Article

J. Biol. Regul. Homeost. Agents. 2024; 38(1): 137–148
https://doi.org/10.23812/j.biol.regul.homeost.agents.20243801.10

Antioxidant Activity of the *Chrysanthemum* Family and Quantitative Analysis of Phenolic Compounds by HPLC/UV

Tham Thi Mong Doan[1,†], Neil Patrick Uy[2,†], Gia Han Tran[2], Sanghyun Lee[2,3,*], Jin Hee Lim[1,*]

[1]Department of Bio-Industry Resources Engineering, Sejong University, 05006 Seoul, Republic of Korea
[2]Department of Plant Science and Technology, Chung-Ang University, 17546 Anseong, Republic of Korea
[3]Natural Product Institute of Science and Technology, 17546 Anseong, Republic of Korea
*Correspondence: slee@cau.ac.kr (Sanghyun Lee); jinheelim@sejong.ac.kr (Jin Hee Lim)
[†]These authors contributed equally.
Published: 1 January 2024

Background: This research focused on evaluating the antioxidant properties and phenolic compound content of three different *Chrysanthemum* species from various regions in South Korea. Phenolic compounds play crucial roles in plant defense; they also attract pollinators and have applications in diverse industries, such as cosmetics, food supplements, and food packaging. Moreover, their radical-scavenging abilities make them promising in combating diseases like diabetes, Alzheimer's, and cancer.
Methods: The study encompassed a total of eight samples from three *Chrysanthemum* species, including *Dendranthema indicum*, *Dendranthema boreale* (*D. boreale*), and *Aster spathulifolius* (*A. spathulifolius*). These samples were collected from distinct regions in South Korea, namely Jeju Island, Pohang, Busan, and Gubong Island, and were extracted using methanol (MeOH). High-performance liquid chromatography (HPLC) analysis was conducted using a Waters Alliance system and a YMC Pack Pro C18 column. To prepare the samples, extracts were dissolved in MeOH, and stock solutions were created for standard compounds. Antioxidant activity was assessed using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-casino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays, and HPLC was used to identify phenolic compounds in the plants.
Results: The study found strong antioxidant activity in all samples, with *D. boreale* samples from Jejudo Sanguk Flower (JSF) showing the highest potential for medicinal use. In the DPPH assay, JSF exhibited the most potent scavenging activity with a half-maximal inhibitory concentration (IC_{50}) of 5.8 mg/mL. In the ABTS assay, JSF also scored highest at 2.8 mg/mL. Further research is needed to explore their medicinal applications due to their exceptional antioxidant properties. HPLC analysis successfully detected ten out of twelve standard phenolic compounds.
Conclusions: These findings underline the significant antioxidant potential of these *Chrysanthemum* samples, with JSF showing the most promise. The study underscores the need for further investigation into their potential medicinal and therapeutic applications, given their remarkable antioxidant properties. Overall, this study can help improve the market value of the *Chrysanthemum* family in various industries.

Keywords: ABTS; DPPH; high-performance liquid chromatography; phenolic compounds; quantitative analysis; *Chrysanthemum*

Introduction

The *Chrysanthemum* family includes many species of ornamental mums, as well as certain species which are cultivated for medicinal and culinary purposes such as *Chrysanthemum indicum* and *Chrysanthemum morifolium* [1]. *Chrysanthemum* has been the subject of extensive research due to its cultural, ornamental, and potential medicinal significance [2]. The medicinal properties of these plant species have garnered considerable interest in recent years [3]. These bioactive properties are attributed to the phenolic compounds present in these plants [4]. *Chrysanthemum* species are known to contain various phenolic compounds, which contribute to their medicinal properties [5]. These compounds can include flavonoids (e.g., quercetin and luteolin), sesquiterpene lactones, and other polyphenols.

