



## Antioxidant and Anti-inflammatory Activity of Six Halophytes in Korea

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**Abstract** – The aim of this study was to measure and compare polyphenol content, antioxidant, and anti-inflammatory activity of six halophytes (*Limonium tetragonum*, *Suaeda glauca*, *Suaeda japonica*, *Salicornia europaea*, *Triglochin maritimum*, and *Sonchus brachyotus*). Depending on the total polyphenol content, the plants were categorized into two groups: (1) a high total polyphenol content group that included *L. tetragonum*, *S. brachyotus*, and *S. europaea*, and (2) a low total polyphenol content group consisting of *S. glauca*, *T. maritima*, and *S. japonica*. Antioxidant activity was evaluated using DPPH and hydroxyl radical scavenging assays, and by measuring ROS. Anti-inflammatory activity was evaluated by measuring NO and PGE<sub>2</sub>. *L. tetragonum* and *S. brachyotus*, that have high polyphenol content, also showed strong antioxidant activity. In addition, *L. tetragonum*, *S. brachyotus*, and *S. europaea* showed good anti-inflammatory activity. Consequently, the total polyphenol content was thought to be related to antioxidant and anti-inflammatory activity. Therefore, *S. brachyotus* and *L. tetragonum* are good candidates for use in pharmaceuticals and functional foods.

**Keywords** – Halophytes, DPPH, Polyphenol, NO, PGE<sub>2</sub>

### Introduction

The salt-tolerant halophyte is native to saline habitats (coastal sand dunes, salt marsh, and mud flats) where the soil mixture contains organic nutrient material and inorganic nutrient salts flowing from land and ocean.<sup>1</sup> Halophytes that inhabit such hostile habitats have developed mechanisms to adapt to extreme environments and are likely to have various bioactive compounds. Halophytes have been used in traditional medicine, food sources, cosmetics, functional foods, and natural seasoning.

Among the halophytes in Korea, *Limonium tetragonum* (Plumbaginaceae), *Suaeda glauca* (Chenopodiaceae), *Suaeda japonica* (Chenopodiaceae), *Salicornia europaea* (Chenopodiaceae), *Triglochin maritimum* (Juncaginaceae), and *Sonchus brachyotus* (Asteraceae) are commonly distributed along the western coast of Korea. These halophytes have been reported to possess many bioactive properties. *L. tetragonum* showed protective effects on diethylnitrosamine-induced liver fibrosis in rats.<sup>2</sup> The biological activities of *S. glauca* result in hepatoprotective, anti-oxidative, and anti-neuroinflammatory effects.<sup>3,4</sup> *S. japonica* has antioxidant and anti-inflammatory effects.<sup>5,6</sup>

*S. europaea* showed cytotoxic activity against *Artemia salina* LEACH and *Daphnia magna* STRAUS and anti-neoplastic activities in the potato disk assay.<sup>7</sup> *T. maritimum* showed strong inhibition of fungal growth.<sup>8</sup> *S. brachyotus* had antioxidant, anti-bacterial, and peroxynitrite-scavenging activity.<sup>9,10</sup>

Many phytochemicals, pyrocatechol, syringic acid, apigenin, isorhamnetin, kaempferol, dihydroferulic acid, vanillic acid, 4-hydroxybenzoic acid, homooriodictyol, naringenin, quercetin, luteolin, 9-epiblumenol C, scopoletin, dihydroisorhamnetin, chrysoeriol from *S. japonica*, myricetin-3-*O*-β-D-galactopyranoside, myricetin-3-*O*-α-L-rhamnopyranoside, quercetin-3-*O*-β-D-glucopyranoside, quercetin-3-*O*-β-D-galactopyranoside from *L. tetragonum*, lignoceric acid, β-sitosterol, daucosterol, quercetin, luteolin, luteolin-7-*O*-β-D-glucopyranoside, isorhamnetin, scopoletin, stigmasterol from *S. glauca*, isorhamnetin-3-*O*-β-D-glucopyranoside, quercetin-3-*O*-β-D-glucopyranoside, 3-caffeoyl, 4-dihydrocaffeoyl quinic acid, quercetin, isorhamnetin, quercetin-3-*O*-β-D-glucopyranoside, quercetin-3',4'-di-*O*-β-D-glucopyranoside, rutin from *S. europaea*, triglochinin from *T. maritimum*, and chlorogenic acid, luteolin-7-*O*-β-D-rutinoside, luteolin-7-*O*-β-D-glucopyranoside, and luteolin from *S. brachyotus* have been reported from halophytes.<sup>11-20</sup> Of these, most compounds were reported that were polyphenolic compounds. Polyphenols have been reported to have various biological activities

