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RESEARCH ARTICLE

A study on the nutritional and biochemical analysis of *Selaginella tamariscina* powder

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Abstract This study reports the various nutritional components of *Selaginella tamariscina,* which is traditionally used in folk or Chinese medicine. The iron nutrient content in *S. tamariscina* powder was 0.94±0.06 mg/100 g powder, whereas selenium was present in a small amount, which showed strong antioxidant power. The total phenolic content of *S. tamariscina* powder was 8.65–11.61 mg gallic acid equivalents/g. *S. tamariscina* showed antioxidant activity in 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. The ferric reducing antioxidant power of *S. tamariscina* powder was higher in the ethanol extract. Additionally, the ethanol extract demonstrated antimicrobial activity against *Bacillus subtilis* KCTC 2189. The level of high-density lipoprotein-cholesterol in the blood of ICR mice was significantly higher in the HF 20% +*S. tamariscina* 20% group than in the other groups ($p<$ 0.05). The present study demonstrates that *S. tamariscina,* an abundantly existing plant, possesses antimicrobial, antioxidant, and anticytotoxic activities. *S. tamariscina* powder has potential as a functional food.

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Keywords *Selaginella tamariscina* powder · Antibacterial activity · Antioxidant activity · Anticytotoxic activity · Blood lipid level

Introduction

With an improvement in income levels and a changing diet, physiological benefits such as antioxidant, anticancer, and antifungal activities from plants have spurred great interest among both consumers and researchers, and the elucidation of these properties in plants has become an area of active research. Therefore, there has been increasing interest in studying the functional aspects of commercial vegetables (Lee et al., 2005).

Selaginella tamariscina [*Selaginella tamariscina (P. Beauv.) Spring*] is commonly called Kwonbaek due to its resemblance with a fist-like pine tree. In addition, it has been described as "hand," "butcher hand," "boson hand," "fireweed," and as a perennial pine (Chu et al., 2016). *S. tamariscina* is an evergreen perennial plant native to mountain rock walls and belongs to the order *Selaginellales,* the family *Selaginellaceae,* and the genus *Selaginella.* They reach a height of approximately 20 cm. They have spores that are egg-shaped triangles with serrated edges hang one by one at the end of their small twigs, and there have four rows of scaly leaves. *S. tamariscina* contains flavones, phenols, polysaccharides such as amino acids and trehalose, and small amounts of tannins, such as apigenin, amentoflavone, hinokiflavone, and isocryptomerin, which have also been reported as flavones (Jung et al., 2006; Lee et al., 2009; Shin and Kim, 1991). *S. tamariscina* is used in folk medicine to treat the side effects of mental instability, tumor prevention and healing, renal function enhancement, stones, asthma, bronchial disease, and radiation therapy. *S.*

tamariscina is also used to treat bleeding and maintain hemostasis (Chu et al., 2016; Park and Rhee, 1994). It is also used to alleviate menstrual pain or bruising, improve blood circulation, and has been known to have a negative metabolic effect (Chu et al., 2016; Park and Rhee, 1994). In previous studies (Chu et al., 2016; Hsin et al., 2013; Kim et al., 2015; Nguyen et al., 2015; Yang et al., 2013), *S. tamariscina* was found to inhibit acetylcholinesterase and pharyngeal cancer cell activity; biflavonoids isolated from *S. tamariscina* extracts reported impact. It has also been reported to decrease the secretion of matrix metalloproteinase (MMP)-2 and MMP-9 in osteoblasts, as well decrease the expression of genes associated with the p38 and Akt signaling pathways (Chu et al., 2016; Hsin et al., 2013; Kim et al., 2015; Nguyen et al., 2015; Won et al., 2018; Yang et al., 2013). In addition, amentoflavone, an active ingredient of *S. tamariscina*, inhibits beta-amyloid formation in PC-12 neurons and inhibits antioxidant activity and apoptosis in hippocampal tissues (Chu et al., 2016; Sasaki et al., 2015; Zhang et al., 2015).

As pointed out in the study by Kim et al. (2019), *S. tamariscina* is actively used as a medicinal herb in Korea. A difference in the biological activity according to the processing of foams has been reported (Lee et al., 2006), but research on their biological activity following different processing conditions such as extraction, drying, and cooking for practical use as a food is lacking. Various studies on *S. tamariscina* have been conducted worldwide, primarily in China. *S. tamariscina* has been widely used in folk medicine since ancient times, but research has been conducted in the fields of oriental medicine and pharmacology. However, this plant has not been scientifically studied in the field of food and nutrition in Korea. Recently, as it is known as a health food through broadcast media, continuous research is required regarding its nutritional quality in Korea. Therefore, in this study, *S. tamariscina* was tested to determine its usefulness as a functional food through general component analysis, as well as through antibacterial, antioxidant, and cytotoxicity assays. In addition, its biochemical activity in mice was observed by adding *S. tamariscina* powder to their high-fat diet.