Phenolic compounds or phenolics, are a group of secondary metabolites synthesized through the plant's shikimic acid and pentose phosphate through the metabolism of phenylpropanoid [6–8]. They take on various forms, including phenolic acids, simple flavonoids, and more complex flavonoid derivatives [7,8]. Previous studies have explored the phenolic compounds in some species of the *Chrysanthemum* family [9–12]. Such efforts demonstrate that the scientific community is showing great interest in the phytochemical constituents of this plant family. One study

Copyright: © 2024 The Author(s). Published by Biolife Sas. This is an open access article under the CC BY 4.0 license.
Note: *J. Biol. Regul. Homeost. Agents.* stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

identified phenolic compounds in the extracts of *Chrysanthemum morifolium* [9]. Among these compounds, fifteen are caffeic acid derivatives and another fifteen are flavonoids including 3-methoxyoxal-1,5-di-caffoeylquinic acid, luteolin 7-O-rutinoside and quercetin 3-O-galactoside. Another study profiled the phenolic compounds in the Chinese *Chrysanthemum* (Huangshan Gongju) and successfully identified chlorogenic acid, apigenin-7-O-rutinoside, and apigenin-7-O-6″-acetylglucoside [10]. This study also investigated the antioxidant activity of the *Chrysanthemum* extract and correlated its results with the phytochemical constituents present in the extract. Similar methods were employed in another study wherein the luteolin levels of two *Chrysanthemum* spp. cultivars and its neuroprotective activity were assessed [11]. Luteolin has already been proven to be an antioxidant polyphenol due to its structure which is essential for scavenging free radicals [13].

Dendranthema indicum, *Dendranthema boreale* (*D. boreale*), and *Aster spathulifolius* (*A. spathulifolius*) are all species belonging to the *Chrysanthemum* family [14,15]. These plant species are more commonly known under the umbrella term "Daisy" [16]. The former and middle are characterized by bright yellow coloration while the latter by purple coloration. These plants are commonly used to cure a variety of illnesses in Chinese herbal medicine [17]. The biological activity of these plants can be attributed to their rich phenolic compound contents [18–21]. A study showed that *D. indicum* flowers and buds have antimicrobial properties against common pathogenic bacteria [18]. Particularly, *D. indicum* showed the strongest inhibition activity against *Staphylococcus aureus* which was comparable to that achieved by known commercial drugs. Another study investigated the sedative and anticonvulsant activities of *D. boreale* flowers and leaves *in vivo* using a pentobarbital-induced sleeping assay and pentylenetetrazole-induced convulsion assay [19]. It was found that the floral extract exhibited stronger activities than the stem and leaf extracts. Another study using *D. boreale*, otherwise known as *D. boreale* showed that essential oils from this plant show promise as a bioactive material for the treatment of atopic dermatitis due to its anti-inflammatory and skin barrier-enhancing properties, as demonstrated in both *in vitro* and *in vivo* experiments [22]. Lastly, *A. spathulifolius* extracts were tested for their ability to prevent obesity in rats that had been given a high-fat diet [20]. Supplementation of *A. spathulifolius* extract significantly reduced body weight gain, visceral fat pad weights, blood cholesterol levels, and hepatic lipid levels in the treated rats, according to the study's findings.

Numerous bioactivities remain unexplored within the scientific community, and more characterizations are needed in order to identify which compounds are responsible for these bioactivities. Hence, in this study, methanol (MeOH) extracts of the three aforementioned species grown in various regions of South Korea were tested for their antioxidant activity using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-casino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical-scavenging assays. In addition, to determine which compounds were responsible for the antioxidant activity, high-performance liquid chromatography (HPLC) analysis was conducted. The findings of this research could be used in pharmaceutical applications and also serve as a reference for future studies.

Table 1. List of *Chrysanthemum* samples grown in different regions in Korea.

