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Original Research Article

Pharmacological activities of extracts from different parts of *Cirsium japonicum* var *spinossimum*

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Abstract

Purpose: *To evaluate the antioxidant, anti-inflammatory, inhibitory and cell proliferation activities of Cirsium japonicum (C. japonicum) var. spinossimum.*
Methods: *Phytochemicals (polyphenols, flavonoids, and silymarin) in leaves, roots, and seeds of C. japonicum var. spinossimum were quantified using high-performance liquid chromatography (HPLC). Antioxidant activity was investigated on 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. Anti-inflammatory activity was investigated on nitric oxide (NO) production in lipopolysaccharide (LPS)-induced RAW264.7 cells. Immunostimulant and hair loss prevention was confirmed through 3'-{1-((phenylamino)-carbonyl)-3, 4-tetrazolium}bis(4-methoxy-6-nitro)benzenesulfonic acid hydrate (XTT)-based cell proliferation assays using Jurkat cells and human follicle dermal papilla cells (HFDPC).*
Results: *Extract from seeds exhibited the highest total polyphenol content (610.08 ± 32.40 mg GAE/g), as well as the highest total flavonoid content, while the total polyphenol content of leaves and root extracts were similar. The RC50 values for seed extract against ABTS and DPPH radicals were 8.20 ± 0.02 µg/mL and 40.13 ± 2.47 µg/mL, respectively. In particular, all six Silymarin derivatives were found to be highest in the seed extract. The C. japonicum var. spinossimum seed extract (CSE) showed significant ($p < 0.05$) nitric oxide (NO) inhibitory activity in RAW264.7 cells induced by LPS at all concentrations (12.5, 25, 50, 100 and 200 µg/mL) and exhibited significant ($p < 0.05$) cell proliferation activity in Jurkat and hair follicle dermal papilla cells (HFDPC) at 25 µg/mL.*
Conclusion: *Extract of C. japonicum var. spinossimum seeds contain polyphenols and flavonoids and show significant NO inhibitory and cell proliferation activity in Jurkat and HFDPC. Therefore, Cirsium japonicum is a promising source of anti-inflammatory and anticancer agents.*

Keywords: *Cirsium japonicum var. spinossimum, Jurkat, Human follicle dermal papilla cells (HFDPC), Silymarin, Seed*

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INTRODUCTION

Cirsium miller is a group of plants belonging to the tribe Carduinae, subtribe Cynareae, in the family Asteraceae. It is distributed worldwide. *Cirsium japonicum* var. *ussuriense* (*C. japonicum* var. *spinossimum*) or *Cirsium japonicum* Fisch ex DC, also known as Korean thistle, is a biennial

plant. The tender stems can be eaten raw or cooked as a vegetable, and it is known to contain a component called silymarin, which has an excellent antioxidant and hepto-protective effect [1]. Some examples of species in this genus include *Cirsium pendulum*, *Cirsium setidens*, and *Carduus crispus*. *Silybum marianum*, also known as milk thistle, contains a high concentration of silymarin and is used as a natural liver protectant. It is utilized in the production of healthy functional food supplements, particularly for hepatoprotective drugs in Europe and other regions.

The *Cirsium* genus of plants contains about 78 species of flavonoids with excellent pharmacological activities, including apigenin, luteolin, myricetin, kaempferol, pectolinarin, 5,7-dihydroxy-6,4'-dimethoxyflavone, and hispidulin-7-O-neoheperioside [2,3]. Apigenin has been shown to have anticancer and neuroprotective effects, as well as anti-inflammatory, antispasmodic, and antimicrobial effects [4-8]. *Cirsium* plants also have liver-protective effects by inhibiting lipid peroxidation, increasing the activity of glutathione reductase, and promoting alcohol detoxification. They can also decrease serum lipid levels in cases of hyperlipidemia and delay liver damage by reducing the concentration of total cholesterol and neutral lipids in the liver [9]. Silymarin found in *Silybum marianum* has been reported to have hepatoprotective effects against alcohol-induced lipid peroxidation, alcohol-induced liver fibrosis, and other liver diseases due to its flavolignan content [10]. Cirsimaritin has been detected in *Cirsium pendulum*, and linarin has been found in the roots of *Cirsium xanthocanthum* [11].

