■ Brief Communication ■

Changes in the Compound and Bioactivity of *Suaeda japonica* Makino Extract by Different Harvesting Time

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This study was the change of compound and bioactivity were analyzed by different harvesting time (May, August, and November) of *Suaeda japonica* Makino. The total polyphenol and flavonoid contents of *S. japonica* were the highest at about 22.81 mg GAE/g and 4.56 mg QE/g, respectively, in the *S. japonica* harvested in Nov. Also, the contents of quercetin, showed the highest content in Nov harvested *S. japonica*. In addition, the antioxidative activity of each extract from *S. japonica* changed depending on harvesting time. For *S. japonica* harvested in Nov showed the highest DPPH and ABTS radical scavenging activity. From the NO inhibition assay, the *S. japonica* harvested in Nov had shown the highest anti-inflammatory effects. Therefore, consideration of the optimal harvesting time for *S. japonica* could be an important factor attributing to its natural antioxidant and anti-inflammatory properties and the optimal harvesting time was confirmed especially to be in Nov.

Key Words: Different harvesting time, *Suaeda japonica* Makino, Polyphenol, Anti-inflammatory, Antioxidant

Suaeda japonica Makino is a continuous annual herbaceous plant belonging to the family Chenopodiaceae and is a salt-tolerant plant capable of growing at high salt concentrations along with *Salicornia* and *Altriplex* (Larcher, 1995). It is seen abundantly on muddy seashores along the coast of Korea, Japan, Europe, and Iran (Han et al., 2003). The plant of the genus *Suaeda* to which this species belongs is a haophytaloplant (Jung et al., 2008), which refers to a plant naturally selectively grown in a saline environment, and is a plant with high adaptability against excessive levels of ion concentration (Greenway and Munns, 1980). The *S. japonica* is considered to be rich in secondary metabolites with high bioavailability different from other organisms because it can grow in high salt and humid regions as well

as contains a large amount of natural minerals (Jung et al., 2008). Therefore, it has the potential to be utilized as a functional material. In addition, *S. japonica* is used as natural salt, and in herbal medicine, it is used as a medicinal material including antioxidant and antipyretic effect (Choi et al., 2009).

Recently, as consumers who natural preferences and interest in well-being have increased, many attempts have been made to develop bioactive compound contained in natural raw materials as new materials for health functional foods. Particularly, polyphenolic compounds such as phenolic acid, cinamic acid, resveratrol, lignan, tannic acid, and flavonoids are well known as functional raw materials (Lee et al., 2005). These polyphenolic compounds exhibit strong

biological activity. Moreover, they are better alternatives to several synthetic antioxidants that have side effects (Ito et al., 1983; Kahl and Kappus, 1993). Antioxidant compounds extracted from natural raw materials exhibit protective effects in cells against reactive oxygen species (ROS). They consist of peroxynitrite, superoxide, hydroxyl radicals, peroxyl radicals, and singlet oxygen, which induce oxidative stress, and thereby cause cellular damage (Bayr, 2005). Therefore, it is important to continue to evaluate the antioxidant activity and free radical scavenging ability of natural raw materials.

There is no study yet on the compound and bioactivity of *S. japonica* according to the different harvesting time. Therefore, in this study, the contents of polyphenol, flavonoid, and quercetin were analyzed by different harvesting time (May, August, and November) of *S. japonica* from the coastal area of Suncheon, Jeollanam-do, Korea. In addition, antioxidant and anti-inflammatory effect of *S. japonica* of different harvesting time were investigated.

The *S. japonica* samples were harvested in the period of May, August, and November 2019 at Suncheon-si (Jeollanam-do, Korea). The *S. japonicaa* was dried, ground to a fine powder (~20 mesh) using a milling machine, and then extracted with 70% ethanol three times by stirring for 24 h at RT. After filtration the solvent was removed using a rotary vacuum evaporator, and the freeze-dried extract stored deep freezer (-80 ℃) until used for further analysis.

Total polyphenol content was analyzed according to the Folin-Denis method (Singleton and Rossi, 1965). After the *S. japonica* extract was prepared at a concentration of 1 mg/mL, Folin & Ciocalteau's phenol reagent 60 μL was added to sample solution 60 μL. After 3 min, 60 μL of a 10% sodium carbonate (Na_2CO_3) was added, allowed to stand at RT for 1 h, and then, was measured at 700 nm using a microplate reader (Bio-Rad Inc., Hercules, CA, USA). The total polyphenol content was calculated based on a standard curve using gallic acid (Sigma Chemical Co., St. Louis, Mo. USA).