Materials and methods

Materials and reagents

Selaginella tamariscina was collected from rocks near Mt. Bulam between June and September 2017. Samples were freeze-dried (FDTL-4504, Operon, Gyeonggi-do, Korea) between −50 and −40 °C, and then used as samples while stored in a−20 °C freezer. Twenty grams of each ground material was added to 500 mL of deionized water (DIW) and ethanol, respectively, and then extracted twice using a heating mantle with a reflux condenser for 3 h. The extract was centrifuged after compression filtration (6000 rpm, 30 min, 4 °C), the supernatant was concentrated under vacuum at 45 °C, and then lyophilized (LYPH-LOCK 12, Labconco, Kansas City, Mo, USA) to obtain a powder sample. Dry materials (1 g of natural product) were sonicated with 10 mL of 80% methanol for 1 h to prepare a 100 mg/mL stock solution. The samples were then cooled to room temperature for 24 h. The extract was centrifuged at 8000 rpm for 10 min. The supernatants were collected at 4 °C prior to use within 24 h. Both 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Fluka (Buchs, Switzerland). Folin-Ciocalteu reagent (FC reagent), potassium persulfate, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Gallic acid, sodium carbonate, sodium acetate, acetic acid, and $FeCl_3{\cdot}6H_2O$ were obtained from Riedel-de Haën (Seelze, Germany). The other chemicals and solvents used in this study were of analytical grade (Han et al., 2019; Lee et al., 2017).

General composition analysis

The moisture content of the *S. tamariscina* powder was determined using the atmospheric pressure drying method (FS-620, Tokyo Seisakusho Co., LTD., Osaka, Japan), and the crude protein was analyzed via the micro-Kjeldahl method using a crude protein automatic analyzer (Kjeltec TM 2300, FOSS, Höganäs, Sweden). Ash content was measured using the direct painting method (KL-160, Toyo Seisakusho Co., LTD., Osaka, Japan), and crude fat content was measured using the Soxhlet method (SOX606, LABTECH, Seoul, Korea) (Choi et al., 2016; Lee et al., 2008).

Mineral content analysis

The content of certain minerals, including copper, zinc, iron, selenium, and manganese, were analyzed according to the method suggested by Kim et al. (2007). Sample pretreatment was carried out via dry decomposition and the resulting product was filtered to obtain a test solution of up to 100 mL with distilled water. The pretreated test solution was analyzed using an inductively coupled plasma spectrometer (ICP-AES, inductively coupled plasma-atomic emission spectrophotometer, Z6100, Hitachi, Tokyo, Japan) (Choi et al., 2016).

Preparation of bacterial cultures and antibacterial assay

All microorganisms were purchased from the Korean Collection for Type Culture (Daejeon, Korea). The purchased microorganisms were inoculated into a broth and incubated for 12 h at 30 °C and 37 °C. Stock cultures were maintained at −80 °C in a broth containing glycerol (20% v/v). The antimicrobial activity of the extract was determined using the disk diffusion method (Seul and Yang, 2017; Kim et al., 2016). Briefly, after 18 h of culture, a bacterial suspension in saline was diluted to a density of 1–2×10^8 CFU/mL (McFarland standard of 0.5). The bacterial suspension was gently distributed onto the surface of Mueller–Hinton agar (MHA) using sterile cotton-tipped swabs. Each sample extract (20 µL) was added to a paper disc (6 mm in diameter) that was placed on the MHA. A disk containing ceftazidime, gentamycin, cefotaxime, or chloramphenicol was used as a positive control, and the diameter of the inhibition zone (mm) was measured after incubation at 35 ± 1 °C for 16–20 h.

Total phenolic content (TPC) assay

TPC was determined with Folin-Ciocalteu reagent, using gallic acid as a standard. A 20 µL aliquot of a diluted sample (the stock solutions were further diluted with distilled water) was added to 100 µL of FC reagent and mixed well. After 5 min, 300 µL of 20% sodium carbonate solution was added, and the mixture was vortexed. The samples were incubated for 2 h at room temperature in the dark. The absorbance was measured at 765 nm using a UV–vis spectrophotometer (Ultrospec 3100 Pro, Amersham Bio., Cambridge, UK). The results are expressed as gallic acid equivalents (mg GAE/g dry weight [DW]). All experiments were performed in triplicate (Waterhouse, 2002).