In recent times, there has been a growing interest in health-related issues such as disease prevention and treatment, which has led to the development of various drugs and functional foods. Various pharmacological activity experiments have been conducted to demonstrate the functionality of *C. japonicum* var. *spinossimum* as a basic material for the development of functional products. In this study, phytochemicals such as polyphenol and silymarin content were quantified, the extract with the most significant activity was selected, and its pharmacological activity was investigated on inflammatory T cell (Jurkat cell), and human follicle dermal papilla cells (HFDPC) proliferation.

Therefore, this study was aimed at investigating the antioxidant, anti-inflammatory, and immunostimulant activities of the leaves, roots, and seeds of *C. japonicum* var. *spinossimum*.

EXPERIMENTAL

Materials and extraction

Cirsium japonicum var. *spinossimum* was cultivated in Osu-myeon, Imsil-gun, Jeollabuk-do, Korea. It was taxonomically identified by a plant biotechnologist, Dr. Jong Bo Kim (College of Biotechnology, Konkuk University Glocal Campus, Chungju, Republic of Korea). A voucher specimen was deposited at the College of Health Science, Dankook University, Korea. The leaves and roots were dried at 55 ℃ and stored at ≤ 4 ℃, while the seeds were frozen.

Each sample was prepared by grinding 100 g of the material into powder and extracted three times with 70 % ethanol (1 L) for 24 h. The extracts were filtered using a 0.45 µm filter and then freeze-dried (Eyela FDU-2100, Tokyo Rikakikai Co., Tokyo, Japan) into powder form, which was stored at -20 ℃ for use. The extraction yields were 12.92 % for leaves, 15.68 % for roots, and 16.77 % for seeds.

Measurement of total polyphenol content

Total polyphenol content was measured using the Folin-Denis method [12]. Each extract was dissolved in distilled water (DW) at a concentration of 1 mg/mL, and then 1 mL of Folin-Ciocalteau reagent and 1 mL of 10 % sodium trioxocarbonate IV (Na_2CO_3) (Sigma Co., Ltd., St. Louis, MO, USA) solution were added sequentially. The mixture was left at room temperature (RT) for 1 h and absorbance was measured at 700 nm.

The total polyphenol content of the extract was determined by preparing a standard calibration curve using gallic acid (Sigma Co.) at concentrations ranging from 0 to 100 µg/mL and analyzing it using the same method as the extract.

Measurement of total flavonoid content

Total flavonoid content was measured using the method of Moreno *et al* [13]. A sample solution of 1 mg/mL was prepared by adding 0.1 mL of 10 % aluminum nitrate (Sigma Co.), 0.1 mL of 1 M potassium acetate (Sigma Co.), and 4.3 mL of ethanol in sequence, and then allowed to stand at RT for 40 mins. Absorbance was measured at 415 nm. Total flavonoid content was determined from standard calibration curve obtained from the range of 0 - 100 µg/mL using quercetin (Sigma Co.) as the standard reference.

2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity

2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity was measured using the method of Re *et al* [14]. A solution (7.4 mM) of ABTS (Sigma Co.) and 2.6 mM potassium persulfate (Sigma Co.) were mixed and left at RT for 24 h to form radicals. The ABTS solution was then diluted with methanol to an absorbance of 0.70 ± 0.00 (mean ± SE) at 732 nm just prior to the experiment. Thereafter, 50 µL of the extract was added to 950 µL of prepared ABTS solution, and the mixture was left for 1 min before measuring the absorbance at 732 nm. The sample was analyzed by determining the RC_{50} value, which is the concentration required to reduce the activity of the negative control by 50 %.

2,2-diphenyl-1-picrylhydrazyl radical scavenging activity

The DPPH radical scavenging activity was measured using Blois' method [15]. The samples were dissolved in methanol (MeOH) to a final concentration of 15, 30, 60, 125, 250, and 500 µg/mL and quantified. Then, 100 µL of each sample was added to a 96-well plate along with 100 µL of 0.3 mM DPPH to make a total volume of 200 µL. After incubating at RT for 30 min, the absorbance was measured at 540 nm using an ELISA reader (Molecular Devices, USA). The DPPH radical scavenging activity was expressed as the percentage difference in absorbance between the sample and control groups. Samples were compared and analyzed by calculating the RC_{50} value, which is the concentration that reduces the activity of negative control group by half.

