

## The Efficacy of Amaranth Extract as an Ingredient in Multi-Functional Cosmetics

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**Abstract:** This study assessed the anti-oxidative and anti-aging efficacies of amaranth extract and measured its applicability as a natural ingredient for multi-functional cosmetics. For this, the leaves and flowers of Korean native amaranth were extracted with 80% ethanol and two samples were obtained. After performing DPPH and ABTS assays, both collagenase and elastase inhibitory activities were measured. The data were then statistically analyzed. The results found the following: In terms of the antioxidant capacity of the two substances, both DPPH and ABTS radical scavenging activities revealed similar results which were dose-dependent. In collagenase and elastase inhibitory activities, the results were better than other natural materials that have recently been studied such as *Eleutherococcus senticosus* and *Paeonia suffruticosa*. Therefore, it was confirmed that amaranth extract would be suitable as an anti-aging ingredient that can prevent skin aging from ultraviolet rays or free-radicals. The said results prove the possibility of amaranth extract as a natural ingredient for multi-functional cosmetics. With the recent heightened interest in inner beauty, the development of an ingredient for cosmetics using food would be more meaningful.

**Key words:** Amaranth, multi-functional cosmetics, anti-oxidative, anti-aging inner beauty, *Eleutherococcus senticosus*, *Paeonia suffruticosa*

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### INTRODUCTION

Both “antioxidants” and “anti-aging” have recently emerged as prominent keywords in various sectors. The anti-aging trends in society, regardless of age and gender, coincide with the industrial activities of each sector and have become more evident. When it comes to the cosmetics industry, companies have spurred the development of functional products for anti-wrinkle, whitening and anti-aging effects (Valko *et al.*, 2007). According to the Global Industry Market Research Report, the market value of anti-aging products could reach US\$275.2 billion in 2020 (Kwon, 2013). The anti-aging product market is expected to expand globally into Asian and African regions beyond Europe and the US and market growth appears to be further accelerated in response to the global aging phenomenon (Chung *et al.*, 2003).

The local cosmetics industry has also helped Korea grow into the fourth largest cosmetics exporting country in 2017, promoting active research in various cosmetic ingredients (Omaye *et al.*, 1997). Also, as the scope of functional skin care products increased from 3-11

categories, screening and regulatory requirements for safety and effectiveness have been reinforced (Lee *et al.*, 2001). Amaranth belongs to the *Amaranthus* spp. L. family and is used as an ingredient for a wide variety of foods due to its significant nutritional properties including protein, squalene and polyphenol (Kim and Ryoo, 2002). Amaranth seeds are high in protein and minerals including iron, calcium, zinc, magnesium and lysine. The leaves are known to have large amounts of protein and ascorbic acid as well as flavonoid and anthocyanin pigments (Choi, 2011). In the wake of controversial events in relation to a toxic humidifier disinfectant accident, cancer causing chemicals in sanitary pads and heavy metals in skin care products, consumer reluctance to accept chemical products is increasing. On the other hand, the demand for natural ingredients and beauty products is growing, thereby boosting the number of chemical-free consumers who advocate for naturally occurring compounds with no hazardous chemicals (Han *et al.*, 2013). In line with this trend in consumer markets, the grading and labelling system has been introduced to control patent applications and the safety of functional ingredients (Park *et al.*, 2014). The present study,

therefore, aimed to evaluate the antioxidant and anti-aging effects of extracts from amaranth flowers and leaves which are widely used for functional foods and to develop a natural functional ingredient for skin care products.

## MATERIALS AND METHODS

Extracts were obtained from Amaranth Leaves (AL) and Flowers (AF) grown in Pyeongchang, Gangwon Province, Korea through a preparation process in which the collected samples were thoroughly cleaned, cut into small pieces, naturally dried and ground. The 30 g of the prepared sample were placed into each extraction flask connected to a vertical reflux condenser and 250 mL of 80% ethanol was added to carry out extraction at 1.23 bar for 15 min in an autoclave (SJ-220A100, Sejong Scientific Co. Ltd., Bucheon, Korea). Vacuum filtration of the extract was then performed. The filtered liquid was condensed using a vacuum rotary evaporator (R-114, Buchi Co., Flawil, Switzerland) and dried using a freeze dryer (Ilshin Co., Seoul, Korea). Powdered samples were refrigerated until ready to use in the experiment.