Ferric reducing antioxidant power (FRAP) assay

The antioxidant capacity of *S. tamariscina* powder was estimated according to the procedure described by Pulido et al. (2000). The FRAP reagent included 25 mL of 300 mM acetate buffer (pH 3.6), 2.5 mL 10 mM TPTZ solution in 40 mM HCl, and 2.5 mL 20 mM $FeCl_3 \cdot 6H_2O$. FRAP reagent (900 µL), prepared fresh and warmed to 37 °C, was added to 90 µL of distilled water and 30 µL of sample, as with the reagent blank. The final sample dilution in the reaction mixture was 1:34. The absorbance at 595 nm was measured every 15 s, and the reaction was monitored for 30 min. The results were corrected for dilution and were expressed as µmol Trolox/g DW of material. All measurements were performed in triplicate (Lee et al., 2017).

ABTS radical scavenging activity and DPPH radical scavenging activity

The ABTS radical cation decolorization assay described by Lee et al. (2017) was performed, with slight modifications. An $ABTS^{\bullet+}$ solution was prepared by reacting 7 mM aqueous ABTS solution with 140 mM (2.45 mM final concentration) potassium persulfate ($K_2S_2O_8$). After storage in the dark for 12–16 h, the radical cation solution was further diluted in phosphate buffered saline (PBS, pH 7.4) to an absorbance of 0.7 (±0.02) at 734 nm and equilibrated at 30 °C. PBS was used as a blank solution. Diluted samples (10 µL) were mixed with 1 mL of the $ABTS^{\bullet+}$solution and the decrease in the absorbance was measured after 15 min. Measurements were performed in triplicate compared with the blank solution. The ABTS radical cation scavenging capacity was calculated using the following equation:

$$\text{ABTS scavenging activity}(\%) = [(\text{ABTS}_{\text{Control}} - \text{ABTS}_{\text{Sample}})/\text{ABTS}_{\text{Control}}] \times 100$$

The DPPH assay was performed according to the method described by Thaipong et al. (2006). The stock solution was prepared by dissolving 24 mg DPPH in 100 mL methanol and then stored at −20 °C until use. The working solution was obtained by mixing 10 mL stock solution with 45 mL methanol to obtain an absorbance of 1.1 ± 0.02 units at 515 nm using a spectrophotometer. Diluted samples (50 µL) were added to 2 mL of DPPH solution and mixed. The absorbance of the remaining DPPH was determined after 30 min at 515 nm. The blank sample contained methanol. The percentage of DPPH radical scavenging capacity was calculated using the following equation:

$$\text{DPPH radical scavenging activity}(\%) = \left[\left(\text{ABS}_{\text{Control}} - \text{ABS}_{\text{Sample}}\right)/\text{ABS}_{\text{Control}}\right] \times 100$$

EC_{50} values were calculated from data using a mathematical method based on the principle of the right-angled triangle: EC_{50}=D−[(A-50% max response)·(D–C)]/(A−B), where A is the immediate higher response of 50% of the maximum response, B is the immediate lower response of 50% of the maximum response, D=log concentration corresponding to the A response, and C=log concentration corresponding to the B response. The Trolox equivalent antioxidant capacity (TEAC) values were expressed as µmol Trolox/g DW of material. All measurements were performed in triplicate.

Cell viability assay

Ethyl alcohol (Sigma-Aldrich, 459844, St. Louis, MO, USA) was added to the *S. tamariscina* powder sample to create a 100 mg/mL solution. MRC5 cells, a human-derived normal lung cell line, were purchased from ATCC (American Type Culture Collection) and were cultured in minimum essential medium (MEM) in a 5% CO_2 incubator at 37 °C. After seeding and culturing MRC5 cells (1×10^4 cells/well) in a 96-well plate, twofold serial dilutions were performed at concentrations of 12.5, 25, 50, 100, 200, and 400 μg/mL, and cultured for 24 h. Cell viability was measured using an MTT assay kit (Abcam, ab211091, Cambridge, UK), and the absorbance at 590 nm was measured using a microplate reader (Synergy H1 Hybrid Multi-Mode Microplate Reader, BioTek, Winooski, VT, USA).

Experimental animals and breeding conditions

Male Institute of Cancer Research (ICR) mice (6–7 weeks old) were purchased from Hallim Experimental Animal Co., Ltd., and were used per experimental group. The mice were acclimatized with a commercially available solid diet (PicoLab® Rodent Diet) for 1 week, then completely randomly grouped according to their weight, and reared while supplying water and food ad libitum for 60 days. During the experiment, the breeding conditions were maintained at a room temperature of 20±2 °C and humidity of 40–60%, and the contrast was adjusted every 11±1 h. The experiment was conducted from October 11, 2017, to December 30, 2017. All animal experiments were approved by the Committee of Laboratory Animals in accordance with the institutional guidelines of Sahmyook University, Republic of Korea (SYUIACUC 2017-017).

