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RESEARCH NOTE

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Antimicrobial, Anti-inflammatory, and Anti-oxidative Activities of *Scilla scilloides* (Lindl.) Druce Root Extract

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Abstract The root extract of *Scilla scilloides* (which has been used as a traditional folk medicine in Korea) was evaluated with regard to antimicrobial, anti-inflammatory, and anti-oxidative activities. The roots of *S. scilloides* were minced and extracted with 95% ethanol (root:ethanol=25:75, w/v). The inhibitory effects of *S. scilloides* root extract on the growth of *Staphylococcus aureus* ATCC 35556, *Salmonella enteritidis* ATCC 12021, *Escherichia coli* O157:H7, and *Candida parapsilosis* KCCM 35428 were tested. The results indicate that the antimicrobial effects of both 0.1 and 1.0% extract of *S. scilloides* were greater against the growth of *S. aureus* ATCC 35556 and *C. parapsilosis* KCCM 35428 than the growth of *S. enteritidis* ATCC 12021 and *E. coli* O157:H7. The anti-inflammatory effects were evaluated by measurement of the inhibition of hyaluronidase activity *in vitro*. It appears that both 0.1 and 1.0% concentrations of extract have inhibitory effects on hyaluronidase relative to the control. Finally, the anti-oxidative effect of 1.0 and 10% extract solutions were measured according to the thiocyanate method and were compared with 1.0% BHT. The results indicate that the anti-oxidative effect of 10% *S. scilloides* root extract (anti-oxidative index (AOI); 72.3±4.2) is not significantly different from that of 1.0% BHA (AOI; 76.8±3.5) ($p<0.05$). However, it appears that the anti-oxidative effect of *S. scilloides* root extract is at least three-fold greater than that of BHA when accounting for the amount of dissolved solids in each.

Keywords: *Scilla scilloides*, antimicrobial effect, anti-inflammatory effect, anti-oxidative effect

Introduction

The medical use of various plants has led to increased interest in their chemical composition and biochemical function. For example, many researchers have isolated and evaluated the major components with therapeutic activities from various natural herb products and propolis (1-3).

Scilla scilloides (Lindl.) Druce is considered a source of new materials for natural medicines. It is from the Liliaceae family and is a wild plant ubiquitously grown in rural regions of Korea and Japan. This plant is perennial and around 50 cm in height. As shown in Fig. 1, the root is oval-shaped, much like garlic. The blooming season for this plant is July through August and the inflorescence is racemiform with light-violet colored flowers. *S. scilloides* root extract has been used as a folk medicine for many years because of its anti-inflammatory and analgesic effects (4). In addition, *S. scilloides* root extract has been recently reported to have an inhibitory effect on the growth of cancer cell lines such as HeLa and MCF, and nortriterpenoid oligosaccharides in the extract were identified as the major components having this effect (5). Although this root extract has been valuable as a traditional medicine, information about its major components, their bio-functional effects and the biochemical mechanisms involved has not been reported.

Recently, the antimicrobial effects of plant-extracts and propolis on pathogens such as *Staphylococcus aureus, Salmonella enteritidis, Listeria monocytogenes, Helicobacter pylori* and so on, have been actively studied, however little is known about their antimicrobial mechanisms (2, 6, 7).

The anti-oxidative effects of natural extracts are also well known (2, 8, 9). The main compounds shown to have anti-oxidative effects are various flavonoids and other polyphenols (9). It is well understood that polyphenols scavenge peroxide radicals at the beginning of lipid auto-oxidation, which stabilizes the resonance structure and delays the oxidative reaction.

The anti-inflammatory effects of plant-extracts have

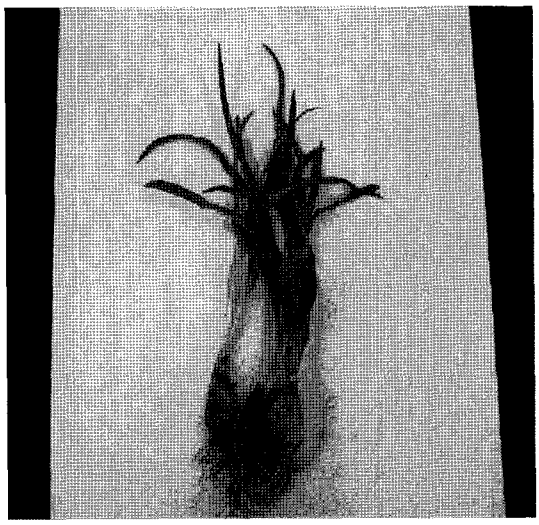

Fig. 1. A picture of *Scilla scilloides* (Lindl.) Druce.