Total flavonoid content was measured according to the Nieva Moreno et al. (2000) method. After diluting the *S. japonica* extract (100 μL) was mixed with 20 μL of 1 M potassium acetate and 10% aluminum nitrate adding 860 μL of 80% ethanol. The absorbance was then measured at 415 nm after incubation for 40 min. The total flavonoid content was calculated based on a standard curve using Quercetin (Sigma Chemical Co.).

The content of quercetin in *S. japonica* extract was analyzed by Yang et al. (2017) method. The *S. japonica* extract were dissolved in 99% methanol to prepare solutions at 10 mg/mL. And then, solutions were filtered through 0.22 μm filters. Using a 20 μL sample loop, the sample was analyzed using a reversed-phase HPLC system (Waters 2690, MA, USA), a quaternary pump, and a vaccum degasser. Quercetin separation was performed by a prepacked ZORBAX C_{18} column (XDB-C_{18}, 4.6×150 mm, 5 μm). Two mobile phases were used: solvent A, 5% acetic acid; B, 100% acetonitrile. The gradient for HPLC analysis was linearly changed as follow (total 50 min): 5% B at 0 min, 25% B at 20 min, 100% B at 21 min, 100% B at 35 min, 5% B at 36 min, 5% B at 50 min. Flow rate was set to 1.0 mL /min.

Measurement of ABTS radical scavenging activity was performed by the previously described method (Re et al., 1999). For ABTS radical formation, 14 mM 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS; Sigma Chemical Co.) and 4.9 mM potassium persulfate were mixed at RT for 24 h. It was diluted with phosphate buffered saline (PBS, pH 7.4) so that the absorbance value at 732 nm was 0.70 (±0.02). 20 μL of the sample was added to 180 μL of the diluted solution, and then absorbance was measured at 732 nm after 1 min.

DPPH radical scavenging activity was analyzed according to the method of Blois (1958). After adding 40 μL of 0.15 mM DPPH solution to 160 μL of the sample solution and stirring, the absorbance was measured at 517 nm after standing in the dark for 30 min. The radical scavenging activity was calculated by the following equation.

Radical scavenging activity (%) = 100-[(O.D. of sample/ O.D. of control)×100]

RAW 264.7 mouse macrophage cells were purchased from Korea Cell Line Bank (KCLB). The cells were cultured in Dulbecco's Modified Eagle's Media (DMEM; Gibco, Carlsbad, CA, USA) containing 10% (v/v) fetal bovine serum (FBS; Gibco) and 1% penicillin-streptomycin (Gibco)
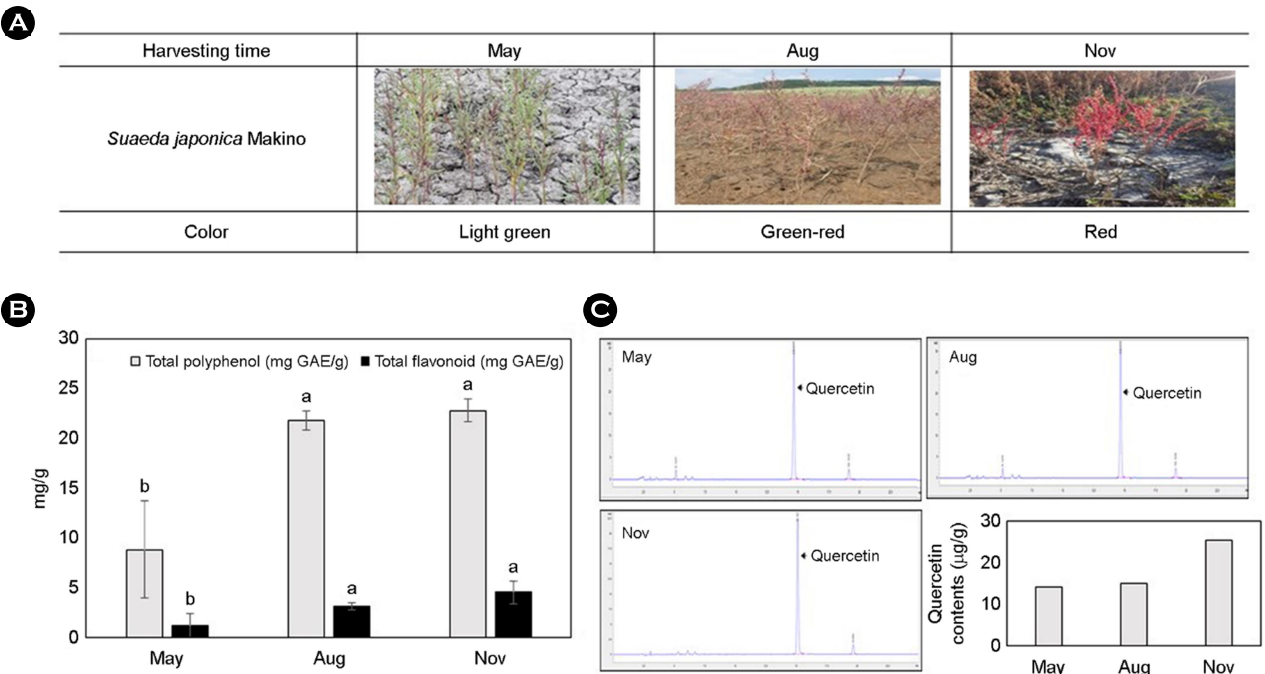
at 37℃ in a humidified atmosphere of 5% CO_2.