**DPPH assay:** The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay proposed by Brand-Williams was used to assess antioxidant capacity (Starkey *et al.*, 1997). Two kinds of extracts were prepared at concentrations of 125, 250, 500 and 1000 mg/mL and the DPPH solution was prepared at concentrations of  $1 \times 10^{-4}$ M,  $2.5 \times 10^{-4}$ M,  $5 \times 10^{-4}$ M and  $6.5 \times 10^{-4}$ M. First, 1 mL of the sample solution was added to 1 mL of the DPPH solution for each concentration and absorbance at 540 nm was measured at 10 sec intervals for 5 min using a UV/VIS spectrophotometer. After 25 min of incubation in the dark and standing for 5 min, absorbance values were measured. Three replicates were prepared for each sample.

**ABTS assay:** Each of the AL and AF solutions was diluted to obtain final concentrations of 1, 10, 100 and 1,000  $\mu$ g/mL. The ABTS solution was prepared by mixing 7.4 mM ABTS with 2.6 mM potassium persulphat and left to stand for one day in the dark to generate ABTS radical cation (ABTS<sup>+</sup>). Then, the reaction mixture was diluted to obtain an absorbance of 1.5 or less at 732 nm. After mixing 150  $\mu$ L of the diluted ABTS<sup>+</sup> solution with 5  $\mu$ L of each AL and AF sample and incubated at room temperature for 10 min and then absorbance was measured at 732 nm. Distilled water was added to the control sample and its ABTS<sup>+</sup> radical scavenging activity was calculated.

**Elastase inhibition activity:** Each AL and AF sample was diluted to make final concentrations of 1, 10, 100

and 1,000  $\mu$ g/mL. A test solution was then prepared by dissolving elastase and N-succinyl-(L-Ala) 3-p-nitroanilide in a 50 mM Tris-HCl buffer (pH 8.6) at each concentration level. Then, 40  $\mu$ L of 2.5 U/mL elastase was added to 5  $\mu$ L of each AL and AF sample and 80  $\mu$ L of 0.5 mg/mL N-succinyl-(L-Ala) 3-p-nitroanilide was added to each mixture which was then incubated at 37°C for 30 min. After reaction, absorbance was measured at 445 nm.

**Collagenase inhibition activity:** Each AL and AF sample was diluted to make final concentrations of 1, 10, 100 and 1,000  $\mu$ g/mL. To prepare a test solution, 4-phenylazobenzoyloxycarbonyl-Pro-Leu-Gly-Pro-D-Arg was dissolved in 0.1 M Tris-HCl buffer (pH 7.5) containing 4 mM CaCl<sub>2</sub>. Then, 250  $\mu$ L of 0.3 mg/mL 4-phenylazobenzoyloxycarbonyl-Pro-Leu-Gly-Pro-D-Arg was added to 100  $\mu$ L of each AL and AF sample. The reaction was then initiated by adding 150  $\mu$ L of 0.2 mg/mL collagenase for 20 min at room temperature. After 500  $\mu$ L of 6% citric acid and 1.5 mL of ethyl acetate were added, absorbance was measured at 320 nm.

**Statistical processing:** Experiment results were analyzed using an unpaired Student's t-test and ANOVA in SPSS 18.0 and statistical significance was determined with cutoffs at  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$

## RESULTS AND DISCUSSION

### Results of the experiment and considerations

**DPPH assay:** Total antioxidant capacity of Leaf and Flower extracts of amaranth (AL and AF) is presented in Fig. 1. The DPPH radical scavenging ability of the AL extract was  $22.2 \pm 0.6$ ,  $43.0 \pm 0.4$ ,  $69.3 \pm 0.2$  and  $79.5 \pm 0.2\%$ ,