Animal feed composition

The feed composition was based on the weight ratio, and the feed of the control group consisted of 60% carbohydrates (starch + sucrose + glucose + fructose + lactose), 21% proteins, 13% lipids (beef oil), 1% vitamins, 3% minerals, and 2% fiber. The feed of the high-fat diet group consisted of 53% carbohydrates (starch + sucrose + glucose + fructose + lactose), 21% proteins, 20% lipids (beef oil), 1% vitamins, 3% minerals, and 2% fiber. The feed composition of the experimental group with *S. tamariscina* powder added to the high-fat diet consisted of 33% carbohydrates (starch + sucrose + glucose + fructose + lactose), 20% *S. tamariscina* powder, 21% proteins, 20% lipids (beef oil), 1% vitamins, 3% minerals, and 2% fiber.

Blood collection

Experimental animals were fasted for 12 h on the last day of rearing, anesthetized with CO_2 gas, their thoracic cavities surgically opened, and blood was collected from their hearts. Each blood sample was placed in a refrigerator at 4 °C for approximately 1 h. Serum was separated via centrifugation at 3000 rpm for 15 min at 5 °C. The separated serum was stored in a −70 °C freezer until use, where an aliquot of 100 μL was placed into a microfuge tube and used in subsequent experiments.

Analysis of blood lipid concentration

Serum cholesterol content was measured using the o-phthaldehyde method(Rudel and Morris, 1973). Samples were aliquoted at 0.1 mL, 0.3 mL of 33% KOH solution and 3.0 mL of 95% ethanol were added, the solution was mixed well and was heated in a water bath at 60 °C for 15 min and then cooled. To this, 5.0 mL of nucleic acid was added and mixed, 3.0 mL of distilled water was added, and then mixed well for 1 min, and the layers were separated, and 1.0 mL of the nucleic acid layer was separated. The nucleic acid layer was concentrated and dried with nitrogen, and 2.0 mL of o-phthaldehyde reagent was added, mixing well. After 10 min, 1.0 mL of concentrated sulfuric acid was added as a color developing reagent, and the solution was mixed well. After the addition of sulfuric acid, a spectrophotometer (Human Corporation, Seoul, Korea) was used to measure the absorbance of the solution at 550 nm, and the cholesterol content was quantified according to a standard calibration curve. High-density lipoprotein (HDL)-cholesterol (HDL-C 555, Eiken Co., Tokyo, Japan) and low density lipoprotein (LDL)-cholesterol (BLF, Eiken Co., Tokyo, Japan) kit reagents were used according to the corresponding manufacturer's instructions. To measure the HDL-cholesterol content, 0.3 mL was placed in a test tube, and 0.3 mL of precipitation reagent was added, mixed well, and incubated at room temperature for 10 min, followed by centrifugation at $700 \times g$ for 10 min. Then, 50 μL of the supernatant, 50 μL of the prepared standard solution (100 mg/dL), and 3.0 mL of the HDL coloring reagent were added to 50 μL of distilled water as a blank, mixed well, and heated in a water bath at 37 °C for 5 min. The HDL-cholesterol content was quantified by measuring the absorbance at 555 nm using a blank as a control. To measure the LDL-cholesterol content, 0.1 mL of serum, 0.1 mL of standard serum, and 4.0 mL each of BLF kit reagents I and II, respectively, were added to a test tube, and mixed well for 5 s, then incubated at room temperature (25±3 °C) for 25 min. Within 10 min of standing, the LDL-cholesterol content was quantified by measuring the absorbance at 650 nm

using a spectrophotometer with distilled water as a control. Serum triglyceride levels were analyzed using a TG kit reagent (Sigma chemical Co., St. Louis, MO, USA). Ten microliters of serum, 10 μL of standard solution (300 mg/dL), and 1.0 mL of TG kit reagent were added to 10 μL of deionized water as a blank, mixed well, and then reacted in a water bath at 37 °C for 5 min, using the blank as a control. The TG content was quantified by measuring the absorbance at 540 nm using a spectrophotometer.

Biochemical analysis of liver function

Alkaline phosphtase (ALP) concentrations were measured using a commercial kit (Asan Pharmaceutical Co., Ltd., Seoul, Korea), and serum alanine transminase (ALT) and aspartate transminase (AST) concentrations were measured using the Reitman and Frankel method (1957). serum lactate dehydrogenase (LDH) was measured using cytotoxicity assay kit (Thermo Scientific, Rockford, IL, USA).

Statistical analysis

All data were analyzed using the SPSS package version 18.0 (Statistical Package for Social Science, SPSS Inc., Chicago, IL, USA) program to calculate the means and standard deviations. All samples used in the experiment were repeated three times. Comparison of mean values was performed using the t-test and one-way ANOVA, and significance analysis ($p<0.05$) of the differences between means was performed using Duncan's multiple test method. Correlations between the antioxidant activities of *S. tamariscina* powder were analyzed using Pearson's correlation analysis ($p<0.01$).