Analysis of silymarin content

High liquid performance chromatography (HPLC) analysis was performed to determine the content of 6 silymarin standards (Sigma Co.), including silybin A & B, isosilybin A & B, silychristin, and silydian in extracts from different parts of *C. japonicum* var. *spinossimum*. Samples and silymarin standards were prepared by dissolving in MeOH to a concentration of 1 mg/mL and filtered through a 0.2 µm filter prior to analysis. An HPLC system (Shimadzu Corporation, Kyoto, Japan) consisting of a CBM-20A communications bus module and SPD-M20A diode array detector was used. A C18 column (250 × 4.6 mm, 5 µm) was selected for analysis, and the column temperature was maintained at RT. Solvent A was 0.01 % acetic acid and solvent B was MeOH, and the solvent gradient was programmed as follows: 0 - 3 min, 47 % B; 3 - 13 min, 50 % B; 13 - 25 min, 60 % B; 25 - 35 min, 60 % B; 35 - 35.01 min, 47 % B; 35.01 - 45 min, 47 % B. The total analysis time for all samples was 45 min. The flow rate was 1.0 mL/min, and the sample injection volume was 10 µL. Quantitative analysis was performed at 288 nm absorbance.

Cell culture

RAW264.7 macrophages and Jurkat cell line (T cell) were obtained from Korean Cell Line Bank (KCLB), and human follicle dermal papilla cells (HFDPC) were purchased from ABM Inc (Richmond, British Columbia, Canada). RAW264.7 cells and HFDPC were cultured in Dulbecco's Modified Eagle Medium (DMEM), and Jurkat cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium with L-glutamine (Lonza, Walkersville, MD, USA), each supplemented with fetal bovine serum (FBS), penicillin G (100 IU/mL), and streptomycin (100 µg/mL). Cultures were maintained in a CO_2 incubator at 37 °C.

MTT assay

To measure cell toxicity of *C. japonicum* var. *spinossimum* seed extract (CSE), a 3-(3,4-dimethyl-thiazolyl-2)-2,5-diphenyl tetrazolium bromide (MTT) assay was performed, according to the method described previously by Carmichael *et al* [16]. The RAW264.7 cells were seeded at a density of 1×10^5 cells/well in a 96-well plate and cultured for 24 h prior to the experiment. The plate was filled with 90 µL of medium and cells and pre-treated with 12.5, 25, 50, 100, and 200 µg/mL concentrations of CSE. After 1 h, lipopolysaccharide (LPS) was added at a concentration of 100 ng/mL and the plate was incubated in a 5 % CO_2 incubator for 24 h. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each well (10 µL MTT and 90 µL fresh medium) and incubated for 3 h. After removing the medium, Dimethyl sulfoxide (DMSO) was added (100 µL) to dissolve the formazan crystals and the absorbance was measured at 540 nm.

Nitric oxide (NO) content

Concentration of nitric oxide (NO) was measured by using the Griess Reagent System to determine nitrite concentration in the culture medium. RAW264.7 cells were seeded at a density of 1.0×10^5 cells/well in a 96-well plate and cultured for 18 h. The cells were then pre-treated with CSE at concentrations of 12.5, 25, 50, 100 and 200 µg/mL for 1 h, followed by

treatment with LPS at a concentration of 100 ng/mL and further incubation for 24 h. An equal volume of Griess reagent was added to the culture medium, and after a 10-min incubation at RT, the absorbance was measured at 540 nm [17]. The concentration of NO in the culture medium was determined using a standard curve of sodium nitrite (Sigma Co.) at various concentrations.

Cell proliferation

Cell proliferation of Jurkat cells and HFDPC in response to the test samples was measured using the XTT kit (XTT II, Boehringer Mannheim, Mannheim, Germany) [18]. Cells were seeded at a density of 1×10^4 cells/well in a 48-well culture plate and treated with CSE at concentrations of 0, 12.5, 25, 50, 100, and 200 µg/mL, divided into 3 treatment groups. After 24 and 48 h, 50 µL of XTT reagent (sodium 3'-(1-(phenyl-aminocarbonyl)-3, 4-tetrazolium)-bis (4-methoxy-6-nitro) benzenesulfonic acid hydrate N-methyl dibenzopyrazine methyl sulfate; mixed at a ratio of 50:1) was added, and after 4 h, the absorbance was measured at 490 nm using an ELISA plate reader. The experiment was independently performed in triplicate and the average was taken.