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that result in antioxidant, anti-hyperlipidemic, anti-cancer, and anti-inflammatory effects.<sup>21,22</sup> Therefore, the six halophytes tested in this study were expected to contain a variety of biological activities. The objective of this study was to compare the antioxidant and anti-inflammatory properties of six halophytes in Korea and to assess their possible importance for use in bio-products.

## Experimental

**Plant materials and extraction** – Six halophytes [*L. tetragonum* (H1), *S. brachyotus* (H2), *S. glauca* (H3), *S. japonica* (H4), *T. maritima* (H5), and *S. europaea* (H6)] were collected at Ganghwa-gun, Incheon, Korea. The plants were separately lyophilized, pulverized, and stored at -80 °C. The lyophilized halophytes were extracted with 70% EtOH for 1 h (5 times) using a sonicator. The extracts were then lyophilized, pulverized, and stored at -80°C for later use in experiments.

**Determination of total polyphenol content (TPC)** – The total polyphenol content of halophyte extract was determined using the Folin-Ciocalteu method.<sup>23</sup> Briefly, 20 µL of each extract were added to 100 µL of Folin-Ciocalteu reagent (Sigma chemical Co., St. Louis, MO, USA), allowed to react in the dark for 3 min at room temperature. This mixture was added to 80 µL of 7.5% Na<sub>2</sub>CO<sub>3</sub> and placed in the dark for 20 min at room temperature. The absorbance was determined in triplicate samples at 765 nm using a microplate reader (Power-Wave XS2, BioTek Instruments, Inc., USA). The total polyphenol content was based on a calibration curve obtained with gallic acid and expressed as gallic acid equivalents per gram of dry weight (mg/GAEg).

**DPPH radical scavenging assay** – The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of halophyte extracts was measured using the method described by Blois with a slight modification.<sup>24</sup> 100 µL of each extract of concentration (9.765–625 µg/mL) were added to 100 µL of 0.4 mM DPPH (Sigma chemical Co., St. Louis, MO, USA) in a 96-well plate. The mixture was shaken vigorously and left standing in the dark at room temperature for 30 min, after which absorbance was measured at 517 nm. The DPPH radical scavenging activity was expressed as the half maximal inhibitory concentration (IC<sub>50</sub>) value.

**Hydroxyl radical scavenging assay** – The hydroxyl radical scavenging activity of halophyte extract was measured using the method described by Rosen and Rauckman with some modification.<sup>25</sup> Twenty µL of each extract, 0.625 and 1.250 mg/mL, were added to 20 µL

each of 0.3 M 5,5-dimethyl-1-pyrroline N-oxide (DMPO), 10 mM FeSO<sub>4</sub>, and 10 mM H<sub>2</sub>O<sub>2</sub>/0.1 M phosphate buffer (pH 7.4). This mixture was allowed to react at room temperature for 2.5 min. The hydroxyl radical scavenging activity was expressed as a percentage (%).

**Cell culture** – RAW 264.7 macrophages were cultured in RPMI1640 supplemented with 10% FBS, penicillin (100 U/mL), and streptomycin (100 µg/mL) at 37 °C in a humidified incubator with 5% CO<sub>2</sub>. Cells were washed with DMEM and treated in serum-free medium for at least 4 h prior to treatment.

**MTT cytotoxicity assay** – The cell viability was measured using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) assay described by Hansen et al.<sup>26</sup> The halophyte extracts and lipopoly-saccharide (LPS)-treated cells were added to 1 mg/mL and incubated for 4 h at 37 °C. The resulting formazan crystals were dissolved in DMSO (dimethyl sulfoxide) (150 µL) and absorbance was measured at 540 nm using a microplate reader.