Results and discussion

General composition analysis

The results of the general composition analysis of *S. tamariscina* powder are presented in Table 1. The freeze-dried *S. tamariscina* powder contained 2.61±0.39% water, 17.39±0.38% protein, 1.23±0.06% fat, and 7.67±0.54% ash. In a previous study (Sheo and Lee, 1989), ferns belonging to the same category contained 2.7% water, 19.8% crude protein, 2.9% crude fat, 7.9% ash, and 31.7% dietary fiber. The results of general composition analysis of *S. tamariscina* powder in this study showed similar content to ferns. Also, The Japanese royal fern was reported to contain 90.6 g water, 2.4 g protein, 0.1 g fat, and 0.7 g of ash (RDA, 2006).

Mineral content analysis

The mineral content of *S. tamariscina* powder is shown in Table 1. Per 100 g, the freeze-dried *S. tamariscina* powder contained 0.94±0.06 mg of iron, 0.53±0.02 mg of manganese, 0.44±0.01 mg of zinc, and 0.26±0.01 mg of copper. In addition, there was a very small amount of the strong antioxidant selenium (0.01±0.001 mg). In particular, when the iron content was compared with *P. aquilinum var. latiusculum* (2.5 g) (Sheo and Lee, 1989) and Japanese royal fern (1.7 g) (RDA, 2006), the *S. tamariscina* powder in this study showed a low level.

Antibacterial activity assays

The antimicrobial activity of *S. tamariscina* powder is shown in Table 2. DIW extracts of *S. tamariscina* powder did not show antibacterial activity against *Bacillus subtilis* KCTC 2189, *S. aureus* KCTC 3881, *P. aeruginosa* KCTC 2004, *E. aerogenes* KCTC 2190 or *E. coli* KCTC 1682. However, the ethanol extract of *S. tamariscina* powder showed antibacterial activity against *B. subtilis* KCTC 2189. Therefore, *S. tamariscina* powder ethanol extract, which showed antimicrobial activity against gram-positive bacteria, could be effectively used as an antimicrobial agent. A previous study (Lee et al., 2009) reported that isocryptomerin isolated from *S. tamariscina* showed strong antibacterial activity against gram-positive and gram-negative bacterial strains, including *Staphylococcus aureus*. In particular, *S. tamariscina* powder has been reported to have synergistic effects when combined with cefotaxime. These data suggest the potential of *S. tamariscina* as a therapeutic compound against infectious diseases. Amentoflavone is a biflavonoid compound with antioxidant, anticancer,

Table 1 General composition and mineral content of *S. tamariscina*

Composition (%)	*S. tamariscina*	Composition (%)	*S. tamariscina*
Moisture	2.61±0.39[1)]	Copper	0.26±0.01
Crude protein	17.39±0.38	Zinc	0.44±0.01
Crude fat	1.23±0.06	Iron	0.94±0.06
Crude ash	7.67±0.54	Selenium	0.01±0.001
–	–	Manganese	0.53±0.02

[1] Values represented mean±SD of three parallel measurements

Table 2 Antibacterial activity of different common natural extracts concentrations and antibiotics against pathogenic bacteria using paper disc agar diffusion method

Solanum nigrum L	*B. subtilis* KCTC 2189	*S. aureus* KCTC 3881	*P. aeruginosa* KCTC 2004	*E. aerogenes* KCTC 2190	*E. coli* KCTC 1682
DIW	–	–	–	–	–
EtOH	+[a]	–	–	–	–

[a] Growth inhibition size of clear zone; –: not detected, +: 8□12 mm

antibacterial, antiviral, anti-inflammatory, and sunscreen properties that can be isolated from *S. tamariscina* (Oh et al., 2013). Amentoflavone extracted from *S. tamariscina* showed strong antifungal activity against several pathogenic strains and could be used as a major compound in the development of antifungals; additionally, it has a weak hemolytic effect on human red blood cells (Jung et al., 2006).

