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Evaluation of *Achyranthes japonica* Ethanol Extraction on the Inhibition Effect of Hyluronidase and Lipoxygenase

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The 1, 1- diphenyl 2-picrylhyorazyl (DPPH) is a well-known radical and a trap (scavenger) for other radicals. Hyaluronidase (HAase) is an enzyme that depolymerizes the polysaccharide hyaluronic acid (HA) in the extracellular matrix of connective tissue. Lipoxygenase (LOX) enzyme was reported to convert the arachidonic, linoleic and other polyunsaturated fatty acid into biologically active metabolites involved in the inflammatory and immune responses. The purpose of the present study is to evaluate plant extracts as sources of natural antioxidants and to examine whether *Achyranthes japonica* having significant DPPH, HAase and LOX inhibitory activity. The inhibitory effect of HAase by *A. japonica* was assayed using a Morgan microplate assay. The antioxidant activity of the *A. japonica* extracts was measured on the basis of the scavenging activity of the stable 1, 1- diphenyl 2-picrylhyorazyl (DPPH) free radical. DPPH scavenging activity of matured roots of *A. japonica* was evaluated at 4.0 mg/ml was 87.8% and that of young roots was 86.2% at same concentration. The roots of *A. japonica* showed maximum inhibition of HAase activity (IC_{50} = 27.7 μg/ml). The highest LOX inhibition was recorded in the root extract among three vegetative parts. Inhibition of HAase activity of roots may contribute towards the development of herbal medicines. Although percent inhibition of lipoxygenase by *Achyranthes japonica* for all young and matured groups for leaves, stems, and roots at different concentrations, there were not show a statistically significant difference (p<0.05).

Key words : 1, 1- diphenyl 2-picrylhyorazyl (DPPH), *Achyranthes japonica*, hyaluronidase, lipoxygenase

Introduction

Antioxidants can be found naturally in plants and their compounds in food play an important role as a health protecting factor [10]. Antioxidant scavenge free radicals are very important in inhibiting oxidative mechanisms that lead to degenerative some diseases. The 1, 1- diphenyl 2-picrylhyorazyl (DPPH) is a well-known radical and a trap (scavenger) for other radicals [4].

Hyaluronan (HA, also known as hyaluronic acid or hyaluronate) is one of the important matrix components of the ground substance of the subcutaneous tissues and plays important roles in development, growth, and repair of tissues [26]. Hyaluronidase (HAase, EC.3.2.1.35) is an enzyme that depolymerizes the polysaccharide hyaluronic acid (HA) in the extracellular matrix of connective tissue. The enzyme is found both in organs (testis, spleen, skin, eye, liver, kidney, uterus and placenta) and in body fluids (tears, blood and sperm) [6, 19].

Lipoxygenase (LOX) enzyme was reported to convert the arachidonic, linoleic and other polyunsaturated fatty acid into biologically active metabolites involved in the inflammatory and immune responses [3]. 15-Lipoxygenases have been found in plants as well as in animal tissue. Commercially obtainable 15-1ipoxygenase is isolated from soybeans as the type 1 isoenzyme. There is a good correlation between inhibitory activity towards the mammalian and the soybean-derived enzymes [16, 21].

46: 8.*Achyranthes japonica* (Miq.) Nakai (Amaranthaceae) is a perennial plant growing to 1.0 m tall with thickened roots. Stems are glabrous or slightly pubescent and shape quadrangular and branched. Its nodes are dilated. The root of the plant is used in the traditional medicine of Korea to treat oedema, rheumatism, delayed menses and as a contraceptive and abortifacient. The root contains triterpenoid saponins and has been shown to have analgesic, antiallergic, antiinflammatory, antispasmodic, diuretic, hypotensive, and uterine stimulant properties. In addition, it contains protocatechuic acid, which has antioxidant properties, and also

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inhibits the aggregation of platelets [23].

A. japonica is native of eastern and southeastern Asia including Korea and Japan. The plant contains several substances which can be used medicinally. It is one of two species in the genus *Achyranthes* found in the United States (the other is *A. aspera*, an exotic species found in upland environments of the Southeast coastal plain) [13]. *A. japonica* was first discovered in North America 30 years ago on the banks of Tug Fork of the Big Sandy River, Martin County, Kentucky [18].