Statistical analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS software, ver. 25, IBM Co., Armonk, NY, USA). Results are presented as mean ± standard deviation (SD). Analysis of variance (ANOVA) was conducted to test statistical significance. The statistical significance of the mean values was analyzed using Duncan's multiple range test at a significance level of $p < 0.05$.

RESULTS

Total polyphenol, flavonoid content, and antioxidant activity

Total polyphenol content was highest in the seed extract (610.08 ± 32.40 mg GAE/g), followed by the leaf (19.88 ± 0.91 GAE/g) and root extracts (19.40 ± 0.67 mg GAE/g). Total flavonoid content was also highest in the seed extract (153.48 ± 13.32 mg QE/g), followed by the leaf (11.73 ± 0.36 QE/g) and root extracts (1.25 ± 0.01 mg QE/g) (Table 1). The seed extract showed the highest ABTS radical scavenging activity (RC_{50} of 8.20 ± 0.02 µg/mL), followed by the leaf (RC_{50} of 108.21 ± 4.93 µg/mL) and root extracts (RC_{50} of 352.64 ± 10.21 µg/mL). Ascorbic acid, used as a positive control, showed an RC_{50} value of 4.40 ± 0.01 µg/mL. Furthermore, seed extract showed the highest DPPH radical scavenging activity (RC_{50} of 40.13 ± 2.47 µg/mL), followed by the leaf (RC_{50} of 112.21 ± 10.10 µg/mL) and root extracts (RC_{50} of 890.24 ± 25.32 µg/mL) (Table 1).

Silymarin content

The chromatogram of the silymarin standard substance showed six major peaks (Figure 1). The *C. japonicum* var. *spinossimum* seed extract showed a higher content of all silymarin derivatives than the leaf and root extracts (Table 2). Particularly, the content of Silydianin and Silybin B, among silymarin derivatives in the seed extract, were 240.48 and 220.48 µg/mg, respectively. Therefore, the *C. japonicum* var. *spinossimum* seed extract (CSE), which contains excellent antioxidant and pharmacological active components, was used for anti-inflammatory activity, Jurkat cell proliferation, and HFDPC proliferation tests.

Anti-inflammatory activity of CSE

Before confirming the anti-inflammatory effect of CSE on RAW264.7 cells, cell viability was checked (Figure 2 A) and the results showed that the cell survival rate was over 90 % at all concentrations of CSE (12.5, 25, 50, 100, and 200 µg/mL), indicating no toxicity. Furthermore, levels of NO in CSE were examined (Figure 2 B), and the results revealed that the dose-dependent increase ($p < 0.05$) in NO-induced by LPS treatment was significantly reduced ($p < 0.05$) at a concentration of 12.5 µg/mL of CSE.

Effect of CSE on T cell proliferation

To investigate the effect of CSE on Jurkat cell proliferation, cells were treated with CSE at concentrations of 12.5, 25, 50, 100, and 200 µg/mL, and cell viability was measured after 24 and 48 h of incubation. From the result obtained after 24 h incubation, CSE showed cell proliferation of 101.84 ± 1.16, 106.95 ± 0.21, 114.78 ± 0.50, 130.80 ± 2.40 and 161.97 ± 1.21 % at 12.5, 25, 50, 100, and 200 µg/mL, respectively ($p<0.05$) to control group (100 ± 0.60 %). After 48 h incubation, cell proliferation was 98.48 ± 1.57 %, 102.37 ± 0.63 %, 113.74 ± 2.78 %, 130.74 ± 3.63 %, and 164.03 ± 7.22 % at 12.5, 25, 50, 100, and 200 µg/mL respectively (Figure 3).

Effect of CSE on HFDPC proliferation

To investigate the effect of CSE on human follicular dendritic cells (HFDPC) proliferation,

cells were treated with CSE at concentrations of 12.5, 25, 50, 100, and 200 µg/mL, and cell viability was measured after 24 and 48 h of incubation. After 24 h of incubation, CSE showed cell proliferation of 103.82 ± 0.82, 103.83 ± 0.95, 110.93 ± 1.71, 133.33 ± 0.47 and 150.82 ± 0.82 % at 12.5, 25, 50, 100, and 200 µg/mL respectively compared to control group (100 ± 0.25 %). While after 48 h incubation, cell proliferation was 98.71 ± 0.96, 109.01 ± 0.84, 126.29 ± 1.10, 137.87 ± 3.07 and 149.63 ± 0.84 % at 12.5, 25, 50, 100, and 200 µg/mL respectively (Figure 4).