TPC assay

The TPC of *S. tamariscina* powder is shown in Table 3, which ranged from 8.65 to 11.61. The TPC of the DIW extract was 8.65±0.61 GAE mg/g extract, whereas it was 11.61±0.30 GAE mg/g in the ethanol extract, which was significantly higher ($p<0.05$). It has been reported a higher total phenolic compound content is associated with higher antioxidant activity (Lee et al., 2018). In a previous study (Jang et al., 2014; Kim et al., 2019), phenolic compounds were reported to have excellent anticancer, anti-inflammatory, antioxidant, and antibacterial effects. In particular, they have also been shown to inhibit the accumulation of various lipids. Kim et al. (2019) reported the yield of *S. tamariscina* as 10.3%, with a TPC as 60.29 GAE mg/g and a total flavonoid content of 14.90 quercetin equivalent (QE) mg/g. Lee et al. (2006) reported that the TPC of *S. tamariscina* was 99.3 GAE mg/g and its total flavonoid content was 37.2 QE mg/g. As pointed out in the study by Kim et al. (2019), there may be differences in the reported values depending on the cultivation environment of the sample or due to differences in the methods of measuring the total flavonoid and phenolic content. Shin et al. (2016) reported that the TPC of *H. plumaeforme*, *T. kanedae*, and *L. juniperoideum* was 96.53, 59.20 and 119.87 mg GAE/g, respectively, and the TPC of *S. tamariscina* was similar to that of mosses and ferns. In a study by Lee et al. (2005), a total polyphenol content of 130.22, and 120.69 μg/mg *Cirsium nipponicum (Maxim.) Makino* leaf and *Athyrium acutipinnulum Kodama ex Nakai* leaf extracts, respectively, were reported. *Achyranthes japonica (Miq.) Nakai* root and *C. nipponicum (Maxim.) Makino* seeds and stems reported very low levels of total polyphenol content, at 16.74, 29.20, and 27.27 μg/mg, respectively. There were differences in TPC depending on the plant variety and test method used. Berries are known to have a high TPC. The TPC content of berries was 9.03 GAE mg/g for blueberry, 5.34 GAE mg/g

Table 3 Total phenolic content, FRAP, ABTS and DPPH radical scavenging activity of *S. tamariscina*

S. tamariscina	Total phenolic content (GAE mg/g)		FRAP (TEAC μmol/g dry weight)	
	DIW[1]	EtOH[2]	DIW	EtOH
	8.65±0.61[b3,4]	11.61±0.30[a]	139.14±1.73[4,b]	161.08±0.93[a]

S. tamariscina	ABTS radical scavenging activity				DPPH radicals scavenging activity			
	TEAC (μmol/g dry weight)		EC50 values (mg/mL)		TEAC (μmol/g dry weight)		EC50 values (mg/mL)	
	DIW	EtOH	DIW	EtOH	DIW	EtOH	DIW	EtOH
	187.05± 2.59[b]	261.19 ± 4.64[a]	5.41± 0.074[a]	3.88± 0.07[b]	52.36± 0.124[b]	65.29 ± 0.74[a]	15.10± 0.044[a]	12.11 ± 0.14[b]

[1] Deionized water extract

[2] Ethanol extract

[3] Values represented mean±SD of three parallel measurements

[4] Significant at $p<0.05$ by t-test

for raspberry (Jeong et al., 2008), 73.66 GAE mg/g for maquiberry, 59.26 GAE mg/g for aronia, and 43.70 GAE mg/g for blackcurrant (Chung, 2016). A comparison of the TPC of *S. tamariscina* powder with berries showed that the phenolic content of *S. tamariscina* powder was even higher than that of certain berries.

FRAP assay

Table 3 shows the reducing power of the DIW and ethanol extracts of *S. tamariscina* powder as determined via FRAP. The total FRAP of *S. tamariscina* powder was found to be 139.14–161.08 TEAC μmol/g. The FRAP of the *S. tamariscina* powder was higher in the ethanol extract (161.08±0.93 TEAC μmol/g) than in the DIW extract (139.14±1.73 TEAC μmol/g) ($p<0.05$). Lee et al. (2018), the FRAP value of cherry was 86.94 TEAC μmol/g, and the value of *S. tamariscina* powder in this study showed a higher values. Therefore, in this study, the FRAP value of *S. tamariscina* powder showed a similar tendency in antioxidant effect when compared to cherry or berries (Lee et al., 2018).