Various antioxidant activity methods have been used to screen and compare the antioxidant in vegetables, fruits, tea, and wine. The purpose of the present study is to evaluate plant extracts as sources of natural antioxidants for DPPH and to examine whether the herbal medicine (*A. japonica*) having significant HAase and LOX inhibitory activity.

Materials and Methods

Sample extract

I divided the plants of *A. japonica* into three parts: leaves, stems, and roots. Each vegetative organism was divided two additional groups. One is young group and the other is matured group. Each sample (100 g) of *A. japonica* was ground with pestles and liquid nitrogen at -70℃ and homogenized prior to beginning extraction experiments. The extraction solvent was ethanol. The sample was treated with ultrasound at room temperature for a given duration. The ultrasound extraction was carried out using an ultrasonic bath (5510, Branson, USA). The mixture was further stirred with a magnetic bar at 65°C for 2 hr. Extracted sample was filtered. The sample was evaporated to remove solvent under reduced pressure and controlled temperature by using rotary vacuum evaporator (N-1001S-W, Eyela, Tokyo, Japan). To get dry powder, samples placed in a low temperature vacuum chamber.

DPPH free radical

The antioxidant activity of the *A. japonica* extracts was measured on the basis of the scavenging activity of the stable 1, 1- diphenyl 2-picrylhyorazyl (DPPH) free radical according to the method described by Brand-Williams et al. [4] with slight modifications. 1 ml of 0.1 mM DPPH solution in ethanol was mixed with 1 ml of plant extract solution of various concentrations (0.1, 1.0, 2.0 and 4.0 mg/ml). To determine the IC_{50} value of the active component, the technique using 96-well microplates was employed [14]. DPPH was added to the solutions prepared with plant extracts and standard antioxidant substances and stirred. A solution of DPPH was prepared by dissolving 5 mg DPPH in 2 ml of ethanol, and the solution was kept in the dark at 4°C. A stock solution of the compounds was prepared at 1 mg/ml in DMSO. The stock solution was diluted to varying concentrations in 96-well microplates. Then, 5 μl of ethanol DPPH solution (final concentration 300 μm) was added to each well. The plate was shaken to ensure thorough mixing before being wrapped with aluminum foil and placed into the dark. After 30 min, the optical density (OD) of the solution was read using the UVmini-1240 Reader (Shimadzu, Kyoto, Japan) at the wavelength 517 nm. Absorbance changes are measured at 517 nm. Corresponding blank sample was prepared and L-Ascorbic acid (1.0 μg/ml) was used as reference standard (positive control). The inhibition % was calculated using the following formula.

Percentage inhibition was calculated using the following formula: % Inhibition = [1 - OD (DPPH + sample)/OD (DPPH)] ×100%.

A dose response curve was plotted to determine the IC_{50} values. IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity.

Hyaluronidase inhibition assay

The inhibitory effect of HAase by *A. japonica* was assayed using a Morgan microplate assay. HAase (Type I-S from bovine testis, Sigma-Aldrich Co., England) is dissolved in 0.1 M acetate buffer (pH 3.5) and mixed with extracts of *A. japonica*. The resulting solution was applied to a microplate. A negative control (0.1 M acetate buffer) to serve as a reagent blank was also applied to another wells with enzyme. The plate was put in water bath for 20 minutes at 30°C. 12.5 mM $CaCl_2$ was added to the plate and incubated for 20 minutes at 37°C.

HA (6 mg/ml) which was dissolved in a 0.1 M acetate buffer was added to HAase complex solution and incubated for 40 minutes at 37°C. 0.4 N NaOH and 0.4 M potassium tetraborate were added to terminate the enzymatic reaction for 3 minutes at 100°C. After cooling the mixture until room temperature, 180 μl DMAB solution (0.04 g/5 ml p-dimethyaminobenzaldehyde, 100% 3.5 ml acetic acid, and 10 N 5.0 ml HCl) were added to each well and incubated for 20 minutes at 37°C. The color change was measured spectrophotometrically at a wavelength of 540 nm.

HAase assay was validated by demonstrating that pure tannic acid (0.07 mg/ml Sigma-Aldrich Co., England) as a positive control, a known HAase inhibitor [7], gives 76-80% enzyme inhibition [8]. All experiments were done in triplicate.

