Evaluation of Biological Activity of Natural Composite Extracts

Hye-Jin Kwon, *Professor, Dept. Chemical Engineering, Soongsil Univ., Dongjak-gu Seoul, Korea, 06978,*
kwonhj0070@ssu.ac.kr
*Corresponding author

Abstract. To increase the value of using natural plants, we developed antioxidant and anti-aging cosmetic ingredients using *Portulaca oleracea*(PO) and *Agrimonia pilosa Ledeb*(APL), known as natural medicinal plants. Both PO and APL were extracted with ethanol as solvents and thecomposite extract was prepared in a 1:1 ratio of PO and APL extracts. The final samples were compared and evaluated based on three extracts, i.e., PO extract, composite extract, and APL extract. The antioxidant effects of these samples were evaluated by measuring DPPH, ABTS, and hydroxyl radical scavenging activity, whereas the anti-aging effects were evaluated via Collagenase and Elastase inhibition rates. In evaluating the antioxidant activity of the APL extract was the highest, whereas that of the composite extract showed inhibitory activity similar to thatof the single extracts but still lower than that of individual extracts. Moreover, the anti-aging effect of the composite extract demonstrated higher inhibitory activity than that of the single
tract. The highest inhibition rate was shown in a 2:1 ratio of PO and APL composite. The results to date demonstrate that the composite extract of APL and PO has an efficacy to be used as a functional cosmetic material. With increased interest in anti-aging and well-being around the world, the development of natural materials for herbal medicine is expected to have a considerable effect on the cosmetic industry in future.

Keywords: Portulaca oleracea; Agrimonia pilosa Ledeb; anti-aging; antioxidant; natural composite extracts

INTRODUCTION

The global cosmetics market is continuously growing. In particular, with the elderly population increasing, the "anti-aging cosmetics" are becoming a global trend. Moreover, the 'composite functional cosmetics' with combined functions are attracting considerable attention along with "'functional cosmetics" that are differentiated from ordinary cosmetics [1]. The popularity of natural cosmetics is primarily high in the Asia–Pacific region. In particular, natural cosmetic products containing plant extracts, organic ingredients, yeasts, and herbal medicine plants are being developed, as well as the demand for cosmetics that use natural ingredients is significantly increasing among composite functional products [2,3]. In Korea, natural herbs have been used for their medicinal effects based on Donguibogam and several natural medicinal plants are already exploited as cosmetic ingredients [4]. *Portulaca oleracea*(PO) is a whole plant in Portulacaceae. Its pharmacological effects have been reported for antimicrobial, antioxidant, and anti-inflammatory activities; therefore, in oriental medicine, the plants are used in several inflammatory diseases. The major components of PO are coumarin, flavonoids, and glutamic acid; furthermore, its leaves and stalks were reported to contain high levels of essential fatty acids and nutritional components such as vitamin B1, B2, and malic acid [5]. However, Agrimonia pilosa Ledeb(APL) is used as a medicinal herb by drying the agrimony, the perennial herb of the Rosaceae family and the turning of the homologous plant. Generally, these plants are distributed in the roadsides and fields of the mountains in Korea. They have been used in folk medicine as hemostatic, anti-inflammatory, hypoglycemic, hematopoietic agents, and pain reliever. Moreover, it is being considered as having anti-inflammatory, antibacterial, anti-cancer, and antiviral effects [6,7], other critical healthcare solutions [18-34]. As part of basic research to explore antioxidant and anti-aging materials derived from natural products, we herein developed raw materials for antioxidant and anti-aging composite herbal cosmetics using PO and APL; moreover, their biological activities were compared and evaluated.

1. Experimental setup
Sample extraction
Portulaca oleracea (PO) and *Agrimonia pilosa Ledeb* (APL) used in this experiment were purchased at Kyungdong market in Seoul. Each sample (50 g) was extracted with 80% ethanol (500 mL) for 24 h at 60 °C, centrifuged, filtered twice with filter paper, and concentrated under reduced pressure. The concentrated sample was freeze-dried at −80 °C for 24 h. The composite was prepared by mixing PO and APL in a 1:1 ratio and PO single extract, composite extract, and APL single extract were analyzed and compared for final samples.