**Determination of ROS generation** – The intracellular ROS (reactive oxygen species) of halophyte extract were measured using the method described by Engelman et al. with some modification.<sup>27</sup> RAW 264.7 cells were cultured in 96-well plates and allowed to react with 20 µM DCFH-DA (2',7'-Dichlorofluorescein diacetate) (HBSS, Hanks balanced salt solution) in the dark for 30 min. After treatment with halophyte extract and further incubation for 1 h, cells were washed twice with PBS and added to 1 µg/mL of LPS. The fluorescence intensity was measured at an excitation wavelength of 485 nm and an emission wavelength of 528 nm using GENios fluorescence microplate reader (TECAN, Männedorf, Switzerland).

**Measurement of NO production** – The NO (nitric oxide) generated by cells was measured using the Griess reaction method.<sup>28</sup> RAW 264.7 cells were plated in 96-well plates at 1.5 × 10<sup>5</sup> cells/mL and treated with the indicated concentrations (50–500 µg/mL) of each halophyte extract prior to stimulation with 1 µg/mL of LPS for 24 h. One hundred µL of cell culture medium were added to 100 µL of Griess reagent and the mixture was incubated at room temperature for 10 min. The absorbance at 540 nm was measured using a microplate reader. The NO production was expressed as a percentage (%).

**Measurement of PGE<sub>2</sub> production** – The PGE<sub>2</sub> (prostaglandin E<sub>2</sub>) production of halophyte extract was measured using the method described by Moon et al. with minor modification.<sup>29</sup> RAW 264.7 cells were plated in 24-well plates and treated with the indicated concentrations (50–500 µg/mL) of each extract prior to stimulation with

**Table 1.** Total polyphenol content of six halophyte extracts expressed as equivalent of gallic acid (mg of GA/g of extract)

Species	Total polyphenol content (mg/100 g dry weight)
Plumbaginaceae	
H1	14.27 ± 0.26 <sup>a</sup>
Asteraceae	
H2	9.34 ± 0.32
Chenopodiaceae	
H3	4.29 ± 0.15
H4	2.84 ± 0.46
H6	8.71 ± 0.29
Juncaginaceae	
H5	3.33 ± 0.37

<sup>a</sup>Data are expressed as the mean ± SD ( $n = 3$ ).

**Table 2.** DPPH radical scavenging activity of six halophyte extracts expressed as half maximal inhibitory concentration (IC<sub>50</sub>)

Species	IC <sub>50</sub> (mg/ml)
Plumbaginaceae	
H1	0.020 ± 0.001 <sup>a</sup>
Asteraceae	
H2	0.056 ± 0.001
Chenopodiaceae	
H3	0.095 ± 0.003
H4	0.510 ± 0.003
H6	0.061 ± 0.001
Juncaginaceae	
H5	0.570 ± 0.009
Control	
Ascorbic acid <sup>b</sup>	0.019 ± 0.004

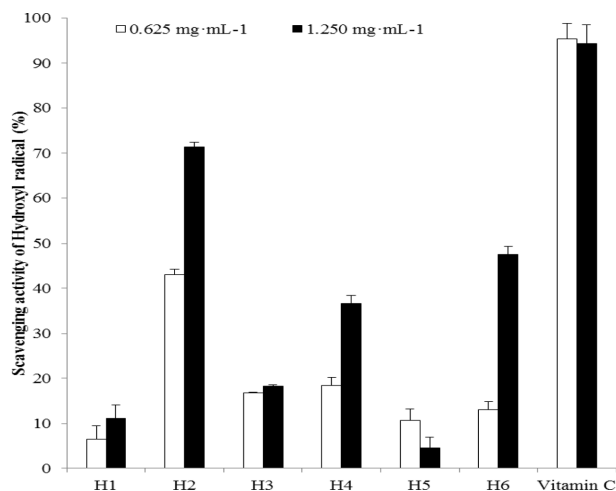
<sup>a</sup>Data are expressed as the mean ± SD ( $n = 3$ ).

<sup>b</sup>Ascorbic acid was used as a positive control.

1 µg/mL of LPS for 24 h. The expression level of PGE<sub>2</sub> was measured using an enzyme immunosorbent assay (ELISA) kit (Cayman chemical, Ann Arbor, MI). The PGE<sub>2</sub> released was expressed as a percentage (%).