Table 1: Total phenolic, flavonoid contents and antioxidant activities of leaf, root, and seed of *C. japonicum* var. *spinossimum*

Sample		Total phenolics (mg GAE/g[1)])	Flavonoids (mg QE/g[2)])	ABTS scavenging (µg/mL, RC_{50}[3)])	DPPH Scavenging (µg/mL, RC_{50})
C. japonicum var. *spinossimum*	Leaf	19.88±0.91[b]	11.73±0.36[b]	108.21±4.93[b]	112.21±10.10[b]
	Root	19.40±0.67[b]	1.25±0.01[c]	352.64±10.21[a]	890.24±25.32[a]
	Seed	610.08±32.40[a]	153.48±13.32[a]	8.20±0.02[c]	40.13±2.47[c]
Ascorbic acid		-	-	4.40±0.01[d]	10.21±0.40[d]

***Note*:** [1)]The total polyphenol content was analyzed as gallic acid equivalents (GAE) mg/g of samples. [2)]The total flavonoid content was analyzed as quercetin equivalence (QE) mg/g of samples. [3)]RC_{50}: Amount required for 50 % reduction of scavenging activity. [abcd]$P < 0.05$ *vs.* control

Table 2: Silymarin derivatives contents from leaf, root, and seed of *C. japonicum* var. *spinossimum*

Part	Silymarin derivatives contents (µg/mg)						
	Silychristin	Silydianin	Silybin A	Silybin B	Isosilybin A	Isosilybin B	Total
Leaf	0.11	1.60	ND	ND	0.23	ND	1.94
Root	1.96	0.50	1.52	2.87	1.34	0.97	9.15
Seed	199.71	240.48	114.30	220.48	87.95	73.88	936.79

Note: ND = Not determined

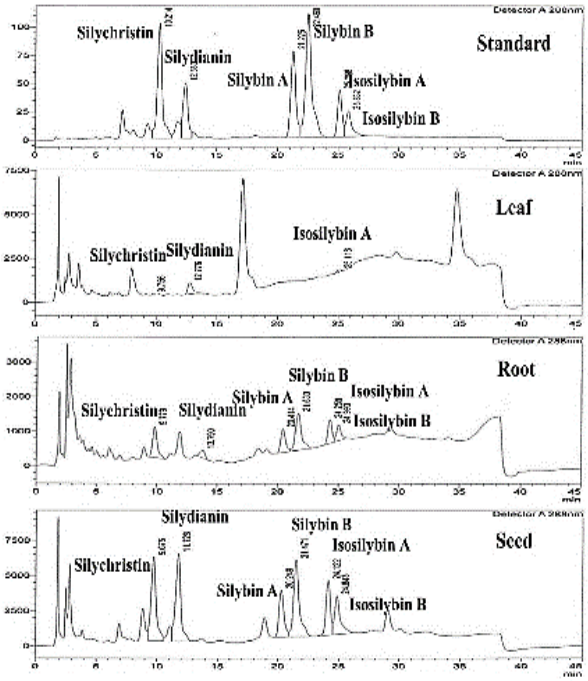

Figure 1: Comparative chromatogram of silymarin derivatives in leaf, root, and seed of *C. japonicum* var. *spinossimum.*