Cell toxicity of *S. japonica* extract was measured by MTT method (Jew et al., 2003). Raw 264.7 cells were seeded in a 96 well plate (5×10^4 cells/mL), with various concentrations (10, 25, 50, 100, 200 μg/mL) of *S. japonica* extract for 24 h. And then, followed by the addition of 0.05% MTT to each well, and the plates were further incubated for 4 h at 37℃. The supernatant was removed, and 100 μL DMSO was added to each well to solubilize the water-insoluble purple formazan crystals. The absorbance at 550 nm was measured using a microplate reader (Bio-Rad Inc.). The percentage of cell viability was estimated compared with control groups.

Measurement of nitric oxide (NO) was measured by reacting the NO_2^- form present in the cell culture by method of Green et al. (1982). RAW 264.7 cells (1×10^5 cell/mL) were seeded in 96-well plates. After incubation for 24 h at 37℃, the cells were treated with *S. japonica* extract (10, 25, 50, 100 μg/mL) and 100 ng/mL of lipopolysaccharide (LPS; Sigma Co.) for 24 h. After 24 h, the supernatants were measured for NO production using the Griess reagent (Sigma Chemical Co.) at 540 nm using a microplate reader (Bio-Rad Inc.).

The results are expressed as the mean ± standard deviation (SD). Significance was determined by a one-way analysis of variance followed by Duncan's multiple range test using GraphPad Prism 7 (GraphPad Software Inc., San Diego, CA, USA). Statistical significance was accepted for P values < 0.05.

Changes in color and compound of *S. japonica* according to the different harvesting time are showed in Fig. 1. Fig. 1A shows the three color, green (May), green-red (Aug) and red (Nov) of *S. japonica* by different harvesting time. It was confirmed that the color of *S. japonica* turned red as the season changed. As a result of measuring the contents of polyphenols and flavonoids in different harvesting time of *S. japonica*, the contents in Nov harvested *S. japonica* were the highest at 22.81 mg GAE/g and 4.56 mg QE/g, respectively (Fig. 1B). The contents of quercetin, the concentrations of 14.19 (May), 15.19 (Aug), and 25.45 (Nov) μg/g, respectively, showed the highest content in Nov harvested *S. japonica*,
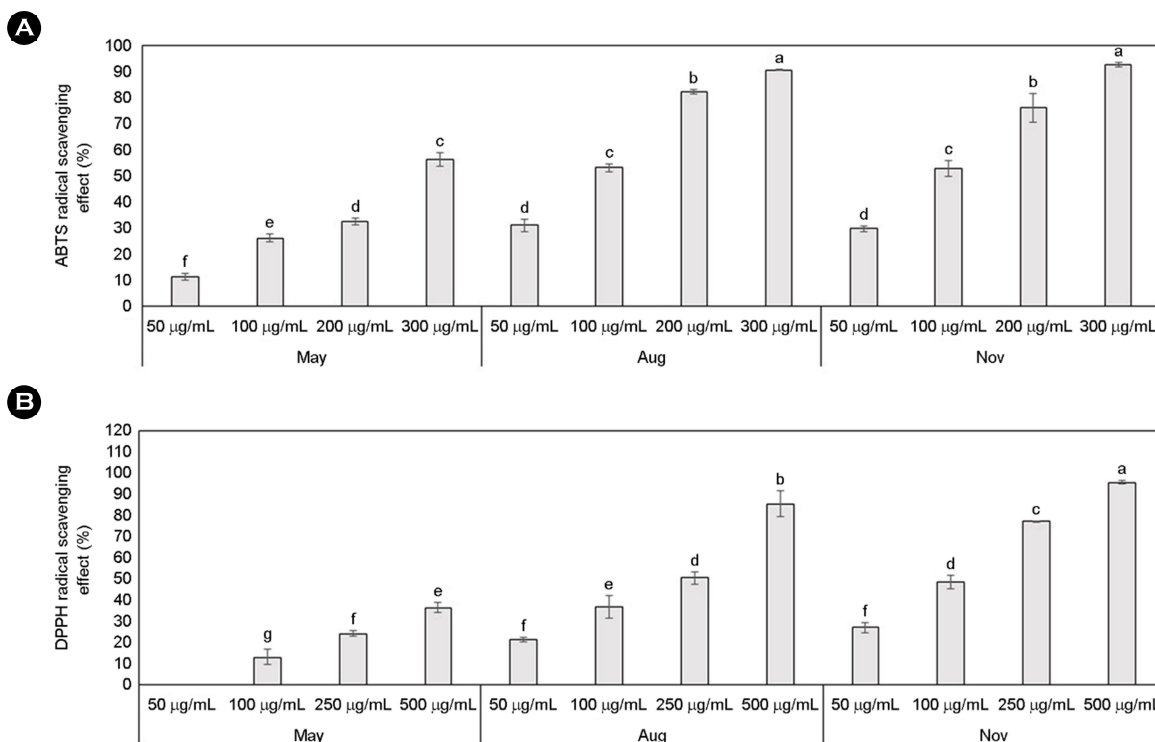

