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Anti-wrinkle Effect of Herbal Medicine Plant and Its Applications in Cosmetics

Young-Ho Park
Department of Pharmaceutical Engineering, International University of Korea
Jinju 52833, Korea. E-mail: bush777@daum.net
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ABSTRACT. Mt. Jiri located in the southwestern part of Korea is a treasure trove of wild medicinal plants. More than 1200 species currently classified as herbs are grown or cultivated in the area. Recently, safflower has attracted interest because of its ability to control fine wrinkle formation on the neck. The objective of this study therefore was to determine whether the active ingredient of safflower could be used in the form of an extract to reduce wrinkle formation in individuals aged 30 to 59 years. In particular, this study was aimed at determining the extract's elastase activity and anti-oxidant effect by using DPPH assay in vitro and evaluating the anti-wrinkle efficacy of different types of safflower extracts in improving fine wrinkles on the neck. This study will provide a basis for future studies to develop safflower extracts using advanced composition technology and contribute to the development of the herbal cosmetics industry.[1]

Key words: Safflower, Polyphenol, Wrinkles

INTRODUCTION

As a part of Korean wave, the K-beauty trend started in the year 2000. The cosmetic industry is expanding its high-value product sales not only to China but also to other Asian, European, and American countries. Amore Pacific, as the representative of Korean cosmetic companies, aimed at a sales target of 12 trillion won to become a global enterprise by 2020. In recent times, Korean herbal cosmetics have achieved continuous market growth. The market size has rapidly expanded to 2 trillion won from that in 2012, accounting for 25% of the total cosmetics market. The current global market for herbal cosmetics is estimated to grow further, with an increasing number of herbal cosmetics being exported every year depending on government export-promotion policies. Korean herbal medicines are mainly produced in the area near Mt. Jiri in Sancheong County, Gyeongnam Province. The area has a typical hot-dry climate ideal for traditional herb production and suitable geographical features for industrialization of these herbs.[1]

Safflower (*Carthamus tinctorius L.*) is an annual herbaceous plant belonging to the chrysanthemum family. This medicinal plant originated in Egypt or the Mesopotamia region and is cultivated in China, Korea, and Japan. The flowers and seeds of the plant have been used as a food or medicine in recent times. Safflower contains 0.3-0.6% carthamin and 20-30% safflower yellow.[1] In particular, studies have been conducted on chemical structure analysis and effective separation of carthamin; physiochemical characteristics of each part of safflower; the complex polyphenol compounds in petal, sprout and safflower seed extracts; and the use of safflower as a bioactive material with antioxidant activity. According to literature, carthamin and safflower pigments are mixed, but these compounds are unstable above 60 °C. Thus, extraction, separation, and concentrated production processes have been optimized by taking advantage of the different physical properties in acidic or basic aqueous solutions.[2] (Hong et al., 1997). In this study, extraction using methanol and an antimicrobial test using the agar diffusion method were performed to increase the concentration during extraction, and to stabilize the chemical properties of natural dyes obtained using the extraction method used for natural pigments from *Sophora japonica* flower in a previous study[3] (Kim and Song, 2000). In addition, the antioxidant effect and skin cell toxicity of the active ingredients of safflower extract were also determined[1] (Park et al., 2010).

An extract of safflower from Mt. Jiri, which has been used as a hemagogue, was prepared. The antioxidant effect of the safflower extract was determined using an in vitro DPPH assay,[1,5] which has been reported in a previous study.[4] The efficacy of the extract was determined using an elastase inhibition test[6–8] and a clinical trial in human subjects was conducted to determine the effect of the safflower extract and an emulsion containing safflower extract on wrinkles on the neck.

EXPERIMENTAL METHOD

Summary of the material extraction experiment

Although it is easy to extract the active ingredients of safflower in alcohol, the extraction yield obtained is lower than that obtained using organic solvents for cosmetic material application. Thus, the extraction was performed using hot water, in line with a previous study, since it affords advantages in terms of stability, extraction device, and production cost.[1] The physicochemical properties of the safflower extract are summarized in *Table* 1. The freeze-dried safflower extract sample (called IUK) was dissolved in the solvent and classified based on density. Subsequently, the elastase inhibition test was conducted in vitro.

Instruments used for analysis

For the elastase inhibition test, an ELISA Reader system (Power wave, Bio-tek Inc., VT) was used as the measurement device while elastase (Sigma, USA), SANA (Sigma, USA), and 0.2 M Tris-HCl buffer (pH 6.8) were used as materials. In addition, a 96-well plate (Nunc, NY) Pipet (Gilson, WI), white tip (Axygen Scientific, Inc, CA), yellow tip (Axygen Scientific, Inc, CA), and blue tip (Axygen Scientific, Inc, CA) were utilized. The sample was labeled as IUK.