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also been reported (10-12). It is well understood that a small molecule, hyaluronic acid, activates tissue inflammation (11, 12). In general, many anti-inflammatory medicines are hyaluronidase inhibitors.

Therefore, the purpose of this study is to evaluate the root-extract of *S. scilloides* as an antimicrobial agent against food pathogens and skin diseases, as an anti-inflammatory agent inhibiting hyaluronidase *in vitro*, and as an anti-oxidative or anti-aging agent. The information provided by this study will be useful for applications within the food industry and dermatologic medicine.

Materials and Methods

Extract preparation *S. scilloides* was taken from the south-eastern area of An-dong (Gyeongbuk, Korea). Dust was removed from the roots with tap water. Twenty grams of roots and 80 mL of 95% ethanol were minced together with a mixer. The mixture was filtered through a Büchner funnel and then a 0.45 μm membrane filter funnel (Durapore HVLP; Millipore Co., Billerica, MA, USA). The filtrate was stored at -10°C until used for research.

Bacterial strains The bacterial strains used for antimicrobial assays were as follows: *S. aureus* ATCC 35556, *S. enteritidis* ATCC 12021, *E. coli* O157:H7, and *Candida parapsilosis*. *S. aureus* ATCC 35556 and *S. enteritidis* ATCC 12021 were purchased from American Type Culture Collection. *E. coli* O157:H7 was obtained from Daegu Haany University and *C. parapsilosis*, a clinical pathogen, was screened from patients afflicted with various skin diseases. All strains were cultured in tryptic soy broth (Difco Laboratories, Sparks, MD, USA) supplemented with 0.6% yeast extract (TSB-YE) at 37°C for 24 hr and used for further studies.

Determination of total solids, fat soluble components, and total carbohydrates The solid contents of *S. scilloides* root extracts were measured after drying at 105 °C. Before drying, 50 mL of extract was put in a drying dish, and water and ethanol were allowed to evaporate until the sample was very viscous. The fat-soluble content was extracted using ethyl ether. Fifty mL of each sample and 50 mL of ethyl ether was mixed in a separatory funnel. After being allowed to stand overnight, the ether layer was removed, washed three times with distilled water, and solvent evaporated in a rotary vacuum evaporator (SB1000; Eyela, Tokyo, Japan). The concentrates were dried in a 105°C dry oven for 2 hr, and the fat-soluble components were weighed. To quantitate the total carbohydrate content of both samples, the Lane-Eynon method (13) was used after acid-hydrolysis in 10 mL of 1 N HCl with refluxing in a boiling water bath for 2 hr.

Determination of antimicrobial activity *S. scilloides* root extracts were prepared at 0.1 and 1.0% of the total broth volume and aseptically filtered through a 0.45 μm membrane filter. One mL of each extract sample was added to 10 mL of TSB-YE media. Each of the four bacterial strains (*S. aureus* ATCC 35556, *S. enteritidis* ATCC 12021, *E. coli* O157:H7, and *C. parapsilosis* KCCM 35428) were inoculated in culture media at 2.0×10^4 CFU/mL, and then incubated at 37°C for 6 hr. The antimicrobial effect on each sample was determined by enumeration of cells. Each experiment was performed in triplicate, and the data for 0 (control), 0.1, and 1.0% root extracts of *S. scilloides* were analyzed using the Student's *t*-test ($p<0.05$).