Fig. 1. **The changes of color and component of *S. japonica* according to the different harvesting time.** (A) three phenotypic categories of colors in *S. japonica*; (B) total polyphenol and flavonoid contents of *S. japonica*. Results are presented as the mean ± S.D. of 3 independent in triplicate. Means with different letter on the same kind or bars are significantly different at *P* < 0.05 by Duncan's multiple range test.; (C) HPLC chromatograms and contents of quercetin of *S. japonica* extract at different harvesting times.
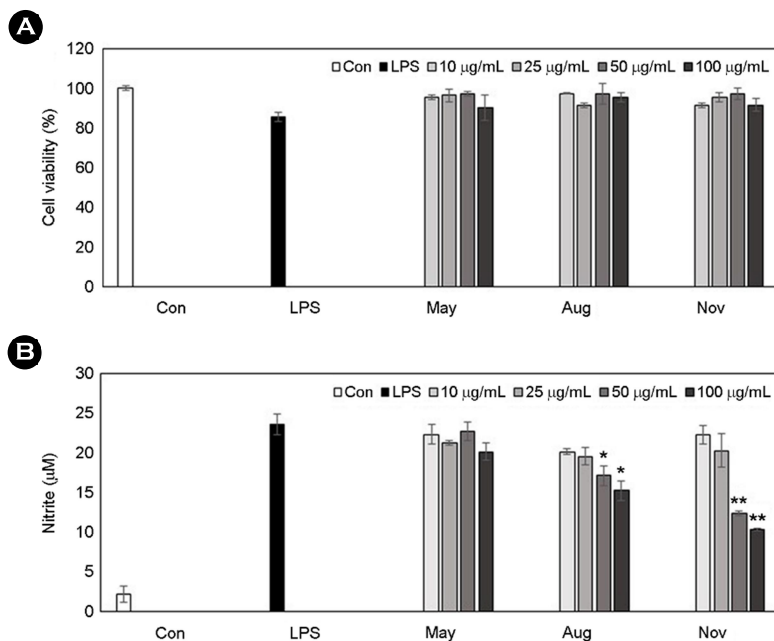
similar to the polyphenol and flavonoid results (Fig. 1C). In the study of Lee et al. (2014) showed a difference in the content of bioactive compound such as epicathechin, catechin, resveratol, and quercetin, which are polyphenol compounds, depending on the harvesting time of grapes. It was reported that this is because complex factors such as the amount of sunlight and growth period affect the content of bioactive compound. It is thought that the content of bioactive compound according to harvesting time of *S. japonica* also showed a lot of difference according to characteristics such as amount of sunlight and growth time.