Measurement method

The experimental procedure was as follows: ① 2.5 ml of reaction mixture was prepared from 50 μl of 10 nM elastase (0.25 μg/ml), the sample, and 0.2 M Tris-Cl (pH 8.0). ② The reaction mixture was incubated at 25 ºC for 10 minutes in a water bath. ③ Thereafter, 20 μl of 125 mM SANA was added. ④ Absorbance was measured at A_{410nm} for 2-3 minutes. The higher the elastase inhibition by the sample, the more effective it was as an elastase inhibitor. The elastase inhibition activity was determined using the following formula.[6–8]

[Elastase inhibition activity (%) = {100-(Absorbance of the sample/(-)average absorbance) × 100}].

In other words, the effectiveness of elastase inhibition increased with an increase in the elastase inhibition activity.

***Table* 1.** Physicochemical properties of safflower extract[1,2]

Condition	Water	2000 ml
	Material	20 g
	Temperature	95 °C
	Time	4 hour
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
Active Ingredient	carthamin	4.4 mg/ml

RESULTS AND DISCUSSION

Elastase inhibition test (in vitro)

The results of the elastase inhibition test in vitro of the safflower extract are shown in *Fig*. 1. Absorbance was measured at 410 nm after adding the sample to a reaction mixture containing elastase and SANA substrate. Higher absorbance was observed as the activity of elastase increased. On the other hand, low absorbance was observed at 410 nm when the sample was added to inhibit the elastase activity. The parameters are as follows (-) : absorbance was measured for the reaction solution containing only elastase and SANA substrate (negative control); Vit C : absorbance measured by adding Vit C, which is known to have an elastase inhibition effect, into the reaction solution containing elastase and SANA substrate (positive control); and BS-# : absorbance was measured at 410 nm by adding Jeju native plant extracts at different concentrations, separately, into the reaction solution containing elastase and SANA substrate. The standard values from statistical measurements were expressed as the average ± standard deviation. The results of the experiment are shown in *Fig*. 1. In addition, the antioxidant efficacy evaluation of safflower extract was conducted by performing DPPH assay, which has been reported in previous studies[1,5].

The elastase inhibition test results showed that the IUK sample (safflower extract) had a significant elastase inhibition effect. All experimental values were compared against

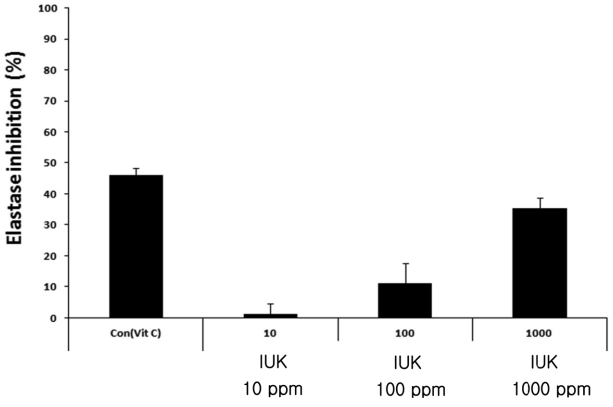

***Figure* 1.** Elastase inhibition activity (%) of IUK sample.[1]

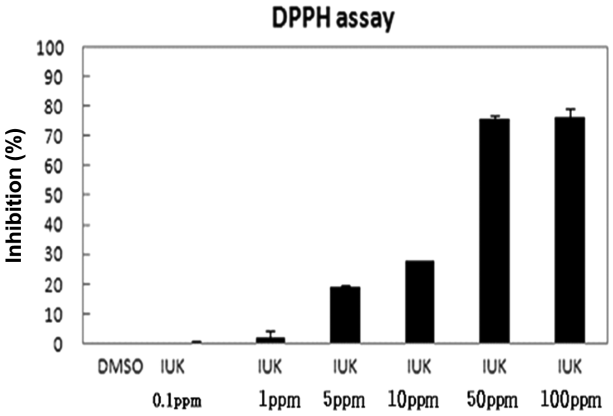

***Figure* 2.** The effect of free radical scavenging activity in DPPH assy.[1] IUK: symbols for safflower flower extract. DMSO (dimethyl sulfoxide): negative control.

