

## Potent Antielastase and Antityrosinase Activities of *Astilbe chinensis*

<sup>1</sup>Seung-Hun Lee, <sup>1</sup>Sandesh Sancheti, <sup>1</sup>Shruti Sancheti and <sup>1,2</sup>Sung-Yum Seo  
<sup>1</sup>Department of Biology, Kongju National University, Kongju 314-701, Korea  
<sup>2</sup>Korean Collection of Herbal Extracts, Inc., Kongju 314-701, Korea

**Abstract: Problem statement:** In the current scenario, photo-aging is a major problem causing skin wrinkling and hyperpigmentation. Therefore, elastase and tyrosinase inhibitors play an important role in the treatment of skin aging and thus, gaining a special attention in cosmetic industries. **Approach:** In the screening of Korean medicinal plants to search the potent elastase and tyrosinase inhibitors, the extract of the rhizomes of *Astilbe chinensis* (*A. chinensis*) exhibited the strongest potential. Further the crude 100% methanolic extract of *A. chinensis* along with its n-hexane, methylene chloride, ethyl acetate and aqueous fractions were investigated for elastase and tyrosinase enzyme inhibition activities. **Results:** The *A. chinensis* extracts showed remarkable elastase inhibitory activities (at a concentration of 20 µg 155 µL<sup>-1</sup> reaction) ranging from 38-92% and tyrosinase inhibition activities (at a concentration of 20 µg 150 µL<sup>-1</sup> reaction) ranging from 2-90%. Among all the fractions, water fraction demonstrated the highest elastase and tyrosinase inhibitory activities (92 and 90%, respectively). **Conclusion:** Based on the noteworthy antielastase and antityrosinase activities by the rhizome of *A. chinensis*, it might prove a strong candidate as an active ingredient in cosmaceutical formulations and further *in vitro* and *in vivo* investigations is needed.

**Key words:** *Astilbe chinensis*, elastase inhibition, tyrosinase inhibition

### INTRODUCTION

The skin is an important barrier that protects the body from damage due to direct contact with the outside environment. Among harmful environmental factors that damage the skin, UV irradiation is the most common and harmful. UV irradiation reduces skin elasticity and the linearity of dermal elastic fibers inducing wrinkle formation<sup>[1,2]</sup>. Moreover, UV irradiation sets in action an integrated mechanism for the formation and delivery of melanin within melanosomes from melanocytes to keratinocytes<sup>[3]</sup>.

Elastase is the only enzyme that is capable of degrading elastin, an insoluble elastic fibrous protein in animal connective tissues. It hydrolyses practically all proteins, including the supporting and structural proteins of the connective tissue, such as collagen and elastin. In UV-irradiated skin, mild inflammation occurs repeatedly in the dermis and it is assumed that connective tissue proteins may be attacked by elastase released from neutrophils, resulting in damage to elastin and finally causing sagging<sup>[4]</sup>.

Tyrosinase is one of the key enzymes in the melanin biosynthetic pathway: Tyrosinase catalyzes the oxidation of L-tyrosine to 3,4-Dihydroxyphenyl-L-Alanine (L-DOPA), followed by the oxidation of L-

DOPA to dopaquinone. Subsequent oxidative polymerizations of several dopaquinone derivatives yield melanin<sup>[5-7]</sup>. Thus, use of tyrosinase inhibitors is becoming increasingly important in the cosmetic industry due to their skin-whitening effects, treatments for hyperpigmentation and solar lentigines by UV-irradiation.

*A. chinensis* is a perennial herb that grows in China, Russia, Japan and Korea. The rhizomes of *A. chinensis* have been traditionally used to treat arthralgia, chronic bronchitis, headache and stomachalgia. Recent studies on the extract of *A. chinensis* rhizomes reported potential anti-inflammatory<sup>[8]</sup> and antitumor activities<sup>[9,10]</sup> of this plant. In this research, we examined the inhibitory effects of *A. chinensis* rhizomes on elastase and tyrosinase enzymes to study the efficacy of it as a cosmaceutical agent.

### MATERIALS AND METHODS

**Plant material:** The rhizomes of *A. chinensis* were obtained from "Korean Collection of Herbal Extracts", a Biotech company in Korea. A collection of voucher specimen is available with the company (Korean Collection of Herbal Extracts, 2000).

**Corresponding Author:** Sung-Yum Seo, Department of Biology, Kongju National University, Kongju, Korea  
Tel: 82-41-850-8503 Fax: 82-41-854-8503

**Extraction:** The rhizomes of *A. chinensis* (2 kg, dry weight) were chopped into small pieces and kept for extensive decoction in 100% methanol for 3 days at room temperature. The extract was then concentrated using rotary vacuum evaporator at 20-30°C to obtain the dried crude extract (150 g).

**Fractionation:** The crude methanolic extract (150 g) was suspended in distilled water (1 L) and partitioned with n-hexane, methylene chloride, ethyl acetate and n-butanol to yield the n-hexane (15 g), methylene chloride (6 g), ethyl acetate (52 g) and aqueous (70 g) fractions, respectively. The enzyme inhibition activity assays were performed at concentration of 2 mg mL<sup>-1</sup> for the crude extract and various fractions.