Sample	Species	Region
GHA	*Aster sphathulifolius* leaves and stems	Pohang
GHF	*A. sphathulifolius* flowers	
GBGF	*Dendranthema indicum*	Gubong Island
GBSF	*D. boreale*	
JGF	*D. indicum*	Jeju Island
JSF	*D. boreale*	
BGF	*D. indicum*	Busan
BSF	*D. boreale*	

GHA, Guryongpo Haegeuk Aerial; GHF, Guryongpo Haegeuk Flower; GBGF, Gubongdo Gamguk Flower; GBSF, Gubongdo Sanguk Flower; JGF, Jejudo Gamguk Flower; JSF, Jejudo Sanguk Flower; BGF, Busan Gamguk Flower; BSF, Busan Sanguk Flower; *D. boreale*, *Dendranthema boreale*.

Materials and Methods

Plant Material

Three different *Chrysanthemum* species (*Dendranthema indicum*, *Dendranthema boreale*, and *Aster sphathulifolius*) were cultivated in four different regions: Jeju Island, Pohang, Busan, and Gubong Island (Table 1). The eight samples were as follows: Guryongpo Haegeuk Aerial (GHA), Guryongpo Haegeuk Flower (GHF), Gubongdo Gamguk Flower (GBGF), Gubongdo Sanguk Flower (GBSF), Jejudo Gamguk Flower (JGF), Jejudo Sanguk Flower (JSF), Busan Gamguk Flower (BGF), and Busan Sanguk Flower (BSF).

Subsequently, the leaves stems, and flowers of these species were freshly harvested between November 2018 and December 2021. All specimens were deposited at the herbarium of the Department of Bio-Industry Resources Engineering, Sejong University, Seoul, Korea (Fig. 1).

Instruments and Reagents

High-performance liquid chromatography (HPLC) analysis was conducted employing an HPLC instrument, consisting of a Waters Alliance e2695 Separations Module and a Waters 2489 UV/Vis Detector from the United States. The setup included a pump and an auto-sampler, with a YMC Pack Pro C18 column (4.6 × 250 mm, 5 µm) (AS12S05-2546WT, YMC Korea Co., Ltd., Seongnam, South Korea) integrated. HPLC-grade solvents were