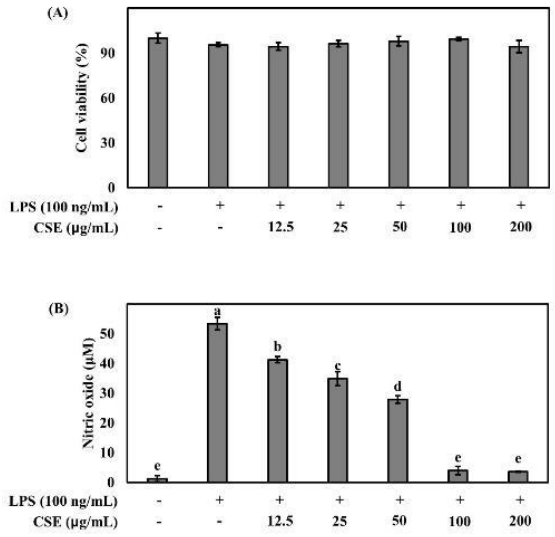

Figure 2: Effect of CSE on the RAW264.7 cell viability and NO inhibition. (A) Cell viability on CSE was determined using an MTT assay in the presence of LPS (100 ng/mL). RAW264.7 cells were treated with CSE at indicated concentrations (12.5, 25, 50, 100, and 200 µg/mL) with LPS (100 ng/mL). (B) NO in the culture supernatant was evaluated using Griess reagent. [abcde]$P < 0.05$ *vs.* control

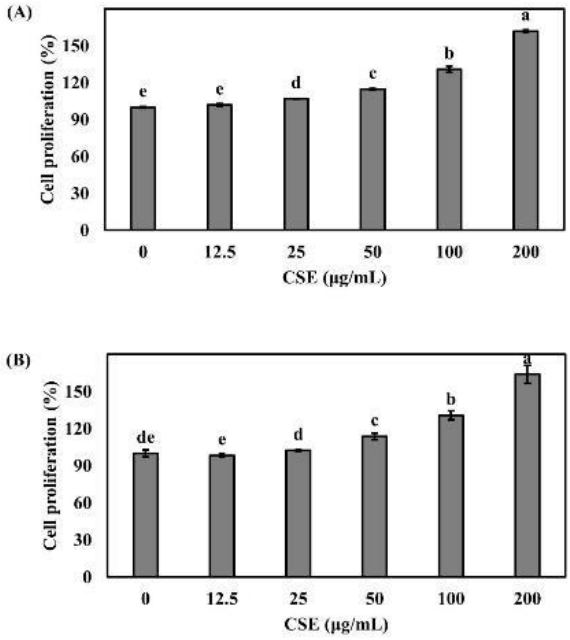

Figure 3: Effect of CSE on the cell proliferation in Jurkat cell line. Cells were incubated with various concentrations of CSE. After (A) 24 h and (B) 48 h of incubation, proliferation was measured by XTT assay. $^{abcde}P < 0.05$ *vs.* control

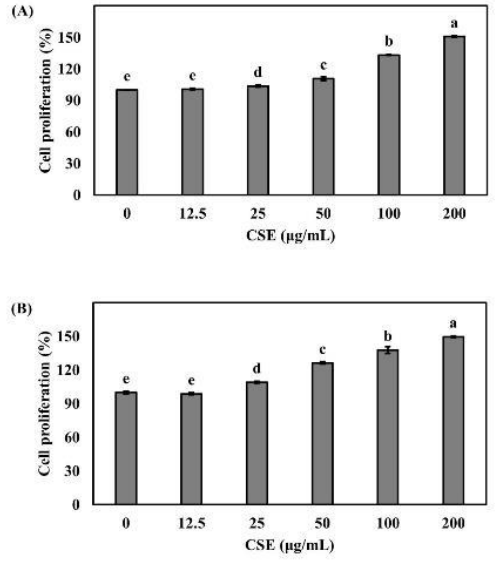

Figure 4: Effect of CSE on the cell proliferation in HFDPC. Cells were incubated with various concentrations of CSE. After (A) 24 h and (B) 48 h of incubation, proliferation was measured by XTT assay. $^{abcde}P < 0.05$ vs. control

DISCUSSION

Recently, due to increase in national income and westernization of lifestyle patterns, dietary habits have been changing, and with increase in interest in health due to high mortality rates from chronic degenerative diseases such as cancer, cerebrovascular disease, and diabetes, there is a growing demand for functional foods with pharmacological functions [19]. Research on food in recent years focused on natural ingredients extracted from natural foods. As a result, studying the pharmacological activity of components proven to be effective in folk remedies and traditional Korean medicine, and applying them to the development of functional foods becomes imperative [19].