**Reagents:** All necessary chemicals and enzymes were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other commercially available reagents and solvents were used as received.

**Elastase assay:** The elastase activity was evaluated according to the method previously reported by Kraunsoe *et al.*<sup>[11,12]</sup> with minor modifications. In order to evaluate the inhibition of elastase activity, the amount of released p-nitroaniline, which was hydrolyzed from the substrate, N-succinyl-Ala-Ala-Ala-p-nitroanilide, by elastase, was assayed by measuring absorbance at 410 nm. In brief, 1.015 mM solution of N-succinyl-Ala-Ala-Ala-p-nitroanilide was prepared in a 0.1 M Tris-Cl buffer (pH 8.0) and this solution (130 µL) was added to the test sample (10 µL) in a 96 well microplate. The microplate was pre-incubated for 5 min at 25°C before an elastase (0.5 Unit mL<sup>-1</sup>) stock solution (15 µL) was added. After enzyme addition, the microplate was kept at 25°C for 30 min and the absorbance was measured at 410 nm using microplate reader. All experiments were carried out in triplicates.

**Tyrosinase assay:** The spectrophotometric assay was performed as described previously by Masamoto *et al.*<sup>[13]</sup> with minor modifications. Ten µL of sample was added to 96 well micro plate. To these, 80 µL of 67 mM phosphate buffer (pH 6.8) and 30 µL of 5 mM L-DOPA was added. After incubating for 10 min at 37°C, 30 µL of mushroom tyrosinase (200 units mL<sup>-1</sup>) was added. The reaction was monitored after 20 min for the formation of dopachrome by measuring optical density at 492 nm using micro plate reader. All experiments were carried out in triplicates.

## RESULTS

The crude extract of *A. chinensis* and its various fractions were evaluated at 20 µg 155 µL<sup>-1</sup> concentrations for elastase enzyme inhibition studies.

All the *A. chinensis* extracts showed remarkable elastase inhibitory activities ranging from 38-92% and are shown (Fig. 1): Water (92%) > crude extract (83%) > n-hexane (78%) > methylene chloride (58%) > ethyl acetate (38%).

Tyrosinase inhibition results (at a concentration 20 µg 150 µL reaction<sup>-1</sup>) are: Water (90%) > crude extract (78%) > ethyl acetate (54%) > n-hexane (5%) > methylene chloride (2%). The water fraction showed the outstanding activity of tyrosinase inhibition (Fig. 2).

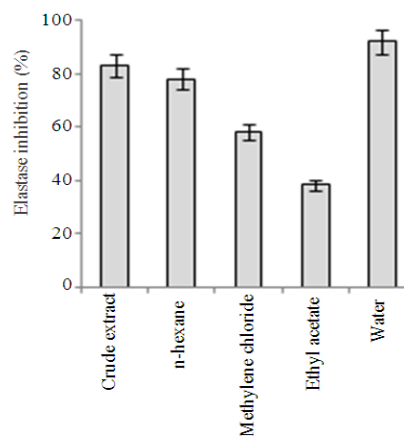


Fig. 1: Elastase inhibition by crude extract of *A. chinensis* and its fractions at 20 µg 155 µL<sup>-1</sup> concentration

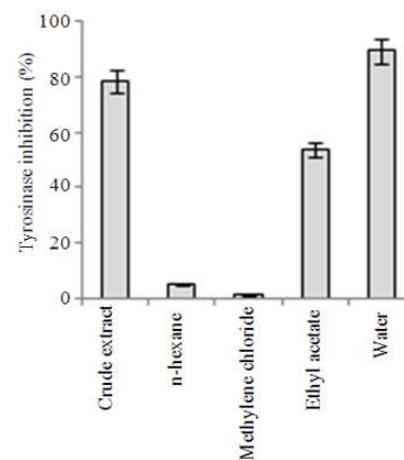


Fig. 2: Tyrosinase inhibition by crude extract of *A. chinensis* and its fractions at 20 µg 150 µL<sup>-1</sup> concentration

## DISCUSSION

In the present study, some of the partitioned fractions of *Astilbe chinensis* extract showed remarkable elastase inhibition and tyrosinase inhibition activities, especially, water fraction showed the strongest inhibition against both the enzymes (Fig. 1 and 2).

Elastase plays a critical role in inflammatory processes<sup>[14]</sup>. Also, it was reported that the *A. chinensis* extract has an inhibitory effect on UVB induced inflammation in human keratinocytes<sup>[15]</sup>. Thus, elastase inhibitors from *A. chinensis* are the important tools for studying the mechanisms of action of elastase and prospective ingredients for cosmetic and pharmacology industries. Furthermore, since the extract of *A. chinensis* showed the outstanding tyrosinase inhibition activities, the findings presented here suggest that extract of *A. chinensis* may have a role as a useful ingredient in cosmetics for wrinkle-care, skin-whitening and as phytopharmaceutical.

## CONCLUSION

The results of the *in vitro* enzyme inhibition studies emphasize the potent effect of the *A. chinensis* rhizomes extract on elastase and tyrosinase inhibitions. Based on this, further pre-clinical and clinical studies can be pursued.

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