Evaluation of antioxidant effects
The antioxidant effects of samples were evaluated by DPPH, ABTS, and OH radical scavenging activities. In the DPPH experiment, 150 μL of each sample was mixed with 100 μL of 0.2 mM DPPH solution in ethanol and reacted at 37 °C for 30 min. After the reaction, absorbance was measured at 517 nm. Note that distilled water was added to the control group of the sample, and ethanol was added to the control group of DPPH solution to obtain a correction value and the result was calculated. In the ABTS experiment, potassium persulphate solution (2.6 mM) and ABTS (7.4 mM) were mixed, and the mixture was stored in the dark for 1 day to form cations (ABTS·+). The absorbance was measured at 732 nm. Next, the absorbance was diluted to its value being <1.5 and 5 μL of each sample was mixed with diluted ABTS·+ solution (150 μL) and reacting at room temperature for 10 min. Moreover, absorbance was measured at 732 nm. The distilled water was added to the control group of the sample; moreover, the result was obtained by measuring ABTS radical scavenging activity against the control group. For the OH radical scavenging activity, 10 mM FeSO4·7H2O-ethylene diamine tetraacetic acid (EDTA; 200 μL), 10 mM 2-deoxyribose (200 μL), 10 mM hydrogen peroxide (200 μL), and 1400 μL of the samples of each concentration were mixed and incubated at 37 °C for 4 h. Note that 1 mL of 1.0% thiobarbituric acid and 1 mL of 2.8% trichloroacetic acid was added to the mixture and boiled for 20 min. After cooling, the absorbance was measured at 490 nm. All experimental results were statistically processed using SPSS 21.0.

Evaluation of anti-aging effects
Experiment on anti-aging was performed on collagenase and elastase inhibition rates. The samples were diluted to 1, 10, 100, and 1,000 μg/mL and used. Both elastase and N-succinyl-(L-Ala)3-p-nitroanilide were dissolved in 50 mM Tris-HCl buffer (pH 8.6) as per the corresponding concentrations. The 40-μL sample was added with 40 μL elastase of 2.5 U/mL. Then, 80 μL of 0.5 mg/mL N-succinyl-(L-Ala)3-p-nitroanilide was added and reacted at 37 °C for 30 min' the absorbance was measured at 445 nm. Moreover, 4-phenylazobenzyloxycarbonyl-Pro-Leu-Gly-Pro-D-Arg was dissolved in 0.1 M Tris-HCl buffer (pH 7.5) containing 4 mM CaCl2 and prepared. We added 250 μL of 0.3 mg/mL4-phenylazobenzyloxycarbonyl-Pro-Leu-Gly-Pro-D-Arg to 100 μL of the sample, followed by adding 150 μL of collagenase (0.2 mg/mL). The resultant mixture was reacted at room temperature for 20 min, and the absorbance was measured at 320 nm after adding 500 μL of 6% citric acid and ethyl acetate (1.5 mL) to the reaction mixture. All results were statistically processed using SPSS 21.0.

2. Result discussions
Antioxidant effects of the plant extracts
Fig. 1, 2 shows the DPPH and ABTS radical scavenging activities of the single extract of PO and APL. At 1, 10, 100, and 1,000 μg/mL, the DPPH radical scavenging activity of PO extract was 0.1% ± 0.5%, 0.9% ± 2.5%, 9.5% ± 2.8%, and 63.5% ± 3.2%, whereas that of APL extract was 2.6% ± 0.4%, 6.5% ± 0.4%, 41.4% ± 2.1%, and 89.8% ± 0.4%, respectively. Both extracts seemed to be concentration-dependent and the APL extract showed more powerful antioxidant effects than the PO extract by concentration. In terms of the ABTS radical scavenging activity at 1, 10, 100, and 1,000 μg/mL, the PO extract was 0.24% ± 0.3%, 2.48% ± 2.3%, 30.0% ± 3.3%, and 84.6% ± 2.9%, whereas that of the APL extract was 0.9% ± 0.1%, 4.7% ± 0.5%, 33.4% ± 0.4%, and 94.7% ± 0.2%, respectively. Similar to the results of the DPPH radical scavenging activity, the ABTS radical scavenging activity of both extracts seemed to be concentration-dependent, whereas the APL extract showed higher antioxidant activity than the PO extract by concentration. Most natural products showed radical scavenging activity depending on concentrations. Thus, this method is the most efficient tool to investigate whether natural products have antioxidant effects [8]. For applying natural products as cosmetic ingredients; however, it is important to determine optimal concentration. The result of the DPPH radical scavenging activity of the 1:1 mixture of PO and APL extracts was 0.05% ± 1.4%, 0.3% ± 3.2%, 2.4% ± 2.2%, and 10.5% ± 0.3%, whereas the ABTS radical scavenging activity was 0.05% ± 1.4%, 1.1% ± 0.4%, 3.6% ± 0.2%, and 24.4 ± 0.3%, indicating the anti-oxi