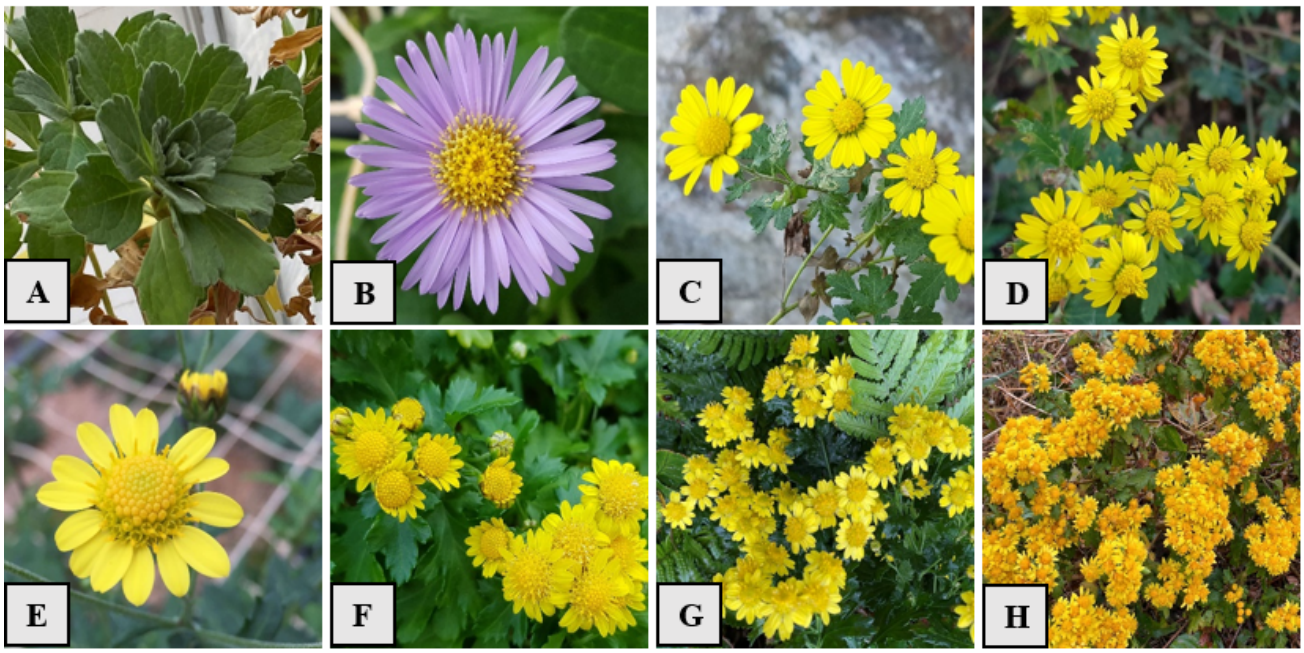

Fig. 1. The plant materials used in this study. (A) Guryongpo Haegeuk Aerial (GHA). (B) Guryongpo Haegeuk Flower (GHF). (C) Gubongdo Gamguk Flower (GBGF). (D) Gubongdo Sanguk Flower (GBSF). (E) Jejudo Gamguk Flower (JGF). (F) Jejudo Sanguk Flower (JSF). (G) Busan Gamguk Flower (BGF). (H) Busan Sanguk Flower (BSF).

procured from J. T. Baker located in Philipsburg, PA, USA. For the solvents, water, acetonitrile (ACN), and methanol (MeOH) were acquired. Additionally, acetic acid was sourced from Samchun Chemicals based in Pyeongtaek, Korea. For the assays, an Epoch microplate spectrophotometer by BioTek (19061216, Winooski, VT, USA) was employed. To determine radical scavenging activity, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-casino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were utilized. Potassium persulfate (7727-21-1) was obtained from Sigma, located in St. Louis, MO, USA. Standard compounds including chlorogenic acid (**1**), schaftoside (**2**), isoschaftoside (**3**), luteoloside (**4**), isochlorogenic acid B (**5**), isochlorogenic acid A (**6**), cosmosiin (**7**), isochlorogenic acid C (**8**), linarin (**9**), luteolin (**10**), apigenin (**11**), and acacetin (**12**) were obtained from the Natural Product Institute of Science and Technology (http://www.nist.re.kr), Anseong, Korea (Fig. 2).

Sample Preparation

Each extract was precisely measured at 25 mg, and separate stocks for the DPPH and ABTS tests were created by diluting the extracts with 1 mL of ethanol (EtOH) and distilled water. The stocks were subjected to several dilution stages after being filtered via a 0.45-µm membrane filter, allowing the creation of calibration curves for each sample. The extract underwent dissolution in methanol (MeOH), followed by suitable dilution, and subsequent formulation in order to be ready for HPLC analysis. The solution underwent filtering using a 0.45-µm polyvinylidene fluoride (PVDF) membrane filter after ultrasonic dissolution to produce the test solution. To create stock solutions (2000 ppm) for each standard, 12 standard compounds were precisely weighed at 2 mg and diluted in 1 mL of MeOH. After complete dissolution was achieved using ultrasonication, a 0.45 µm PVDF membrane filter was used to filter the solutions.