## Result

**TPC** – The TPC of the six halophyte extracts was measured and ranged from 2.84% to 14.27% (Table 1). The TPC was highest in *L. tetragonum* (14.27%), followed by *S. brachyotus* (9.34%), *S. europaea* (8.71%), *S. glauca* (4.29%), *T. maritima* (3.33%), and *S. japonica* (2.84%) respectively. The halophytes were classified into two groups depending on TPC content: (1) the high TPC group consisting of *L. tetragonum* (14.27%), *S. brachyotus* (9.34%), and *S. europaea* (8.71%), and, (2) the low TPC

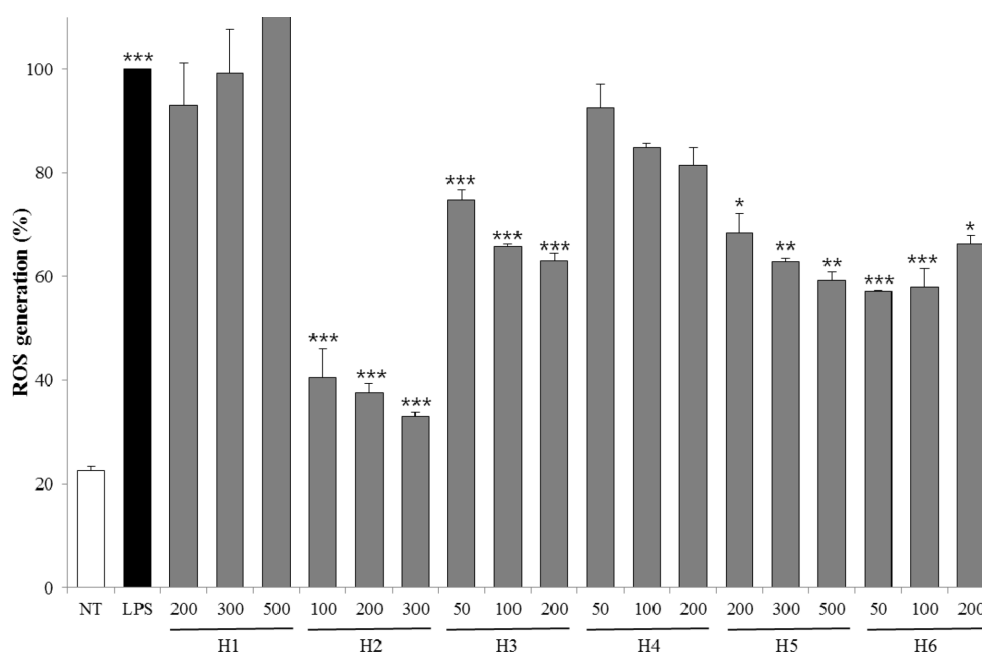
**Fig. 1.** Hydroxyl radical scavenging activity of the extracts from six halophytes. Experiment was performed in triplicate and data are represented as mean ± SD.

group consisting of *S. glauca* (4.29%), *T. maritima* (3.33%), and *S. japonica* (2.84%). Among all six halophytes studied, *L. tetragonum* (14.27%) contained five times more TPC than *S. japonica* (2.84%).