dant activities of two single extracts were more effective than the composite extract. In general, the maximum effect of each compound is expected when two compounds are mixed [9, 10]. However, as shown in this study, its effect may be reduced to a certain extent. Table 1 summarizes the OH radical scavenging activities of the samples. It showed similar tendency to the results of two experiments t hat occurred. The hydroxyl radical scavenging activity of the APL extract was the highest; however, the mixed extract was somewhat reduced.
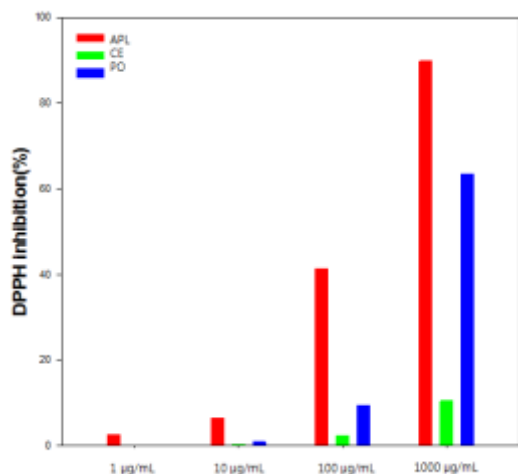

Figure 1.   DPPH inhibition of the extract:
Portulaca oleracea (PO), *Agrimonia pilosa Ledeb* (APL), composite extract (CE)
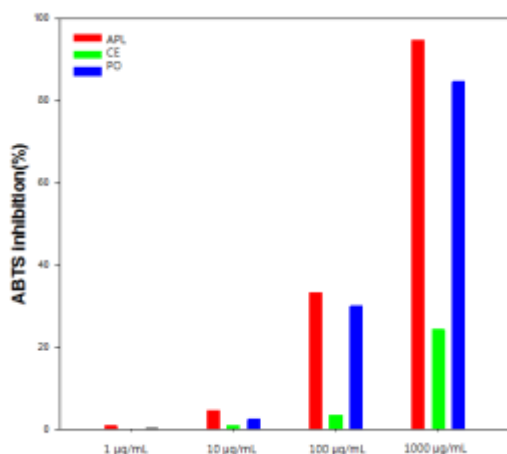

Figure 2.   ABTS inhibition of the extract:
Portulaca oleracea (PO), *Agrimonia pilosa Ledeb* (APL), composite extract (CE)

Table 1.   OH radical scavenging activities of the extracts

concentration	1 µg/mL	10 µg/mL	100 µg/mL	1000 µg/mL
APL	1.04±0.61%	3.92±0.05	38.6±0.03	90.59±0.29
PO	0.1±0.54%	3.16±0.04	37.7±0.03	71.83±0.16
CE	0.6±0.26%	1.5±0.47%,	21.6±7.2%	40.1±0.06

Anti-aging effects of the plant extracts
Fig 3, 4 shows the anti-aging results of the three samples. In the APL extract, elastase inhibition rate was 6.4% ± 0.9%, 19.4% ± 1.0%, 38.0% ± 0.5%, 60.4% ± 1.2%, and collagenase inhibition rate was 2.1% ±

1.4%, 18.5% ± 1.4%, 50.5% ± 0.3%, 73.6% ± 1.9% at 1, 10, 100, and 1,000 μg/mL, respectively. Both inhibition rates were concentration-dependent and higher than those of other recent studies, suggesting that the APL extract may be a promising candidate as a cosmeceutical ingredient with an anti-aging effect [11].