Lipoxygenase activity

Lipoxygenase (LOX) inhibitor Screening Assay Kit (Abnova, CA, USA) was used and measured the hydroperoxides produced in the lipoxygenation using a purified LOX. Stock solutions of the tested samples 15-lipoxygenase standard (Abnova, CA, USA) were prepared by dissolving the extracts in ethanol or methanol. The reaction was initiated by the addition of aliquots (90 μl) soybean LOX solutions (prepared in potassium phosphate buffer, pH 9.0) in a sufficient concentration to give an early measurable initial rate of reaction to 10 μl of arachidonic acid in phosphate buffer. The enzymatic reaction was performed in presence or absence of inhibitor and their kinetics were compared. Quertin was used as positive control.

Nordihydroguaiaretic acid (NDGA) and Rutin used as negative control. Lo inhibition activity was determined using a spectrophotometric method at 490 nm.

The concentration that gave 50% inhibition (IC_{50}) was calculated from the outline of the inhibition percentages as a function of the inhibitor concentration [1]. Aqueous extracts ($IC_{50} \geq 100$ μg/ml) were not taken in this study.

Statistical analysis

All the analysis were carried out in triplicate and the results were expressed as the mean ±SD. Correlation co-efficient (R) to determine the relationship between two or more variables among Radical Scavenging activity tests were calculated using the SPSS software (Release 21.0). Regression analysis was used to calculate IC_{50}, defined as the concentration of inhibitor necessary for 50% inhibition of the enzyme reaction.

The percent inhibition was calculated as the decolourization percentage of the test sample using the following formula:

Inhibition % = (IA - As)/IA ×100

Where IA is the absorbance of the 100% initial and As is the absorbance of the sample. IA and As were the values which were subtracted the average absorbance of the blank wells.

Results

Table 1 was shown the antioxidant activities of the *A. japonica.* Various concentrations of root extracts were higher than those of leaves and stem. DPPH scavenging activity of young leaves of *A. japonica* was evaluated at 4.0 mg/ml was 81.7% and that of matured leaves was 83.2% at same concentration. DPPH scavenging activity of young stems of *A. japonica* was evaluated at 4.0 mg/ml was 72.6% and that of matured stems was 73.4% at same concentration. DPPH scavenging activity of matured roots of *A. japonica* was evaluated at 4.0 mg/ml was 87.8 and that of young roots was 86.2 at same concentration. The high antioxidant activity found on matured roots. The inhibitory activity of leaves (IC_{50} = 24.6 μg/ml) was at the same levels as that of L-ascorbic acid (IC_{50} 23.5 μg/ml), but stem and roots activities were lower than that of L-ascorbic acid (Fig. 3). The all young and matured groups for leaves, stems, and roots did not show a statistically significant difference (p<0.05).

The rates of HAase inhibition of the ethanol extracts were dependent on concentrations (Table 2). Extractions at low concentrations, < 1.0 mg/ml were not shown noticeable HAase inhibition. High concentrations, > 1.0 mg/ml produced significant HAase inhibition. HAase inhibition of matured leaves was 30.2% at 4.0 mg/ml and matured stems and roots was 37.2% and 42.1% at same concentration,

Table 1. Free radical scavenging effects of *Achyranthes japonica* at different concentrations

Concentration (mg/ml)	Leaf		Stem		Root	
	Young	Mature	Young	Mature	Young	Mature
0.1	67.77±3.39	68.37±3.83	54.36±2.18	55.06±2.41	60.15±3.04	61.37±2.26
0.5	70.19±2.29	71.85±1.77	56.98±2.31	58.01±2.02	63.32±0.77	64.31±1.73
1.0	74.17±3.79	75.59±4.09	60.84±2.99	61.31±2.41	73.49±2.32	72.04±1.86
2.0	78.94±5.54	80.64±6.20	67.53±1.74	67.06±3.12	77.23±2.27	78.16±2.28
4.0	81.74±3.37	83.24±4.51	72.55±3.36	73.35±2.34	86.15±3.56	87.84±2.83
t-test	-0.364, p<0.05		-0.107, p<0.05		0.101, p<0.05	

Data represent the mean ± SD from three replicates.

