 30 minutes before sleep once a day for 10 days. The pictures of their skin conditions were compared before and after the application in 10 days after applying mask pack. In addition, the equipment used for clinical trial is DSLR Camera (NS10, Samsung, Korea). It maintained the same light in the designated dark room to keep the conditions before and after the clinical trial and it was preset the picture angle with stanchion to keep the same conditions of picture angle and distance. Also, the subjects were evaluated by the survey form for the history taking on the improvement of wrinkle at the completion of the trial.

RESULTS AND DISCUSSION

The test material IUK (safflower extract) was shown the meaningful effect on the elimination of DPPH free radical in the anti-oxidizing test. All test values were significant compared to the control ($p < 0.01$). Upon the test result of IUK to eliminate DPPH free radical in a way of concentration dependent within the range of 0.1 to 100 ppm, 77% of free radical elimination rate at maximum could be observed at 100 ppm concentration. From the assessment result of IUK anti-oxidizing effect by free radical elimination test, used IUK demonstrated to decrease DPPH free radical significantly within the range of 5 - 100 ppm as shown in Figure 2. After all, this is considered that safflower extract, which has excellent anti-oxidizing effect, may enhance the moisturizing capacity as well as the wrinkle improvement capacity of human facial skin.⁷⁻¹¹

In the facial clinical trial, as shown in Table 2 and Figure 3, 4 out 19 did not show the remarkable improvement, while the other 15 showed more than 70% of improvement rate,

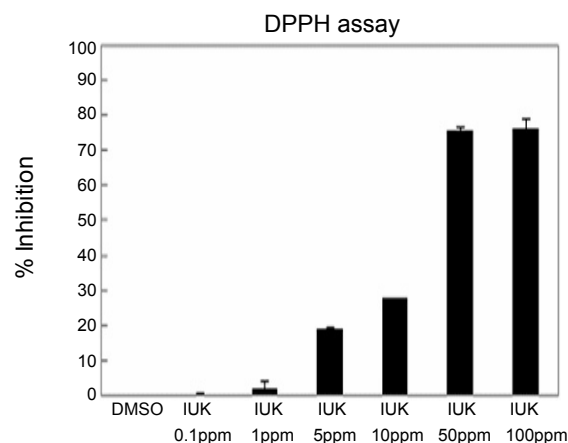


Fig. 2. The effect of free radical scavenging activity in DPPH assay. IUK: symbols for carthamus tinctorius flower extract. DMSO (dimethyl sulfoxide) : negative control.

Table 2. Assessment results by history taking from the subjects on the improvement of wrinkle

No	Sex	Age	Period (Days)	Skin Irritation	Improve-Ment Rate (%)	Remark
1	F	23	10	None	60	Wrinkle
2	F	24	10	Some	50	"
3	F	49	10	None	60	"
4	M	20	10	"	70	"
5	F	19	10	"	50	"
6	F	48	10	"	80	"
7	F	52	10	"	90	"
8	F	50	10	"	90	"
9	M	20	10	"	80	"
10	F	50	10	"	90	"
11	F	19	10	"	80	"
12	M	20	10	"	80	"
13	F	20	10	Some	80	"
14	F	20	10	None	90	"
15	M	20	10	"	90	"
16	F	50	10	"	90	"
17	F	49	10	"	90	"
18	F	40	10	"	90	"
19	M	49	10	"	90	"

and the clinical results also showed the improvement of wrinkle generation inhibition visually as in the pictures, upon the clinical trial and history taking results utilizing mask pack which absorbed the mixture of cosmetic compound, including safflower extract and aroma oil (neroli, lavender, camomile, and tea tree), into non-woven fabric. As a result, the recipe of the above cosmetic compound is considered to have the efficacy on the improvement of skin wrinkle since it has an excellent anti-oxidizing effect partially on the skin.



Fig. 3. Change of skin wrinkle condition about 10 days after using mask pack.

This means that skin application of cosmetic compound with the active ingredient of safflower extract, a natural plant extract, can maintain the skin condition young and lively by enhancing excellent anti-oxidizing effect and skin moisturizing capacity, to the dry and loosened skin due to lowered moisturizing capacity, that is rough and dry skin, which is a major cause of skin aging.

CONCLUSION

From the anti-oxidizing test results of free radical elimination rate with test material IUK, more than 70% was eliminated within the range of 50 - 100 ppm compared to the control. Since high concentration safflower extract contains large amount of polyphenol compound as published before, the active ingredient together with NMF element in the skin are considered to keep the skin moisturizing capacity relatively

maintaining the strong bonding due to chemical hydrogen binding. Also in the clinical trial for human-beings, overall the skin tone of the subjects showed brighter after the usage upon the thorough analysis of pictures before and after in Figure 3, after continuous use of mask pack for 10 days, which is absorbed the mixture of cosmetic compound.

It may be due to the improvement of circulation by even penetration of active ingredients such as collagen including herb extract to the skin as well as glossy skin with relieving stress from mental stabilization and resolving tension, by application of total facial mask pack for 30 minutes every day. Especially, the depth of Chinese character eight shaped long wrinkle by the nose showed relatively lessened visually after using this, which seemed to be a typical phenomenon of improvement for wrinkle generation, and surely this will contribute the improvement of technology development for anti-aging cosmetics if further studies are conducted.

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