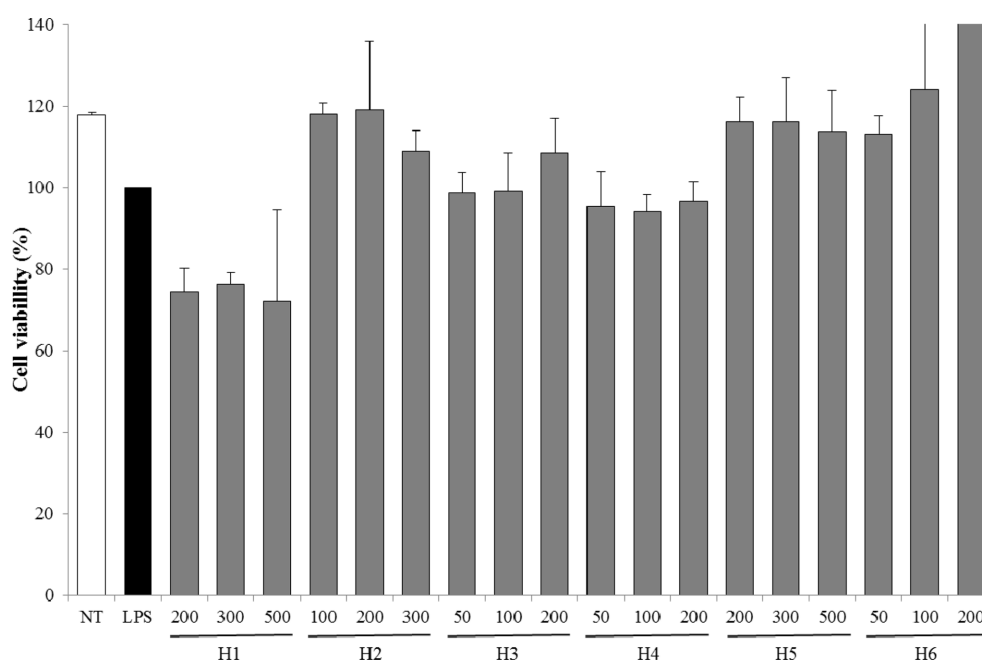
**Antioxidant activities** – Antioxidant activities of halophytes were measured using DPPH, hydroxyl radical scavenging assay and ROS generation (Table 2, Fig. 1 and 2). The DPPH radical scavenging activity was categorized into two groups: (1) the high activity group of *L. tetragonum* (0.020 mg/mL), *S. brachyotus* (0.056 mg/mL), *T. maritima* (0.061 mg/mL), and *S. glauca* (0.095 mg/mL), and, (2) the low activity group of *S. japonica* (0.510 mg/mL), and *S. europaea* (0.570 mg/mL). In the hydroxyl radical scavenging activity test, *S. brachyotus* (43.08 and 71.38%) fared better than other species [*L. tetragonum* (6.48 and 11.13%), *S. glauca* (16.87 and 18.26%), *S. japonica* (18.44 and 36.56%), *T. maritima* (10.71 and 4.51%), and *S. europaea* (13.08 and 47.46%)] using concentrations of 0.625 and 1.250 mg/mL.

To investigate cytotoxicity before inhibition of intracellular ROS activity, we evaluated the effects of halophyte extracts on cell viability. The halophyte extracts did not affect cell viability in LPS-stimulated RAW 264.7 cells (Fig. 3). As shown in Fig. 2, treatment with the extract of halophyte tended to decrease ROS generation. *S. brachyotus*, at a concentration 100 µg/mL, lowered the level of ROS generation (40.50%) compared to the LPS-stimulated RAW 264.7 cells (control). In this study, *L. tetragonum* and *S. brachyotus* showed the strongest antioxidant activity.

**Anti-inflammatory activity** – Anti-inflammatory activity was evaluated by measuring NO production and PGE<sub>2</sub> released from cells after treatment with halophyte extract.



**Fig. 2.** ROS generation of extracts from six halophytes. Experiment was performed in triplicate and data are represented as mean  $\pm$  SD. \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