Determination of anti-inflammatory activity Hyaluronidase activity was determined by measuring the amount of N-acetylglucosamine formed from sodium hyaluronidate with a spectrophotometer (Opron 3000; Hanson Co., Seoul, Korea). Fifty μL of bovine hyaluronidase (5,000 units/mL, Sigma-Aldrich Co., St. Louis, MO, USA) dissolved in 0.1 M acetate buffer (pH 3.5) was mixed with 100 μL of a designated concentration of sample dissolved in 5.0% dimethyl sulfoxide (DMSO). The mixture was then incubated in a water bath at 37°C for 20 min. The control was treated with 100 μL of 5.0% DMSO only. The reaction mixture was added to 100 μL of 12.5 mM calcium chloride, and then incubated in a water bath at 37°C for 20 min. The Ca^{+}-activated hyaluronidase was mixed with 250 μL of sodium hyaluronidate (1.2 mg/mL) dissolved in 0.1 M phosphate buffer (pH 3.5), and then incubated in a water bath at 37°C for 40 min. One hundred μL of 0.4 N sodium hydroxide and 100 μL of 0.4 M potassium borate were added to the reaction mixture. The reaction mixture was then incubated in a boiling water bath for 5 min. After cooling to room temperature, 3 mL of *p*-dimethyl-aminobenzaldehyde solution (4 g of *p*-dimethyl-aminobenzaldehyde dissolved in 350 mL of 100 % acetic acid and 50 mL of 10 N hydrochloric acid) was added to the reaction mixture, which was then incubated in a water bath at 37°C for 20 min. The optical density of the reaction mixture was measured at 585 nm using a spectrophotometer. The inhibitory effect was expressed as follows:

$$\text{Inhibitory effect (\%)} = [(\Delta OD_c - \Delta OD_s)/\Delta OD_s] \times 100$$

(ΔOD_c; optical density of control, ΔOD_s; optical density of sample)

Determination of anti-oxidative activity The thiocyanate method was used to determine the antioxidant properties of *S. scilloides* root extracts (14). Specifically, the extract samples were diluted to 1:9 and 1:99 with 70% ethanol. Additionally, 1.0% butylated hydroxyltoluene (BHT; Sigma-Aldrich Co.) in methanol was used as a standard reagent for comparison. Two mL of linoleic acid (25 mg/mL in ethanol, Wako Co., Osaka, Japan) and 1.0 mL of each sample were mixed, after which 4.0 mL of 40 mM phosphate buffer (pH 7.0) was added to each. The control was treated with 1.0 mL of 70% ethanol. The mixture was incubated at 40°C for 5 days after which 1.0 mL of the mixture was added to 4.0 mL of 70% ethanol, 0.1 mL of 30% ammonium thiocyanate, and 0.1 mL of 20 mM ferrous chloride in 3.5% HCl solution. The optical density of the mixture was measured using a spectrophotometer at 500 nm. The anti-oxidative index (AOI) was calculated using the following equation.

$\Delta OD_s = OD_s - OD_b$, $\Delta OD_c = OD_c - OD_b$,
(OD_s; Optical density of samples, OD_b; Optical density of

Table 1. The composition of total solids, fat-soluble compounds, and carbohydrates in *S. scilloides* roots

Compounds	Values (%)
Solid content	63.6 ± 1.4[1)]
Fat-soluble content	3.1 ± 0.4
Carbohydrates	56.7 ± 1.5
Others	3.8 ± 0.2
Moisture content	36.4 ± 1.2

[1)]The values are mean±SD.

blank, OD_c; Optical density of control)
Antioxidative efficiency (AOE%) $=(\Delta OD_c-\Delta OD_s)/\Delta OD_c \times 100$
AOI = AOE%/solid content, w/v%

Statistical analysis Analysis of variance was performed for triplicate samples using the SAS program. Duncan's test was used to verify the significance of the difference due to each treatment.

Results and Discussion

Solids and fat-soluble contents The composition of total solids, fat-soluble components, and carbohydrates in *S. silloides* root extracts were determined prior to the evaluation of the bio-functionality of each sample. As shown in Table 1, the solid content of *S. silloides* root extract was about 63% and the total carbohydrate content was about 57%. The fat-soluble content, which includes the bio-effective compounds, was 3.1%. When the roots were extracted with ethanol (sample:95% ethanol=20:80, w/v), the solid composition of the extract was about 3.7± 0.3% (data not shown). It is presumed that some of fat-soluble compounds exist as glucosides.