Table 2. Percent inhibition of hyaluronidase by *Achyranthes japonica* at different concentrations

Concentration (mg/ml)	Leaf		Stem		Root	
	Young	Mature	Young	Mature	Young	Mature
0.1	16.60±4.20	17.34±4.38	26.53±1.16	28.14±2.17	28.87±2.04	30.08±2.15
0.5	18.67±2.04	21.01±4.21	28.03±2.88	29.01±2.82	31.17±1.61	32.12±1.93
1.0	20.47±3.93	23.24±3.10	29.43±1.94	32.51±1.29	34.80±0.52	37.27±1.27
2.0	24.80±0.93	26.53±1.59	32.93±1.33	35.33±4.15	37.83±2.46	45.19±3.31
4.0	28.13±1.78	30.18±2.31	34.43±3.21	37.17±2.08	40.30±1.02	51.40±4.73
t-test	-0.632, p<0.05		-0.942, p<0.05		-1.021, p 0.05	

Data represent the mean ± SD from three replicates.

respectively. The all values of HAase inhibition of young leaves, stems, and roots were lower than those of matured vegetative organs. However, the all young and matured groups for leaves, stems, and roots did not show a statistically significant difference (p<0.05). When the tannic acid used as a negative control, extracts for leaves of *A. japonica* were 29.6% inhibitory effects on the activation of HAase and that of stem and root were 21.6% and 46.7% (Fig. 1). The roots of *A. japonica* showed maximum inhibition of HAase activity (IC_{50} = 27.7 μg/ml) (Fig. 3).

Table 3 was shown the LOX activity of *A. japonica* extracts. The highest LOX inhibition was recorded in the root extract among three vegetative parts. LOX inhibition of matured leaves was 21.5% at 4.0 mg/ml and matured stems and roots were 21.9% and 31.9% at same concentration, respectively. The values of LOX inhibition of young leaves and roots were lower than those of matured vegetative organs. However, the value (34.4%) of LOX inhibition of young stems was higher than that (21.9%) of matured stems. When the NDGA used as a negative control, extracts for leaves of *A. japonica* were 16.2% inhibitory effects on the activation of LOX and that of stem and root were 18.3% and 23.5% (Fig. 2). The leaves of *A. japonica* showed maximum inhibition of LOX activity (IC_{50} = 35.4 μg/ml) (Fig. 3). Although percent inhibition of lipoxygenase by *Achyranthes japonica* for all young and matured groups for leaves, stems, and roots at different

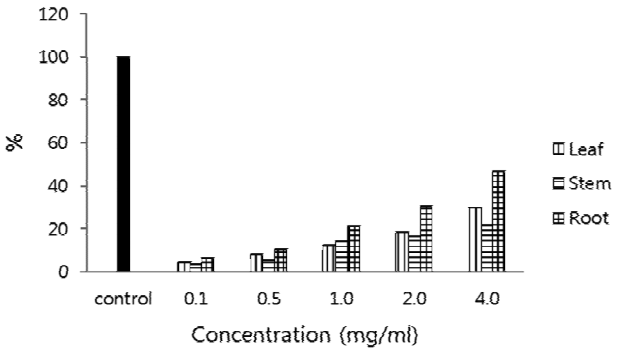

Fig. 1. The rate of hyaluronidase inhibitory of tannic acid (negative control) and relative inhibitory rate of matured plants of *Achyranthes japonica*.

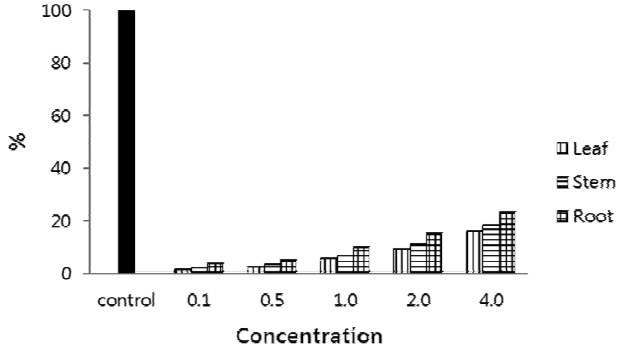

Fig. 2. The rate of lipoxygenase inhibitory of nordihydroguaiaretic acid (negative control) and relative inhibitory rate of *Achyranthes japonica*.

Table 3. Percent inhibition of lipoxygenase by *Achyranthes japonica* at different concentrations

Concentration (mg/ml)	Leaf		Stem		Root	
	Young	Mature	Young	Mature	Young	Mature
0.1	15.08±1.45	15.30±1.89	26.53±1.16	17.37±0.55	22.66±2.10	25.70±2.22
0.5	16.87±0.87	17.74±1.03	28.03±2.88	18.92±1.45	23.75±2.22	26.50±2.34
1.0	18.70±1.02	19.03±1.81	29.43±1.94	19.77±1.71	24.96±1.98	27.78±2.04
2.0	19.24±1.40	19.17±1.88	32.93±1.33	20.07±1.75	26.78±2.47	29.53±1.18
4.0	20.41±2.60	21.47±2.17	34.43±3.21	21.94±2.96	28.72±2.05	31.87±1.26
t-test	-0.349, p<0.05		-1.438, p<0.05		-1.875, p<0.05	

Data represent the mean ± SD from three replicates.