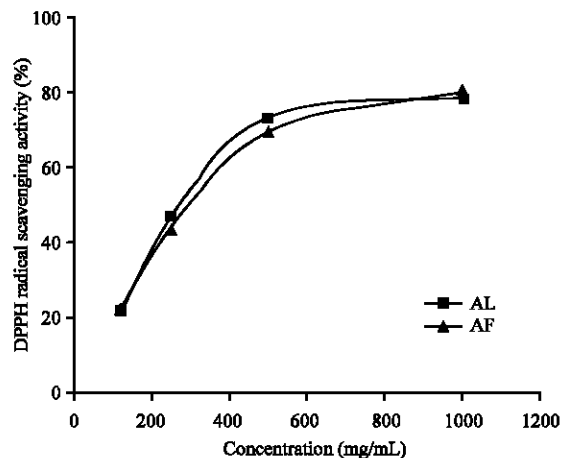


Fig. 1: DPPH radical scavenging activity of AL and AF

respectively, at concentrations of 125, 250, 500 and 1000 mg/m. The AF extract showed 21.2±0.4, 46.2±0.4, 72.9±2.1 and 78.1±0.4%, respectively, at the same concentrations. Thus, the scavenging ability of the two extracts increased with increasing concentration. The IC50 value for the AL and AF extracts was 290.69 and 270.56 mg/mL, respectively, showing a lower IC50 value in the latter. However, there were no significant differences in antioxidant capacity between the two extracts because these extracts exhibited a similar level of antioxidant capacity even at high concentrations. As the free radical scavenging ability of extracts is generally concentration dependent, the IC50 value can be used as a cutoff value when evaluating the antioxidant capacity of extracts.

**ABTS assay:** The ABTS radical scavenging ability is determined by the extent of decolorization of blue/green color and measurable in most types of extract whether hydrophilic or hydrophobic. The higher phenolic compounds lead to the greater disappearance of color, meaning higher ability to scavenge. The ABTS radical scavenging ability of the AL extract was 0.7±0.4, 4.7±0.5, 32.0±0.4 and 93.3±0.1%, respectively, at concentrations of 1, 10, 100 and 1,000 µg/mL. The AF extract showed 0.9±0.1, 4.7±0.5, 33.4±0.4 and 94.7±0.2%, respectively. Both sample extracts exhibited a concentration-dependent increase in radical scavenging ability in the ABTS assay. Thus, the antioxidant capacity of the two sample extracts was similar, according to their scavenging ability against DPPH and ABTS free radicals.

**Elastase inhibition activity:** Elastin as elastic connective skin tissues, decides the thickness and resilience of the dermis along with collagen. With the ability to stretch 3~4 times their length, this elastic protein can form strong elastic structures of cellular tissues. Elastase is an enzyme that degrades not only elastin but also collagen, fibrinogen and other components of immunoglobulin. However, it can also provide regenerating support to the cells damaged or destroyed, making a proper elastase activity important in human body. If elastase activity is unusually increased, elastin fibers in the skin are decomposed as a result of protein breakdown, resulting in decreased skin elasticity (Younes, 1975; Park *et al.*, 2014). Therefore, many studies are conducted to deter the aging process and wrinkle formation through the suppression of excessive elastase activity.

The measured elastase inhibition activity of the AL extract showed 6.5±0.9, 14.4±0.5, 22.2±0.2 and 61.2±0.3%, respectively, at concentrations of 1, 10, 100 and 1,000 mg/mL. The AF extract exhibited 5.9±0.5, 12.5±0.8, 20.6±0.2 and 56.8±0.3% at the four concentrations. Thus, the

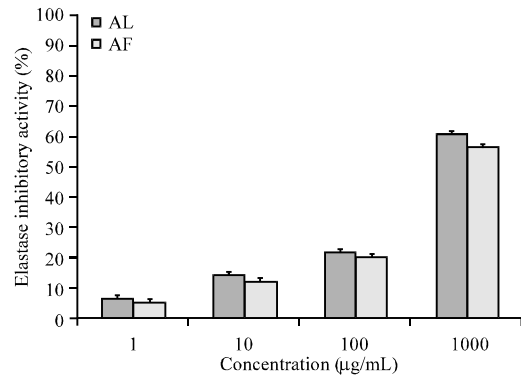


Fig. 2: Elastase inhibitory activity of AD and AF. AD and AF were incubated at 1, 10, 100 and 1,000 mg/ML with elastase,-succinyl-(L-Ala)3-p-nitroanilide for 30 min. Activities were determined by measurement of absorbance at 445 nm. The results were expressed as mean ±SD from three independent experiments

elastase inhibition activities of the two sample extracts increased in a concentration-dependent manner (Fig. 2). These results demonstrated higher overall inhibition activity than those of existing studies in which Kim and Ryoo (2002) used fractions of *Theobroma cacao*. L fractions and Park and Cha (2016) and Lee *et al.* (2011) used active ingredients of *Rosa multiflora* Thunb. In addition, the inhibition of elastase was still higher than those reported by Yuan *et al.* (2007) and Cha *et al.* (2005) in their study using extracts from *Gleditsiae fructus*, *Machilus thunbergii* and *Rhus javanica*. Therefore, amaranth has potential as a functional ingredient for anti-aging beauty products. In particular amaranth flower extract demonstrated great potential showing a percent inhibition of 61.2±0.3%