This study aimed to investigate *C. japonicum* var. *spinossimum* as a functional food. Ethanol was used to extract the leaves, roots, and seeds of *C. japonicum* var. *spinossimum*, and the antioxidant effects of each extract were quantified. The results showed that seed extract contained the highest total polyphenol and flavonoid content. In addition, it was found that the highest ABTS and DPPH activity was observed in the seed extract. Compounds of polyphenol and flavonoid families are known to have excellent antioxidant effects [20]. As a result, the excellent antioxidant activity from *C. japonicum* var. *spinossimum* seed extracts may be due to the large number of flavonoids and polyphenols present. Silymarin is the main component of *Cardus marianus* extract, which is extracted from the fruits of *Cardus marianus* belonging to the Asteraceae family. It is a complex of flavonolignans, consisting of six substances: silychristin, silydianin, silybin A, silybin B, isosilybin A, and isosilybin B. *Cardus marianus* extract is known to restore liver function and cell protection effects, and is used as an adjuvant therapy for liver diseases in Europe and Asia [21-23]. When the silymarin content of *C. japonicum* var. *spinossimum* leaves, roots, and seeds were measured, the highest content was found in the seed extract. Therefore, *C. japonicum* var. *spinossimum* seed extract (CSE) has the highest potential as a functional material. As a result, the cell proliferation activity of T cells and HFDPC was measured using CSE.

When foreign substances enter the body, T cells induce cellular immune responses and B cells produce antibodies to trigger humoral immune responses [24]. To investigate the effect of CSE on T cells, the proliferation of T cells was measured using Jurkat cells. T cells were treated with CSE at 12.5, 25, 50, 100, and 200 μg/mL with a control group that received no treatment and then cultured for 24 and 48 h to determine cell proliferation rate. At 24 and 48 h, there was a significant increase in proliferation rate at 25 μg/mL, indicating that CSE induces T cell proliferation. Hair loss refers to the abnormal shedding of hair at a rate faster than normal hair

growth cycle due to genetics, stress, diet, hair and scalp products, and more. Hair serves a protective function against external stimuli, UV radiation, and temperature changes, and is also used as a decorative means of expressing one's individuality and self-confidence in modern society [25]. Recently, the number of patients suffering from hair loss is estimated to be in the tens of millions, and various studies are underway to develop treatment methods in line with these trends. However, treatment options and cure have not been identified. Therefore, to investigate the potential of CSE as a material for improving hair loss, the cell proliferation ability of HFDPC was measured in a concentration- and time-dependent manner and a significant cell proliferation effect of CSE was observed at 25 μg/mL. Based on these results, the seed extract of *C. japonicum* var. *spinossimum* was considered to be a strong antioxidant and anti-inflammatory agent, and it also has immune-enhancing and HFDPC proliferating effects.

CONCLUSION

The seed extract of *C. japonicum* var. *spinossimum* possesses a high concentration of polyphenols and flavonoids and also shows the best ABTS and DPPH radical scavenging activity with large amounts of silymarin compounds. Furthermore, it significantly inhibits NO production in LPS-stimulated RAW 264.7 cells and promotes HFDPC proliferation. Therefore, *C. japonicum* var. *spinossimum* seed extract may be a valuable candidate for developing immune-enhancing agents and hair loss treatments.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

REFERENCES

1. *Karkanis A, Bilalis D, Efthimiadou A. Cultivation of milk thistle (Silybum marianum L. Gaertn.), a medicinal weed. Ind Crops Prod 2011; 34: 825-830.*
2. *Chung MS, Um HJ, Kim CK, Kim GH. Development of functional tea pro- duct using Circium japonicum. J Korean Food Cult 2007; 22(2): 261-265.*
3. *Liu S, Luo X, Li D, Zhang J, Qui D, Liu W, Yang Z. Tumor inhibition and improved immunity in mice treated with flavone from Cirsium japonicum DC. Int Immunopharmacol 2006; 6(9): 1389-1393.*
4. *Ding YM, Lin JT, Fang LM, Lou ZQ, Liang GN, Zhang XY, Li AQ, Zhang X. The neuroprotective effect of apigenin against OGD/R injury in rat hippocampal neurons. Pak J Pharm Sci 2020; 33(4): 1527–1533.*
5. *Wang W, Yue RF, Jin Z, He LM, Shen R, Du D, Tang YZ. Efficiency comparison of apigenin-7-O-glucoside and trolox in antioxidative stress and anti-inflammatory properties. J Pharm Pharmacol 2020; 72(11): 1645–1656.*
6. *Zhang Y, Yang Y, Yu H, Li M, Hang L, Xu X. Apigenin protects mouse retina against oxidative damage by regulating the Nrf2 pathway and autophagy. Oxid Med Cell Longev 2020; 2020: 9420704.*
7. *Che DN, Cho BO, Kim JS, Shin JY, Kang HJ, Jang SI. Luteolin and apigenin attenuate LPS-induced astrocyte activation and cytokine production by targeting MAPK, STAT3, and NF-κB signaling pathways. Inflamm 2020; 43(5): 1716–1728.*
8. *Surh YJ. Cancer chemoprevention with dietary phytochemicals. Nature Rev Cancer 2003; 3: 768–780.*
9. *Park JC, Hur JM, Park JG, Kim SC, Park JR, Choi SH, Choi JW. Effects of methanol extract of Cirsium*