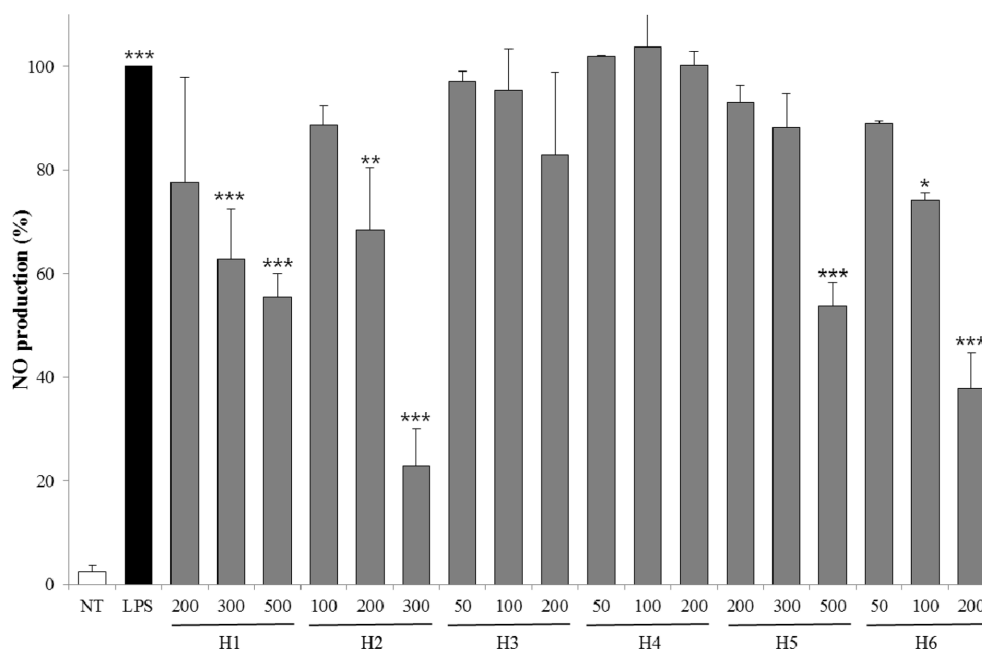


**Fig. 3.** Cell viability of extracts from six halophytes. Experiment was performed in triplicate and data are represented as mean  $\pm$  SD.

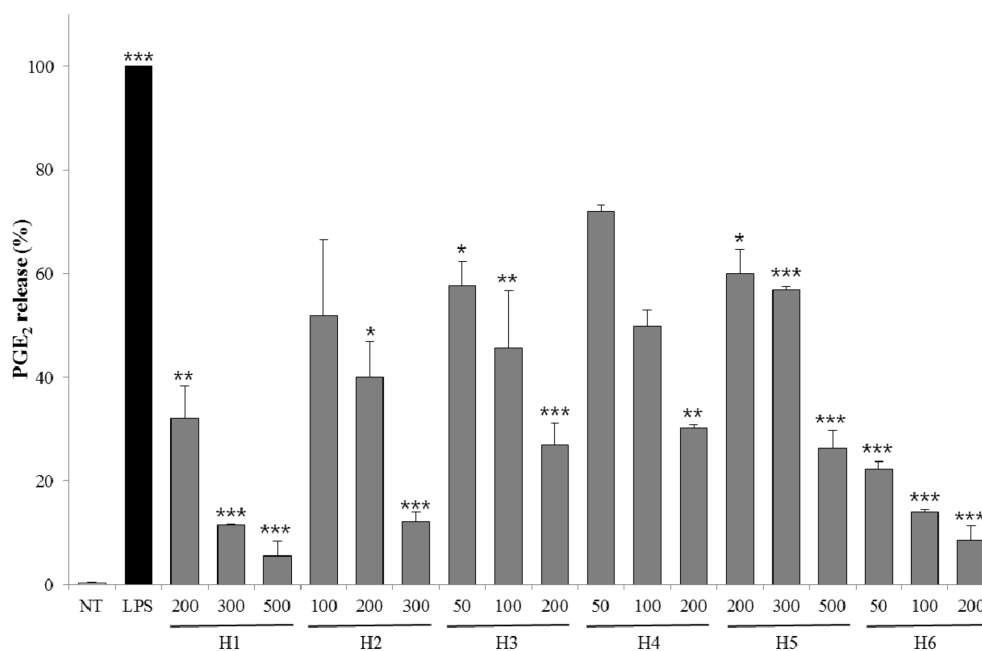
MTT assays were used to rule out the possibility that the concentrations of the six halophyte extracts used did not affect cell viability (Fig. 3). As shown in Fig. 4, the extracts of six halophytes suppressed NO production. Especially, 300  $\mu\text{g/mL}$  of *S. brachyotus* showed the best inhibition of NO production (22.90%) compared to LPS-

stimulated RAW 264.7 cells, and 200  $\mu\text{g/mL}$  of *S. europaea* showed good inhibition of NO production (37.71%). Except for the two aforementioned samples, the other halophyte extract-treated cells showed more than 50% NO production.

In the PGE<sub>2</sub> tests (Fig. 5), all halophyte extract-treated



**Fig. 4.** Inhibitory effects of NO production for extracts from six halophytes in LPS-RAW 264.7 cells. Experiment was performed in triplicate and data are represented as mean  $\pm$  SD. \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .



**Fig. 5.** Inhibitory effects of PGE<sub>2</sub> release for extracts from six halophytes in LPS-RAW 264.7 cells. Experiment was performed in triplicate and data are represented as mean  $\pm$  SD. \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

cells showed less than 50% inhibition of PGE<sub>2</sub> release compared to LPS-stimulated RAW 264.7 cells. *L. tetragonum* inhibited PGE<sub>2</sub> release by 32.04%, 11.53%, and 5.49% at 200, 300, and 500 µg/mL, respectively. *S. brachyotus* inhibited PGE<sub>2</sub> release by 51.84%, 40.04%, and 12.11% at 100, 200, and 300 µg/mL, respectively. In

particular, *S. europaea* showed the best inhibition of the PGE<sub>2</sub> release by 22.27%, 14.03%, and 8.48% at low concentrations (50, 100, and 200 µg/mL). In our study, *L. tetragonum*, *S. brachyotus*, and *S. europaea* showed good anti-inflammatory activity.