**Collagenase inhibition activity:** The measured collagenase inhibition activity of the AL extract revealed 3.7±0.9, 15.9±0.6, 34.5±0.3 and 67.6±0.3%, respectively, at concentrations of 1, 10, 100 and 1,000 (mg/mL). The AF extract exhibited 2.1±0.5, 12.7±1.0, 30.1±0.4 and 55.8±0.3% at these four concentrations. The collagenase inhibition activities of the two sample extracts increased with an increase in concentrations (Fig. 3). These results outperformed inhibition activity reported in existing studies in which Choi (2011) and Park *et al.* (2002) investigated the functional enzymes of *Coriolus versicolor* fruit body and Yuan *et al.* (2007) and Cha *et al.* (2005) used acanthopanacis cortex and moutan cortex. Both collagenase and elastase are Matrix Metalloproteinase (MMPs) that specifically, act on certain proteins. The expression of these enzymes is induced by oxygen free

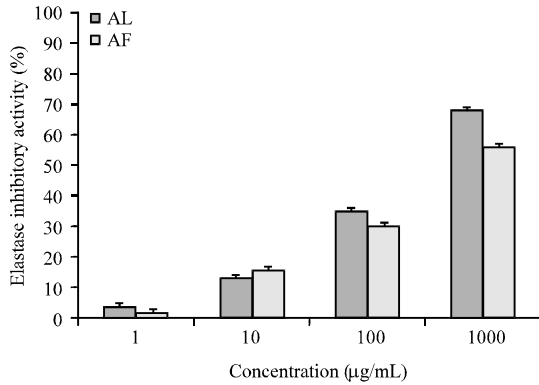


Fig. 3: Collagenase inhibitory activity of AL and AF. AL and AF were incubated at 1, 10, 100 and 1,000 mg/mL with collagenase, 4-phenylazobenzylloxycarbonyl-Pro-Leu-Gly-Pro-D-Arg for 20 min. Activities were determined by measurement of absorbance at 320 nm. The results were expressed as mean  $\pm$ SD from three independent experiments

radicals and UV rays, causing severe protein breakdown or damage which in turn decreases dermis elasticity and induces wrinkle formation and aging (Lee *et al.*, 1999).

In the present study, amaranth extract when compared with other natural plant extracts, exhibited excellent inhibition performance against elastase and collagenase, both of which are mainly used for the evaluation of anti-aging effects. Although, the inhibition activity of the AL and AF extracts showed no significant difference, the AF was more effective in inhibiting elastase and the AL showed better anti-collagenase activity. As part of the aging process, the ability of skin fibroblasts to produce collagen declines, leading to increasing formation of wrinkles. However, the amount of collagen in the skin is not solely determined by collagen synthesis because the extent of MMPs expression is also an important factor influencing skin aging. Thus, blocking UV rays and oxygen free radicals which increase the release of MMPs is an important method to slow down skin aging.

In the present study, Amaranth extract showed high ability to inhibit protein expression and enzyme activity as well as potent anti-oxidant ability. Thus, this extract is considered a possibility to become a key ingredient for anti-aging beauty products. As the facial mask market has been recently expanding globally, a wide variety of products are available. Some masks are developed using storytelling tactics and this trend combines brands of food products such as yogurt and vegetable beverage to create a foodmetics market. Furthermore, the growing

attention of consumers on inner beauty increases the significance of developing food-inspired cosmetic ingredients.

## CONCLUSION

In order to evaluate the potential of amaranth as a functional cosmetic ingredient, this study was conducted to investigate the antioxidant capacity and anti-aging effects of edible amaranth leaves and flowers using two different antioxidant assays and two enzymes, respectively. As a result, the two extracts exhibited IC50 values of 290.69 and 270.56 mg/mL for antioxidant capacity, showing no significant difference, irrespective of concentration level, although, their capacity was higher than those of natural extracts reported in existing studies. In terms of anti-aging potential, higher activity was observed in the AL extract against elastase while there was no significant difference between the two extracts with respect to collagenase inhibition activity. Given the increasing concern over synthetic products, the development of natural ingredients would be a meaningful approach. In light of this fact, this study has provided valuable information on the biological potency of amaranth extract as a functional cosmetic ingredient. material for antioxidant and anti-aging.

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