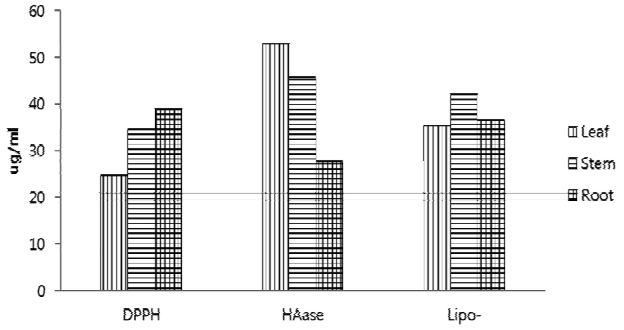

Fig. 3. Inhibitory effects {IC50 (mg/ml)} on DPPH, haluronidase activity, and lipoxygenase by *Achyranthes japonica* at different concentrations.

concentrations, there were not show a statistically significant difference (p<0.05).

Discussion

Herbal medicine is a major part of traditional medicine and has been used in medical practice since antiquity to cure human and other animal. About 60 to 85% of the populations of every country of the developing world rely on herbal or indigenous forms of medicine [22]. World health organization (WHO) notes that 74% of the plant derived medicines are used in modern medicine, in a way that their modern application directly correlates with their traditional use as herbal medicines by native cultures [11]. The herbal plant is a common element of ayurvedic, homeopathic, and naturopathic medicine [2].

Hwang et al. [8] reported that HAase inhibitory compounds extracted from the stem of *Styrax japonica, Deutzia coreana,* and *Osmanthus insularis* might be multifunctional and prevent the degradation of hyaluronic acid and HAase inhibitory rates (%) of three species of medicinal plant extracts, *S. japonica, D. coreana,* and *O. insularis* were 57.3%, 53.5%, and 53.2%, respectively. The strongest inhibition of hyaluronidase was observed for extract from *Lythri herba*, with IC_{50} value 8.1 μg/ml [25]. The methanol extract of *Clitoria ternatea* L. showed significant hyaluronidase inhibition with IC_{50} of 11.70 μg/ml [15].

The *Cucumis sativus* exhibited DPPH-free radical scavenging activity, IC_{50} at a concentration of 14.73 μg/ml and also showed strong anti-hyaluronidase (p<0.001) activity, IC_{50} at a concentration of 20.98 μg/ml [20].

Kwon et al. [12] reported that roots of *A. japonica* showed relatively high antioxidant activities. In this study, DPPH values of *A. japonica* were slightly high (Table 1).

Camellia sinesis, Rhodiola rosea, and *Koelreuteria henryi* had notable significant inhibitory activities towards lipoxygenase [5]. These results show that these plants have some phytochemical constituents which may be active against the lipoxygenase enzyme.

I have shown that 4.0 mg/ml weight of ethanol *A. japonica* extract has inhibitory effect of HAase, lipoxygenase and antioxidants for DPPH. Anti-hyaluronidase and anti-lipoxygenase activity of chosen antioxidant-rich plant materials can support their traditional use in folk medicine [29]. Strong inhibition of both enzymes by extract from *A. japonica* makes this pharmacopeial plant material an interesting topic for further biological and phytochemical examination [27]. The root of *A. japonica* has been used in traditional medicine in Korea for the treatment of various diseases of joint and blood circulation [24]. Marcone et al. [17] reported that *A. japonica* has various physiological effects including the control of blood circulation, the removal of extravasated blood, and the inteneration of joint actions in humans and experimental animals [23]. The roots of *A. japonica* contain various active chemical components such as phytoecdysteroid, saponin, polysaccharide, 20-hydroxyecdysone, and inokosterone and has also been demonstrated to exhibit the highest inhibitory effect against *Clostridium difficile* amongst various herb extracts [9]. Although, *A. japonica* is classified as a herb that activates the blood flow and clears the stagnated blood, many studies will be required for the purification of active chemical groups from the crude extracts and to ascertain the mechanisms of action of these crude extracts [24].