japonicum var. ussuriense and its principle, hispidulin-7-O-neohe-speridoside on hepatic alcohol-metabolizing enzymes and lipid peroxidation in ethanol-treated rats. Phytother Res 2004; 18(1): 19-24.

10. *Wallace SN, Carrier DJ, Clausen EC. Batch solvent extraction of flavonlignans from milk thistle (Silybum marianum L. Gaertner). Phytochem Anal 2005; 16(1): 7-16.*
11. *Kim EM, Won SI. Functional composition and antioxidative activity from different organs of native Cirsium and Carduus Genera. Korean J Food Cook Sci 2009; 25(4): 406-414.*
12. *Peterson DM, Emmons CL, Hibbs AH. Phenolic antioxidants and antioxidant activity in pearling fractions of oat groats. J Cereal Sci 2001; 33: 97-103.*
13. *Moreno DA, Carvajal M, López-Berenguer C, García-Viguera C. Chemical and biological characterization of nutraceutical compounds of broccoli. J Pharm Biomed Anal 2006; 41: 1508-1522.*
14. *Bengmark S, Mesa MD, Gil A. Plant-derived health: the effects of turmeric and curcuminoids. Nutr Hosp 2009; 24: 273-281.*
15. *Blois MS. Antioxidant determination by the use of a stable free radical. Nature 1958; 181: 1199-1200.*
16. *Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell JB. Evaluation of a tetrazolium-based semiautomated colorimetric assay: Assessment of chemosensitivity testing. Cancer Res 1987; 47: 936-942.*
17. *Kang H, Park CH, Kwon SO, Lee SG. ED formula, a complex of Ecklonia cava and Chrysanthemum indicum, ameliorates airway inflammation in lipopolysaccharide-stimulated RAW macrophages and ovalbumin-induced asthma mouse model. Pharmaceuticals 2023; 16(8): 1185.*
18. *Jost LM, Kirkwood JM, Whiteside TL. Improved short- and long-term XTT-based colorimetric cellular cytotoxicity assay for melanoma and other tumor cells. J Immunol Methods 1992; 147(2): 153–165.*
19. *Oh HS, Kim JH. Development of functional soy-based stew sauce including hot water extracts of Cornus officinalis Sieb. et Z. Korean J Food Cult 2006; 21: 550-558.*
20. *Peterson DM, Emmons CL, Hibbs AH. Phenolic antioxidants and antioxidant activity in pearling fractions of oat groats. J Cereal Sci 2001; 33: 97-103.*
21. *Martinelli EM, Morazzoni P, Liviio S, Uberti E. Liquid chromatographic assay of silybin in human plasma and urine. J Liq Chromatogr Relat Technol 1991; 14: 1285-1296.*
22. *Skottova N, Krecman V. Silymarin as a potential hypocholesterolemic drug. Physiol Res 1998; 47: 1.*
23. *Pietrangelo A, Borella F, Casalgrandi G, Montosi G, Ceccarelli D, Gallesi D, Giovannini F, Gasparetto A, Masini A. Antioxidant activity of silybin in vivo during long-term iron overload in rats. Gastroenterol 1995; 109: 1941.*
24. *Raeber ME, Zurbuchen Y, Impellizzieri D, Boyman O. The role cytokines T-cell memory in health and disease. Immunol Rev 2018; 283: 176-193.*
25. *Park JS. Effect of Origanum vulgare extracts on hair regeneration. Kor J Pharmacogn 2013; 43(3): 275-280.*