For the elastase inhibition activity experiment at 1, 10, 100, and 1,000 μg/mL, the PO extract showed 9.1% ± 0.8%, 15.6% ± 0.3%, 48.3% ± 0.5%, and 76.1% ± 0.3% inhibition rates, whereas the composite extract (CE) showed 10.6% ± 1.5%, 17.3% ± 0.3%, 49.7% ± 0.7%, and 80.5% ± 1.6% inhibition rates, respectively, thus being the most predominant inhibitory agent among the three samples. At the same concentrations, the collagenase inhibition rate of PO extract was 14.5% ± 2.5%, 32.6% ± 1.0%, 57.8% ± 0.7%, and 82.3% ± 1.5%; however, that of the CE was 12.4% ± 1.8%, 31.4% ± 0.5%, 56.7% ± 1.0%, and 90.7% ± 2.2%. Moreover, similar to the elastase inhibition activity, CE demonstrated the highest inhibition rate among the three samples. *Portulaca oleracea* (PO) and *Agrimonia pilosa Ledeb* (APL) are used in various fields such as health supplements, medicinal herbal ingredients, and nutritional supplements. APL comprises pyrocatecholic tannin, ellagytannin, phenol, saponin, luteolin, and coumarin, all of which possessed antioxidant effects and saccharides such as glucosides. Moreover, PO contains antioxidant substances such as tannin, saponin, flavonoid, and beta-carotene and essential fatty acids such as omega-3 and alpha-linolenic acid [12]. As reported earlier, these two compounds have excellent antioxidant effects because they contain various nutrients and biologically active substances [13].

Recently, to exhibit synergistic effects with each other, natural products are favored in diverse fields and rather than simply using one natural product, there is an increasing number of cases in which natural products are used as a composite extract [14]. The most important aspect in prescribing these extracts is the mixing ratio [15]. Thus, in these two anti-aging experiments, the anti-aging effects of the two extracts have a synergistic effect because the composite extract demonstrated the best efficiency.
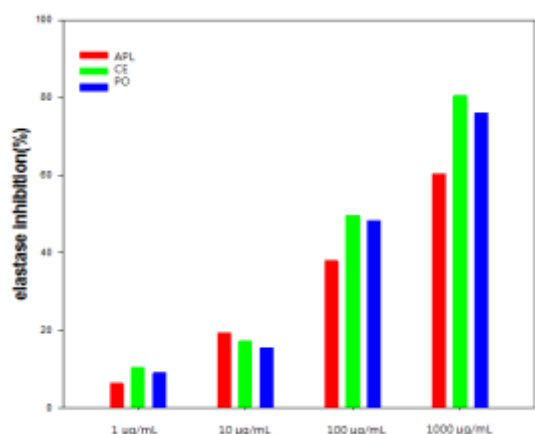

Figure 3. Elastase inhibition of the extract:
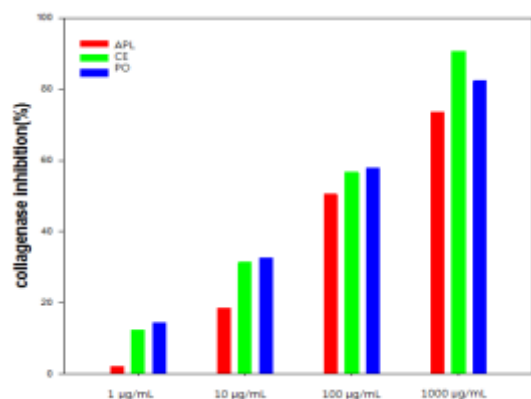*Portulaca oleracea* (PO), *Agrimonia pilosa Ledeb* (APL), composite extract (CE)

Figure 4. Collagenase inhibition of the extract:
Portulaca oleracea (PO), *Agrimonia pilosa Ledeb* (APL), composite extract (CE)

Figure 5, 6 shows the results of elastase and collagenase inhibitory activity at the mixing ratio of 2:1,

1:1, and 2:1 (APL:PO). The mixture of 2:1 ratio showed the highest inhibition rates of 91.2% ± 0.5% and 98.3% ± 1.5% in elastase and collagenase inhibition experiments, respectively. Overall, the mixtures of 2:1 and 1:2 ratio showed improved anti-aging agents compared to the 1:1 mixture. Therefore, in the anti-aging activity, we confirmed that the synergistic effect of the composite extract was evident, which did not appear in the antioxidant activity.