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References

1. Akular, U. S. and Odhav, B. 2008. *In vitro* 5-Lipoxygenase inhibition of polyphenolic antioxidents from undomesticated plants of South Africa. *J. Med. Plants Res.* **2**, 207-212.
2. Amit, S., Saraswati1, B., Kamalesh, U. and Kumud, U. 2013. Formulation and evaluation of a novel herbal gel of *Equisetum arvense* extract. *J. Pharm. Phytochem.* **1**, 80-86.
3. Azila, Y. and Don, M. M. 2012. Evaluation of *Trametes lactinea* extracts on the inhibition of hyaluronidase, lipoxygenase and xanthine oxidase. *J. Phys. Sci.* **23**, 1-15.
4. Brand-Williams, W., Cuvelier, M. E. and Berset, C. 1995. Use of free radical method to evaluate antioxidant activity. *Food*

Sci. Technol. 28, 25-30.
5. Chen, C. H., Chan, H. C., Chu, Y. T., Ho, H. Y., Chen, P. Y., Lee, T. H. and Lee, C. K. 2009. Antioxidant activity of some plant extracts towards xanthine oxidase, lipoxygenase and tyrosinase. *Molecules* **14**, 2947-2958.
6. Duthie, E. S. and Chain, E. A. 1939. A mucolytic enzyme in testes extract. *Nature* **144**, 977-978.
7. Girish, K. S. and Kemparaju, K. 2005. Inhibition of *Naja naja* venom hyaluronidase by plant derived bioactive components and polysaccharides. *Biochemistry (Mosc)* **70,** 948-952.
8. Hwang, S. G., Yang, A., Kim, S. J., Kim, M. K., Kim, S. S., Oh, H. J., Lee, J. D., Lee, E. J., Nam, K. W. and Han, M. D. 2014. Screening of hyaluronidase inhibitor in Korean medicinal plants. *J. Life Sci.* **24,** 498-504.
9. Jung, S. M., Choi, S. I., Park, S. M. and Heo, T. R. 2007. Antimicrobial effect of *Achyranthes japonica* Nakai extracts against *Clostridium difficile*. *Kor. J. Food Sci. Technol.* **39,** 564-568.
10. Kedare, S. B. and Singh, R. P. 2011. Genesis and development of DPPH method of antioxidant assay. *J. Food Sci. Technol.* 48, 412-422.
11. Kumar, V. and Parmar, N. S. 2003. Herbs: A potential source for the development of new pytomedicinals. *The Pharma. Review* **1**, 59-63.
12. Kwon, J. W., Lee, E. J., Kim, Y. C., Lee, H. S. and Kwon, T. O. 2008. Screening of antioxidant activity from medicinal plant extracts. *Kor. J. Pharmacogn*. **39,** 155-163.
13. Ladd, D. and Thomas, J. R. 2015. Ecological checklist of the Missouri flora for floristic quality assessment. *Phytoneuron* **12**, 1-274.
14. Lee, S. K., Zakaria, H. M., Cheng, H. S., Luyengi, L., Gamez, E. J. C., Mehta, R., Kinghorn A. D. and Pezzuto, J. M. 1998. Evaluation of the antioxidant potential of natural products. *Combinat. Chem. High Througput Screen* **1**, 35-46.
15. Maity, N., Nema, N. K., Abedy, M. K., Sarkar, B. K. and Mukherjee, P. K. 2011. Exploring *Tagetes erecta* Linn flower for the elastase, hyaluronidase and MMP-1 inhibitory activity. *J. Ethnopharmacol*. **137**, 1300-1305.
16. Mansuy, D., Cucurou, C., Biatry, B. and Battioni, J. P. 1988. Soybean lipoxygenase-catalyzed oxidations by linoleic acid hydroperoxide: Different reducing substrates and dehydrogenation of phenidone and BW755C. *Biochem. Biophys. Res. Commun*. **151**, 339-346.
17. Marcone, M. F., Jahaniaval, F., Aliee, H. and Kakuda, Y. 2003. Chemical characterization of *Achyranthes bidentata* seed. *Food Chem*. **81,** 7-12.
18. Menzel, E. J. and Farr, C. 1998. Hyaluronidase and its substrate hyaluronan: biochemistry, biological activities and therapeutic uses. *Cancer Lett.* **131**, 3-11.
19. Medley, M. E., H., Bryan, H., MacGregor, J. and John. W. Thieret. 1985. *Achyranthes japonica* (Miq.) Nakai (Amaranthaceae) in Kentucky and West Virginia: new to North America. *Sida* **11**, 92-95.
20. Nema, N. K., Maity, N., Sarkar, B. and Mukherjee, P. K. 2011. *Cucumis sativus* fruit-potential antioxidant, anti-hyaluronidase, and anti-elastase agent. *Arch. Dermatol. Res.* **303**, 247-252.
21. Nuhn, P., Bilge, A., K6hler, T., Lettau, H. and Schneider, R. 1991. Trends bei der entwicklung yon lipoxygenasehemmern. *Pharmazie* **46**, 81-88.
22. Oyetayo, V. O. 2008. Microbial load and antimicrobial property of two Nigerian herbal remedies. *Afr. J. Trad. CAM.* **5**, 74-78.
23. Park, J. H., Kang, S. N., Shin, D., Hur, I. C., Kim, I. S. and Jin, S. K. 2013. Antioxidant activities of *Achyranthes japonica* Nakai extract and its application to the pork sausages. *Asian-Australas J. Anim. Sci.* **26,** 287-294.
24. Park, H. J., Lee, J. S., Hong, M. S., Kim, C. J., Kim, J. W., Lee, H. J. and Lim, S. 2004. The anti-nociceptive and anti-inflammatory effect of *Achyranthes japonica* Nakai. *Kor. J. Orient. Med.* **25,** 8-14.
25. Piwowarski, J. P., Kiss, A. K. and Kozłowska-Wojciechowska, M. 2011. Anti-hyaluronidase and anti-elastase activity screening of tannin-rich plant materials used in traditional Polish medicine for external treatment of diseases with inflammatory background. *J. Ethnopharmacol*. **137**, 937-941.
26. Pogrel, M. A., Low, P. M. and Stern, R. 2003. Hyaluronan (hyaluronic acid) and its regulation in human saliva by hyaluronidase and its inhibitors. *J. Oral Sci.* **45**, 85-91.
27. Son, K. H., Hwang, J. H., Lee, S. H., Park, J. H., Kang, S. J., Chang, S. Y. and Lee, K. S.1999. Isolation and quantitative determination of 20-hydroxyecdysone from *Achyranthis* radix. *Kor. J. Pharmacogn.* **30**, 335-339.
28. Sumantran, V. N., Kulkarni, A., Boddul, S., Chinchwade, T., Harsulkar, A., Patwardhan, B., Chopra, A. and Wagh, U. V. 2007. Chondroprotective potential of root extracts of *Withania somnifera* in osteoarthritis. *J. Biosci.* **32,** 299-307.
29. Wichtl, M. 1994. *Herbal Drug and Phytopharmaceuticals*, pp. 188-191, Medpharm Scientific Publishers: Stuttgart, Germany.