DPPH Assay

The DPPH radical-scavenging assay employed was based on a previously reported method [23]. This assay was used due to it being a rapid, straightforward, cost-effective, and extensively employed approach for assessing the capacity of compounds present in the extracts to function as scavengers of free radicals [24]. The assay commenced with the preparation of a working solution containing 0.2 mM DPPH. This was achieved by diluting the initial DPPH stock solution with 95% EtOH. Subsequently, 10 µL of the plant extracts were combined with 200 µL of the DPPH working solution within the wells of a 96-well plate. This process was repeated three times for accuracy. After thorough mixing on a microplate shaker, the solutions were allowed to incubate in darkness for a duration of 30 minutes. Subsequently, the absorbance was recorded at a wavelength of 514 nm. For comparative purposes, ascorbic acid served as the standard. Calculation of the DPPH radical-scavenging rate facilitated the generation of calibration curves. The calibration curve was plotted by graphing the absorbance values (on the y-axis) against the known concentrations of ascorbic acid (on the x-axis). To estab-

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

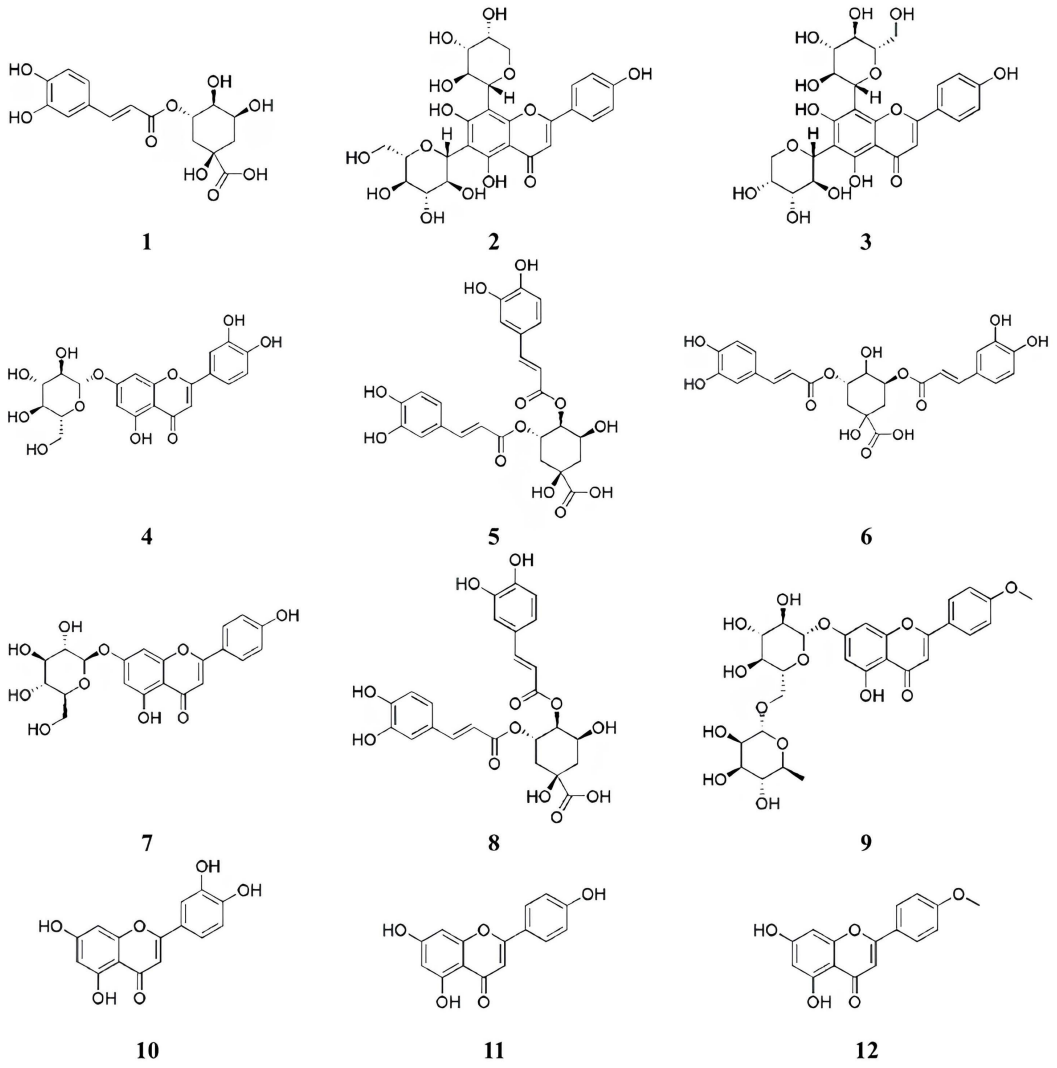

Fig. 2. Chemical structures of phenylpropanoid and flavonoid standards. The four phenylpropanoids used in this study were chlorogenic acid (**1**), isochlorogenic acid B (**5**), isochlorogenic acid A (**6**), and isochlorogenic acid C (**8**). The eight flavonoids used in this study were schaftoside (**2**), isoschaftoside (**3**), luteoloside (**4**), cosmosiin (**7**), linarin (**9**), luteolin (**10**), apigenin (**11**), and acacetin (**12**). The software used was ChemDraw Ultra 8.0 (Perkin Elmer, Shelton, CT, USA).

lish this curve, the absorbance of the DPPH solution without ascorbic acid was used as a reference; this reference value was subtracted from all absorbance measurements before the curve was plotted. Finally, the equation for the calibration curve was determined, typically a linear regression equation.

ABTS Assay