ABTS radical radical scavenging activity and DPPH radical scavenging activity

The ABTS and DPPH radical scavenging activities of *S. tamariscina* powder are shown in Table 3. The ABTS radical scavenging activity of *S. tamariscina* powder was 187.05–261.19 TEAC μmol/g, with an EC_{50} value of 3.88–5.41 mg/mL. The DPPH radical scavenging activity of *S. tamariscina* powder was 52.36–65.29 TEAC μmol/g, with an EC_{50} value of 12.11–15.10 mg/mL. Therefore, the ABTS radical cation decolorization activity and DPPH radical scavenging activity of *S. tamariscina* powder were significantly higher in the ethanol extract than in the DIW extract ($p<0.05$). In a previous study (Lee et al., 2005), the ABTS radical scavenging activity RC_{50} values of *Athyrium acutipinnulum Kodama ex Nakai* leaf and root extracts were 40.93 and 35.39%, respectively, whereas *Solidago virgaurea subsp (gigantea (Nakai) Kitam)* root extract was reported to have an ABTS radical scavenging activity RC_{50} value of 29.08 μg/mL, indicating high scavenging activity. In the study by Lee et al. (2005), DPPH radical scavenging activity showed the highest RC_{50} values of 13.02 and 14.91 μg/mL in *Athyrium acutipinnulum Kodama ex Nakai* leaf and *Solidago virgaurea subsp*, respectively. It was reported that their DPPH radical scavenging activity was not significantly different compared to that of BHA at 5.25 μg/mL. In a previous study (Shin et al., 2016), *H. plumaeforme, T. kanedae,* and *L. juniperoideum* that underwent hydrothermal extraction method based on a tenfold dilution, was found to have an ABTS radical scavenging activity of 2587.33, 1637.00, and 1997.00 μmol Trolox/mg, respectively. DPPH measurements of *H. plumaeforme, T. kanedae,* and *L. juniperoideum* reported values of 298.78, 89.89 and 284.33 μmol Trolox/mg, respectively (Shin et al., 2016). The DIW extract of cherry as presented by Lee et al. (2018) showed high levels of ABTS radical scavenging activity when compared to *S. tamariscina* at 117.00 TEAC μmol/g. The DPPH radical scavenging activity of *S. tamariscina* was found to be as high as that of maquiberry (78.17 TEAC μmol/g) (Lee et al., 2018). Previous studies (Adnan et al., 2021; Kang et al., 1996) reported that DPPH radical scavenging activity and ABTS radical scavenging activity were higher as phenolic compounds increased, and this study clearly demonstrared the antioxidant effect of *S. tamariscina.*

Correlation coefficients among antioxidant activities with *S. tamariscina.*

In the case of ethanol extraction, TPC was positively correlated with ABTS radical scavenging activity ($r=0.961$), DPPH radical scavenging activity ($r=0.927$), and FRAP ($r=0.768$) ($p<0.01$) (Table 4). In this study, TPC and antioxidant activity showed a stronger positive correlation in the ethanol extracts than in the DIW extracts.

Cell viability assay

The cell viability assay results of using *S. tamariscina* powder is presented in Table 5. It was found that the cell viability upon treatment at each concentration of *S. tamariscina* powder was the highest at 98.62±0.56% in the 12.5 μg/mL treatment group, and it was maintained at approximately 96–97% in the groups treated with 25 μg/mL or more of the extract. The results of the MTT assay confirmed that the survival rate of MRC5 cells decreased with an increase in the treatment concentration of *S. tamariscina* powder. Lee et al. (2000a, 2000b) reported that when fibroblast NIH3T3 cells were treated with different concentrations of cadmium, cell viability decreased according to the concentration of *Eoseongcho* extract added, with an MTT_{50} of 33.04 μM. Treatment with *Houttuynia cordata* Thunb. extract was also reported to increase cell number and have a cell regeneration effect on fibroblasts.

Animal blood lipid concentration

The blood lipid concentrations of the rats are shown in Table 6. The total cholesterol concentration in the blood was the lowest in the control group at 152.67±7.72 mg/dL (range, 152.67–213.00 in all groups). However, in the HF 20%+*S. tamariscina* 20% group, the total cholesterol

Table 4 Correlation coefficients among antioxidant activities and functional components with *S. tamariscina*

Factors	DIW[1] TPC	ABTS	DPPH	FRAP	EtOH[2] TPC	ABTS	DPPH	FRAP
TPC	1	0.562**3	0.612**	0.392	1	0.961**	0.927**	0.768**
ABTS		1	0.787**	0.669**		1	0.985**	0.765**
DPPH			1	0.711**			1	0.801**
FRAP				1				1

[1] Deionized water extract

[2] Ethanol extract

[3] Significant at $p<0.01$ by Pearson's correlation analysis

TPC, Total phenolic contents

Table 5 Cell viability assay according to concentration treatment of *S. tamariscina*

S. tamariscina (ug/mL)	Start	12.5	25	50	100	200	400
Cell viability (% of control)	100.01±1.42[1,2]	98.62±0.56[a]	97.60±0.64[b]	97.39±0.71[b]	97.21±0.76[b]	95.17±1.63[c]	96.93±0.47[b]