## Discussion

Polyphenol compounds, including flavonoids, tannins, and lignans, are widely distributed in nature. Polyphenols have been reported to have various biological activities that result in antioxidant, anti-hyperlipidemic, anti-cancer, and anti-inflammatory effects.<sup>21,22</sup>

In our antioxidant test, *L. tetragonum* and *S. brachyotus*, that have high total polyphenol content, showed the strongest antioxidant activity. In previous studies, phytochemicals were reported to contain many polyphenol compounds such as myricetin-3-*O*- $\beta$ -D-galactopyranoside, myricetin-3-*O*- $\alpha$ -L-rhamnopyranoside, quercetin-3-*O*- $\beta$ -D-glucopyranoside, and quercetin-3-*O*- $\beta$ -D-galactopyranoside from *L. tetragonum*, and chlorogenic acid, luteolin-7-*O*- $\beta$ -D-rutinoside, luteolin-7-*O*- $\beta$ -D-glucopyranoside, and luteolin from *S. brachyotus*.<sup>12,20</sup> In addition, *L. tetragonum* and *S. brachyotus* have been shown to have antioxidant activity, which was evaluated by measuring ROS generated in HT-1080 cells and by measuring DPPH and ABTS scavenging and lipid peroxidation inhibition, respectively.<sup>10,12</sup> The antioxidant activity of flavonoids such as myricetin-3-*O*- $\beta$ -D-galactopyranoside, myricetin-3-*O*- $\alpha$ -L-rhamnopyranoside, quercetin-3-*O*- $\beta$ -D-glucopyranoside, quercetin-3-*O*- $\beta$ -D-galactopyranoside, luteolin-7-*O*- $\beta$ -D-rutinoside, luteolin-7-*O*- $\beta$ -D-glucopyranoside, and luteolin have been studied and documented.<sup>14,15</sup> Therefore, the polyphenols of *L. tetragonum* and *S. brachyotus* were thought to contribute to their antioxidant activity.

*L. tetragonum*, *S. brachyotus*, and *S. europaea*, that have high polyphenol content, showed good anti-inflammatory activity. Except for *L. tetragonum* and *S. brachyotus* mentioned above, *S. europaea* contains various polyphenol compounds including isorhamnetin-3-*O*- $\beta$ -D-glucopyranoside, quercetin-3-*O*- $\beta$ -D-glucopyranoside, 3-caffeoyl, 4-dihydrocaffeoyl quinic acid, quercetin, isorhamnetin, quercetin-3-*O*- $\beta$ -D-glucopyranoside, quercetin-3',4'-di-*O*- $\beta$ -D-glucopyranoside, and rutin.<sup>16-18</sup> *L. tetragonum*, *S. brachyotus*, and *S. europaea* have been shown to have anti-inflammatory activity, which was evaluated by inhibition of NO production and suppression of iNOS expression in LPS-activated RAW 264.7 macrophages.<sup>30,31</sup> Specifically, flavonoids are known to have anti-inflammatory activity.<sup>32,33</sup> Therefore, the polyphenols present in *L. tetragonum*, *S. brachyotus*, and *S. europaea* are inferred to be associated with anti-inflammatory activity.

In conclusion, polyphenols of *L. tetragonum*, *S. brachyotus*, and *S. europaea* were inferred to be associated with their antioxidant and anti-inflammatory activity. The differences in the degree of activity among

the six halophytes tested are probably due to the differences in the specific polyphenolic content in each plant. Therefore, additional studies need to be performed to isolate, identify, and measure the anti-oxidant and anti-inflammatory activity of individual polyphenols from *S. brachyotus* and *L. tetragonum*.

## Acknowledgments

This work was supported by National Marine Biodiversity Institute of Korea Research Program 2017M01400.

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Received September 1, 2017

Revised October 26, 2017

Accepted October 27, 2017