The ABTS radical-scavenging assay employed was based on a previously reported method [23]. As in the case of DPPH, this assay was used due to it being a rapid, straightforward, and cost-effective way of assessing the antioxidant activity of the extracts [24]. The assay was conducted through the dilution of the ABTS solution using water, resulting in the creation of the ABTS working solution. Subsequently, within the wells of a 96-well plate, each plant extracts (10 µL) were combined with the ABTS working solution (200 µL). This reaction was repeated thrice to ensure precision. Following thorough mixing facilitated by a microplate shaker, the solutions were subjected to a 30-minute incubation period within a dark environment. Post-incubation, the absorbance was recorded at a wavelength of 734 nm. As a comparative benchmark, ascorbic acid was utilized. By calculating the ABTS radical-scavenging rate, the foundation for constructing calibration curves was laid. Similar to the DPPH assay, the absorbance of the DPPH solution without ascorbic acid was used as a reference; this reference value was subtracted from all absorbance measurements before the curve was plotted. Following that, the equation for the calibration curve was determined, typically a linear regression equation.

Table 2. DPPH radical-scavenging activity.

Sample	Concentration (mg/mL)	DPPH	
		Scavenging activity (%)	IC_{50} (mg/mL)
GHA	6.25	23.60 ± 2.90	17.4 ± 1.5[a]
	12.5	41.86 ± 1.93	
	25.0	65.92 ± 4.67	
GHF	5.0	25.48 ± 3.03	12.7 ± 1.1[b]
	10.0	42.39 ± 2.74	
	20.0	72.42 ± 4.66	
GBGF	3.75	26.08 ± 1.63	9.7 ± 1.2[c,d]
	7.5	42.92 ± 2.41	
	15.0	70.84 ± 7.66	
GBSF	3.75	28.56 ± 0.28	7.7 ± 0.0[d,e]
	7.5	51.37 ± 0.43	
	15.0	86.62 ± 0.56	
JGF	2.5	18.83 ± 1.28	7.8 ± 0.2[d,e]
	5.0	35.21 ± 0.94	
	10.0	61.89 ± 1.53	
JSF	2.5	28.90 ± 0.24	5.8 ± 0.2[e]
	5.0	46.07 ± 0.83	
	10.0	76.33 ± 2.55	
BGF	3.75	20.41 ± 2.64	11.2 ± 0.7[b,c]
	7.5	36.79 ± 5.48	
	15.0	64.49 ± 2.00	
BSF	2.5	25.07 ± 1.08	6.4 ± 0.1[e]
	5.0	38.14 ± 2.23	
	10.0	74.90 ± 2.66	
AA [a]	0.2	61.969 ± 4.817	0.14 ± 0.0
	0.16	59.000 ± 0.751	
	0.12	47.351 ± 0.564	
	0.08	34.611 ± 1.975	
	0.04	18.865 ± 0.456	
	0	0.601 ± 1.242	

[a] AA is ascorbic acid as a positive control.