[1] Values represented mean±S.D. of three parallel measurements

[2] Significant at $p<0.05$ by ANOVA-test

Table 6 Serum lipid levels and liver function test in mice

Variables	Control	HF 20%	HF 20%+*S. tamariscina powder* 20%
Total cholesterol (mg/dL)	152.67±7.72[c1)2)]	162.50±2.50[b]	213.00±3.21[a]
HDL-cholesterol (mg/dL)	128.67±6.94[c]	127.67±14.97[b]	150.00±10.53[a]
LDL-cholesterol (mg/dL)	20.00±4.55	23.00±1.00	25.00±0.11
Triglyceride (mg/dL)	99.00±19.13	98.00±31.00	114.00±8.01
Alkaline phosphatase (U/L)	53.67±11.12[a]	41.00±0.01[c]	45.00±0.01[b]
Aspartate aminotransferase (U/L)	88.00±37.09[c]	149.00±16.00[a]	126.00±18.01[b]
Alanine aminotransferase (U/L)	13.67±0.94	13.50±0.50	13.00±0.71
Lactate dehydronase (U/L)	370.33±97.01[c]	646.00±100.01[b]	967.00±125.92[a]

[1] Values represented mean±S.D. of three parallel measurements

[2] Significant at $p<0.05$ by ANOVA-test

HDL-cholesterol, high density lipoprotein-cholesterol; LDL-cholesterol, low density lipoprotein-cholesterol; HF 20%, high fat diet 20%; HF 20%+*S. tamariscina* 20%, high-fat diet 20%+*S. tamariscina powder* 20%

content (213.00±3.21 mg/dL) was higher than that of the other groups ($p<0.05$). In particular, HDL-cholesterol levels were significantly higher in the HF 20%+*S. tamariscina* 20% group (150.00±10.53 mg/dL) than in the other groups ($p<0.05$). It was reported that the normal blood total cholesterol in mice was 2–20 mg/dL, and that the amount of LDL-cholesterol in the blood was observed to be 10.47–82.7 mg/dL, showing a large difference depending on the measurer (Sheo and Sheo, 2002). In addition, in this study, HDL-cholesterol levels were significantly higher when *S. tamariscina* was administered in addition to a high-fat diet. HDL-cholesterol is a beneficial lipoprotein that reduces the risk of developing cardiovascular diseases. It has been reported that increasing the proportion of HDL-cholesterol in total cholesterol has the potential to not only suppress dyslipidemia but also prevent various types of atherosclerosis, including coronary atherosclerosis (Park et al., 2009). Research is being conducted to lower blood cholesterol and triglyceride levels using various natural extracts, and since this study one of these researches, more comprehensive follow-up studies on the antioxidant and lipid profile improvement effect of *S. tamariscina* are needed in the future.

Biochemical analysis of liver function

The results of biochemical blood analysis in rats are presented in Table 6. Observed ALP levels were in the range of 41.00 to 53.67 U/L and all three groups were within the normal range of 40 to 250 U/L. AST level was 149.00± 16.00 U/L in the HF 20% group, but decreased to 126.00± 18.01 U/L upon the addition of *S. tamariscina* powder in the HF 20%+*S. tamariscina* 20% group ($p<0.05$). Blood LDH was significantly higher in the HF 20%+*S. tamariscina* 20% group (967.00±125.92 U/L) than in the other two groups ($p<0.05$). AST levels in the mice were determined to be 88.00–149.00 U/L, which were higher than the normal range of 7–40 U/L. Previous studies (Choi et al., 2016) have reported that AST plays a central role in supplying amino groups in vivo and is also involved in gluconeogenesis. In addition, it has also been reported that when the level of AST increases, it reflects cellular degeneration and the necrosis of distributed organs, and is widely used as a potent indicator of liver and heart diseases (Choi et al., 2016). In this study, ALT levels were within the normal range of 4–43 U/L in all groups. Abnormalities in blood ALT levels were reported to be associated with metabolic syndrome in a recent study (Kim, 2009). The normal level of LDH in human blood is 250–350 IU/L. In this study, LDH levels were out of the normal range in HF 20%+*S. tamariscina* 20% group compared to the normal range in humans. LDH is an enzyme that acts when the sugar in the body is decomposed and converted into energy; it has been reported that when cells are destroyed, blood LDH levels increase (Choi et al., 2016). In addition, blood LDH is a useful parameter for the screening of malignant tumors, liver diseases, heart diseases, and blood diseases.

In summary, the ABTS radical scavenging activity, DPPH radical scavenging activity, and FRAP reducing activity of *S. tamariscina* powder were higher in the ethanol extract compared to the DIW extract. The ethanol extract showed antibacterial activity against *B. subtilis* KCTC 2189. In addition, *S. tamariscina* powder increased levels of blood HDL-cholesterol, a "good cholesterol". Therefore, *S. tamariscina* powder extract was confirmed as a functional food material. To further utilize *S. tamariscina* as a functional food material, it is necessary to determine the optimal extraction method for enhancing the functionality of *S. tamariscina* powder and to develop it into various foods.

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Author contribution Hyo-Jeong Hwang: Data analysis. Jeong-Yeon Kim: Sample analysis. Kyung-Ok Shin: Conceptualization, Writing – original draft.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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