초록 : 쇠무릎 에탄올 추출물의 DPPH, 히알루로니다아제 및 리폭시게나아제 저해 효과

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우슬(牛膝)은 비름과(Amaranthaceae)의 쇠무릎(*Achyranthes japonica*)의 뿌리를 약용으로 한방에서 사용하고 있다. 1, 1- diphenyl 2-picrylhyorazyl (DPPH)은 잘 알려진 자유 라디칼이다. Hyaluronidase (HAase)은 결합조직의 세포외성 기질에서 히알루론산분해에 관여하는 효소이다. Lipoxygenases (LOX)은 지방산 대사와 면역에 중요한 역할을 수행한다. 본 연구에서는 쇠무릎에서 이 세 작용을 에탄올 추출물을 사용하여 생육 발달 단계(어린 영양기관과 성숙한 영양기관)에 대해 이들 추출물의 활성을 비교 조사하였으며 각 항목에 대해 50% 저해(IC_{50})의 농도를 산출하였다. 항산화 활성은 잎과 줄기, 뿌리 모두에서 탁월되었다. 특히 히알루로니다아제의 저해작용이 현저하였으며 향후 어떤 화합물인지 심도 있는 연구가 필요하다고 판단되었다. DPPH에 대한 항산화 활성과 LOX에 대한 저해작용은 기존 연구자의 결과와 유사하였다. 어린 식물조직과 성숙한 식물조직에서 추출한 발달 단계에 따른 이들 세 가지 활성에서 어린 뿌리보다 성숙한 뿌리에서 약간 더 우수한 작용을 보였으나 통계적으로 유의한 차이가 없었다.