Antimicrobial activity The resistance of microorganisms to *S. scilloides* root extract was studied and the results are summarized in Fig. 2. All strains used in this research are pathogens associated with foods or skin diseases. At the beginning of culture, each strain was inoculated at 2.0×10^4 CFU/mL in the presence of 0.1 and 1.0% concentrations of the extract in TSB-YE broth. The growth of *S. aureus* ATCC 35556 and *C. parapsilosis* KCCM 35428 was inhibited significantly more so than that of *S. enteritidis* ATCC 12021 and *E. coli* O157:H7 with *E. coli* O157:H7 being the most resistant to growth inhibition. However, when tested at extract concentrations below 0.1%, no antimicrobial effects were detected (data not shown). Based on these results, it is expected that *S. scilloides* root extract can be potentially used as an antimicrobial additive or pharmaceutical supplement because of its broad antimicrobial spectrum of effectiveness against various pathogens, although the major components exerting antimicrobial effects have not been identified.

Anti-inflammatory activity Hyaluronic acid is the main component of the extra-cellular matrix and of biological fluids in animal tissue (9, 10). It acts as an important regulator in repairing wounds without scarring (11).

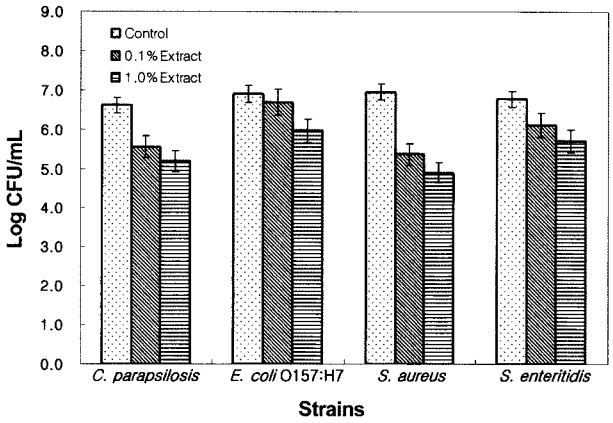

Fig. 2. The antimicrobial effects of *S. silloides* root extract. The samples were diluted with sterilized distilled water (1:9 and 1:99), and 1 mL of each distilled sample was added to 9 mL of TSB-YE, respectively.

However, the degradation of hyaluronic acid to smaller molecules results in an increase in inflammation, angiogenesis, fibrosis, and collagen deposition in the healing of wounds (12). Hyaluronidase is an endo-hexosaminidase which initiates the degradation of high molecular weight hyaluronic acid. In the present study, the anti-inflammatory effect of *S. scilloides* root extract was evaluated by testing for its inhibitory effect on hyaluronidase *in vitro*. The results of this study show that both 0.1 and 1.0% concentrations of the extract have inhibitory effects on hyaluronidase (Table 2). In addition, no inhibitory effects against hyaluronidase were detected at concentrations below 0.1% of extract.

Anti-oxidative activity Anti-oxidative activity was determined by measuring the oxidation of linoleic acid. The results are shown in Table 3 and indicate that 1.0 and 10% concentrations of *S. scilloides* extract have anti-oxidative activity ($p<0.05$). The anti-oxidative activity of 10% *S. scilloides* root extract was similar to that of 1.0% BHA. With regard to the solids in the extract, 10% root extract contains 0.037%(w/v) fat-soluble solids. Adjusting for the amount of solids in each, this indicates that the anti-oxidative effect of *S. scilloides* root extract was at least three-fold greater than that of BHA.

It appears that the major components of *S. scilloides* root extract with antimicrobial, anti-inflammatory, or anti-oxidative effects are fat-soluble and that these are flavonoids or their glucosides. The root extract will be very valuable as new therapeutic material because of its multi-functional activities. The isolation and quantitative

Table 2. The anti-inflammatory effect of *S. scilloides* root extract

Samples	Concentration (%)	Inhibitory effect (%)
Control	0	0.22 ± 0.13[1)]
Extract of *S. scilloides*	0.1	14.85 ± 1.25
	1.0	48.23 ± 0.36

[1)]The values are mean±SD.

Table 3. The anti-oxidative effect of *S. silloides* root extract compared with BHA using the thiocyanate method

Sample	Concentration (%)	AOI[1)]
BHA	1.0	76.8 ± 3.5
Extract of *S. scilloides*	1.0	33.2 ± 5.8
	10	72.3 ± 4.2

[1)]AOI; Anti-oxidative Index.

determination of each flavonoid or major compound in *S. scilloides* root extract as an antioxidant or antimicrobial agent needs to be investigated further.

Acknowledgments

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