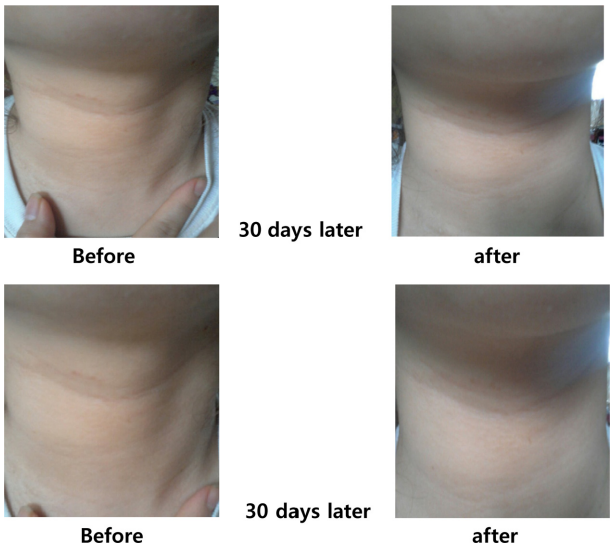

***Figure* 3.** Change in neck skin wrinkle condition 30 days after using IUK emulsion cream.

those obtained for the negative control group ($p < 0.01$). Inhibition of elastase activity was observed as a function of IUK concentration in the range of 100 to 1000 ppm. An inhibition of 35.33% was observed at 1000 ppm. The elastase inhibition test results showed that IUK significantly inhibited elastase activity at concentrations in the range 100 to 1000 ppm. The main ingredients of safflower extract were determined to exhibit excellent wrinkle inhibitory effects. Moreover, they were effective in delaying the aging process of the skin around the neck by increasing the moisture content. The antioxidant effects of safflower extract, measured using the DPPH assay, are shown in *Fig.* 2; the results are in agreement with those of previous studies.[1,9–13]

Neck wrinkle improvement in the clinical trial conducted in human (in vivo)

The emulsion cream prototype was prepared by adding the components of IUK safflower extract (*Carthamus tinctorius* extract) into the main prescription of emulsifier and moisturizer. The anti-wrinkle effect of the IUK cosmetic prototype was evaluated in human subjects using the following method. First, 18 male and female adults with dry skin were selected. The IUK cosmetic prototype was applied on front neck skin 3 times a day for 30 days at an average.

***Table* 2.** Assessment results for wrinkle improvement based on history-taking from subjects

NO	Sex	Age (years)	Period (days)	Skin irritation	Improvement rate (%)	Remark
1	F	45	30	none	60	wrinkle
2	F	50	30	none	60	"
3	F	48	10	none	60	"
4	M	49	10	none	60	"
5	F	52	15	none	60	"
6	M	26	45	none	40	"
7	M	56	45	some	40	"
8	F	53	45	none	80	"
9	M	51	28	none	80	"
10	F	25	28	none	80	"
11	F	24	21	none	80	"
12	M	24	10	none	60	"
13	F	23	28	none	80	"
14	F	21	14	none	60	"
15	M	20	10	none	80	"
16	F	50	20	none	80	"
17	F	46	10	none	80	"
18	F	40	20	none	60	"

The pictures of skin condition before and after using the prototype for 30 days were compared. The results are shown in *Fig*. 3. The device used in human clinical trials was DSLR Camera (NS10, Samsung, Korea). The pictures were taken at the same angle and distance to avoid any bias before and after test. Moreover, a questionnaire was filled out by the participants at the end of the test and a subjective sensory evaluation was conducted simultaneously.

As can be seen from the human clinical trial results in *Fig*. 3 and *Table* 2, 2 of the 18 participants using the IUK emulsion cream prototype did not have significant improvement in wrinkles, but the other 16 participants showed more than 60% improvement. From the actual clinical results in the pictures, the improvement was indicated through fading of neck wrinkles. This cosmetic prototype was effective in delaying skin aging by partially improving skin wrinkle formation along with an antioxidant effect. Rough and impoverished skin loses its moisture and becomes dry, resulting in skin aging. The cosmetic prototype containing the active ingredients from safflower plant with excellent antioxidant effect improved sagging skin. Its application on the neck moisturized the skin and increased vitality to keep the skin looking young.

CONCLUSIONS

In the elastase inhibition test, more than 35.33% inhibition was observed for the IUK sample at 1000 ppm compared to that in the control group. As reported in the literature, the safflower extract contains a large number of polyphenolic compounds. It has been shown that the moisture content of neck skin could be maintained for a relatively long time using these active ingredients since they formed strong cohesive hydrogen bonds with the natural moisturizing factor (NMF) component of the human skin. In addition, the free radical scavenging activity of the IUK sample in antioxidant test through DPPH assay from previous study[1] shows that elimination result by more than 70% was obtained compared to control group even at relatively low concentration of 100 ppm.

The photos obtained before and after continuous application of IUK emulsion prototype in human clinical trials are shown in *Fig*. 3. With careful analysis, lighter skin tone and lighter wrinkles could be observed after using the cosmetic prototype. Through IUK cosmetic prototype application on the entire face 3 times a day, the active ingredients in the safflower extract evenly penetrated the skin. It resulted in better blood circulation by reducing stress, relieving tension, and relaxing the skin. The depth of the wrinkles around the neck relatively reduced after using the cosmetic prototype, suggesting improvement. However, further research is required to improve the technology for developing anti-wrinkle cosmetics.

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