Coumarin is a phenolic secondary metabolite that is extensively distributed in plants and known for its anti-inflammatory, anti-cell aging, and anti-skin aging effects. Essential fatty acids such as omega 3 and alpha-linolenic acid are suggested to protect the skin and prevent skin aging [16]. As the skin ages, the skin loses its ability to produce lipid components, whereas the skin dries because of the lack of such lipid materials. Moreover, it tends to be damaged more easily because there are insufficient intercellular substances between the skin cells. Omega 3 can restore skin health and elasticity by strengthening skin barriers and preventing water loss in the skin [17]. Thus, for antioxidant and anti-aging cosmetics, the composite extracts of various antioxidant agents from APL and anti-aging agents from PO can be a promising functional ingredient.
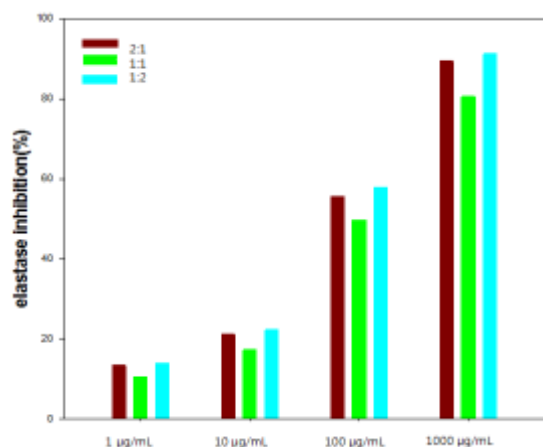

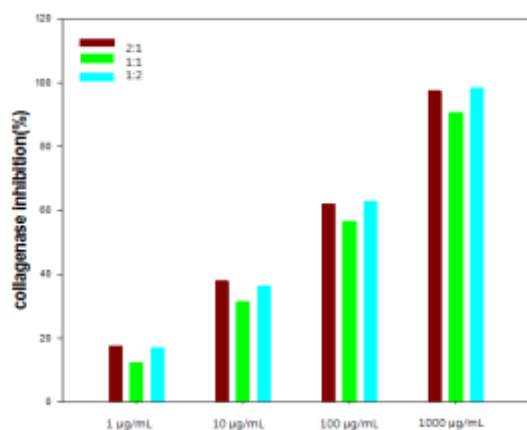**Figure 5.** Elastase inhibition of the composite extract: (2:1, 1:1, 1:2)

Figure 6. Collagenase inhibition of the composite extract: (2:1, 1:1, 1:2)

3. Conclusions

This study aimed to increase the applicability of APL and PO composite extract as an ingredient for herbal cosmeceuticals and these conclusions were obtained. The antioxidant effect of the composite extract show ed inhibition rates similar to those of the single extract. However, for the anti-aging evaluation, the inhibitory effect of the mixed extract was higher than that of the single extract; nevertheless, the highest i nhibitory activity was observed in the 1:2 mixture of APL and PO. Accordingly, for cosmeceutical products, the composite extract was expected to be a suitable natural medicinal material. Recently, as people are increasingly interested in longevity without illness, interest and demand for functional ingredients are increasing. In particular, this trend is becoming more common because everybody is attempting to avoid using synthetic materials. Interests in natural medicinal herbs and their areas of use are continuously growing as both health supplements and raw materials for cosmetics. Most importantly, however, is the

safety issue. These natural products have various advantages; however, they have disadvantages such as toxicity or adverse effects in certain individuals too. Therefore, continuous and systematic research on the stability of raw and herbal medicine from natural materials should be conducted.

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