To examine the antioxidant activity of *S. japonica*, ABTS and DPPH assays were conducted. ABTS is widely used to measure the antioxidant activity of natural materials, and the produced ABTS cation reacts with an antioxidant to decolorize the blue-green color, and the antioxidant activity can be evaluated by measuring its absorbance. The ABTS radical scavenging activity was the lowest in May, and the harvested in Aug and Nov showed similar effect (Fig. 2A). DPPH is

a very stable free radical, and DPPH radical scavenging effect is measured by the principle of decolorizing purple compounds to yellow by the scavenging of radicals through hydrogen donation in phenolic compounds or flavonoids with hydroxy radicals (-OH) (Bondet et al., 1997). Fig. 2B shows the scavenging effect of the DPPH results of *S. japonica* by harvesting time. DPPH radical scavenging activity was showed to be 0% to 95.37% at concentrations of 50, 100, 250 and 500 μg/mL, and it was confirmed that the activity increased in a concentration-dependent manner. At the concentration of *S. japonica* 100 μg/mL, showed to be 12.54 ± 3.24 (May), 36.75 ± 5.32 (Aug), and $48.24\pm3.12\%$ (Nov) respectively, highest DPPH radical scavenging activity in the Nov harvested *S. japonica*. Similar to the research results of Prior et al. (1998), which reported that the antioxidant index increased in proportion to the total phenolic content, the antioxidant activity increased in proportion to the content of flavonoid, vanillic acid, a type of phenolic compound. It was thought to be due to the difference in the content of

Fig. 2. Comparison of ABTS and DPPH radical scavenging activity of *S. japonica* according to the different harvesting time. Results are presented as the mean ± S.D. of 3 independent in triplicate. Means with different letter on the same kind or bars are significantly different at $P < 0.05$ by Duncan's multiple range test.

Fig. 3. Effect of *S. japonica* according to the different harvesting time on cell viability and NO inhibition effect. (A) Cell viability was measured by MTT assay. RAW 264.7 cells were incubated in medium with *S. japonica* at different harvesting times and LPS. After 24 h, MTT reagent was added to the medium. After 4 h of incubation, the absorbance was read at 550 nm; B, Inhibitory effect of *S. japonica* at different harvesting times on the production of NO on LPS-induced inflammation in RAW 264.7 cell. Results are presented as the mean ± S.D. of 3 independent in triplicate. *$P < 0.05$, compared to LPS group. **$P < 0.01$, compared to LPS group.

phenolic compounds in *S. japonica* by different harvesting time.

In order to evaluate of *S. japonica* extract on cytotoxicity by harvesting time, RAW 264.7 cells were treated by various concentration of *S. japonica* extract, and the survival rate of the cells was evaluated through the MTT assay (Fig. 3A). The *S. japonica* extracts by different harvesting time did not show cytotoxicity when treated with concentrations of 10, 25, 50, 100 μg/mL. Therefore, *S. japonica* extracts by different harvesting time were NO inhibition effect tested at concentrations that did not affect cytotoxicity.

Macrophage cells play an important role in the inflammatory response (Iontcheva et al., 2004), and active nitrogen species (RNS) such as NO, HNO_2, and $ONOO_-$ are produced in large quantities due to the immune response of macrophages, neutrophils and other immune cells during the inflammatory reaction (Delanty and Dichter, 1998). Nitric oxide (NO), which is known to play an important role in the induction of inflammation in recent years as a kind of active oxygen, is produced from L-arginine by NOS (Nitric oxide synthase) (Weisz et al., 1996). In particular, iNOS (inducible NOS) is involved in the inflammatory response, and is expressed when inflammatory cytokines such as TNF-α and LPS are stimulated (Moncada et al., 1991). Fig. 3B

shows the results of measuring the effect of the *S. japonica* extracts by different harvesting time on NO production in RAW 264.7 cells using LPS, which is used as an inflammatory inducer. In the control group in which only RAW 264.7 cells were cultured, the concentration of NO was measured to be 2.12 μM, but in the group treated with LPS, the concentration of NO was increased to 23.56 μM. In the experimental group treated with the *S. japonica* extract, the NO concentration was decreased from 50 μg/mL of the *S. japonica* extract harvested in Aug and Nov. The NO inhibitory effect was 12.34 and 10.32 μM respectively at the concentration of 50 and 100 μg/mL of the *S. japonica* extract harvested in Nov. It was confirmed that the most excellent.

Overall, it was confirmed that the extract of *S. japonica* extract showed antioxidant activity and anti-inflammatory activity, and especially, all of *S. japonica* in harvested Nov showed high bioactivity. it is thought to be due to the fact that the antioxidant and anti-inflammatory active ingredients in *S. japonica* change depending on the harvesting time. Therefore, when *S. japonica* is used as an antioxidant and anti-inflammatory functional raw material, it is thought that it should be harvested at the time of highest activity.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication this articles.

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