Mean values followed by different letters indicate that they are significantly different from each other ($p < 0.05$) through Tukey's post hoc test. Hence, GBSF and JGF are not statistically different. Similarly, JSF, and BSF are also, not statistically different but are statistically similar to GBSF and JGF. These samples are similar to one another but significantly different from the rest of the samples. DPPH, 2,2-Diphenyl-1-picrylhydrazyl; IC_{50}, half-maximal inhibitory concentration.

HPLC Condition

All conditions set in the HPLC analysis were derived from a method described in a previous study [23]. A YMC Pack-Pro C18 column (4.6 mm × 250 mm, 5 µm) (AS12S05-2546WT, YMC Korea Co., Ltd., Seongnam, South Korea) was used for the reversed-phase HPLC analysis. The injection volume was 10 µL, and detection took place at a wavelength of 356 nm. The analyses were carried out at a temperature of 30 °C using a gradient elution device with a flow rate of 1 mL/min. ACN and water (A) with 0.25% acetic acid were used as the mobile phase. The elution schedule was as follows: 10% (B) at 0 min maintained until 5 min, 20% (B) between 5 and 10 min, 25% (B) between 10 and 20 min, 30% (B) between 20 and 30 min, 40% (B) between 30 and 35 min, and 100% (B) between 35 and 40 min maintained until 45 min.

Calibration Curve

To ascertain the concentration of the analytes within the sample, it was essential to construct a calibration curve [25]. The 12 standard compounds were initially prepared as stock solutions, and these were subsequently diluted to create six different concentrations (1000, 500, 250, 125, 62.5, and 31.25 ppm) for constructing the calibration curve. The degree of linearity exhibited by the calibration curve was assessed using the correlation coefficient (r^2). By utilizing peak area (Y) and concentration of the standard (X, µg/mL), the calibration equation for the 12 compounds was established, enabling the calculation of a mean value (n = 3) along with its associated standard deviation, and this enabled the quantification of compound content in the extracted samples.

Statistical Analysis

Using the Minitab 16 software (Minitab, LLC, State College, PA, USA), the results were subjected to a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The values were expressed as the mean ± standard deviation.

Results

DPPH Assay

The antioxidant activity of the different samples was measured using the DPPH radical scavenging assay (Table 2). Due to their comparatively high level of stability, DPPH radicals are frequently utilized to assess antioxidant activity [26]. When free radical molecules are snatched up by antioxidants, the color of DPPH, which contains stable free radicals with a deep violet hue in EtOH, fades [27]. The half-maximal inhibitory concentration (IC_{50}) of the eight samples was calculated. Among the eight samples tested, JSF (5.8 mg/mL) demonstrated the best scavenging activity according to its IC_{50} value, followed by BSF (6.4 mg/mL), GBSF (7.7 mg/mL), and JGF (7.8 mg/mL) respectively. This was followed by GBGF (9.7 mg/mL), BGF (11.2 mg/mL), GHF (12.7 mg/mL), and lastly, GHA (17.4 mg/mL).

ABTS Assay

Similarly, the antioxidant activity of the samples was evaluated using ABTS assay (Table 3). The interaction between an antioxidant and the already-created $ABTS^{\bullet+}$ radical cation served as the basis for this experiment [28,29].

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

Among the eight samples tested, JSF (2.8 mg/mL) gave the best activity, followed by GHF (3.7 mg/mL), JGF (4.3 mg/mL), BSF (4.6 mg/mL), BGF (4.6 mg/mL), GBSF (5.7 mg/mL), GBGF (6.0 mg/mL), and GHA (9.4 mg/mL).

Table 3. ABTS radical-scavenging activity.

Sample	Concentration (mg/mL)	ABTS	
		Scavenging activity (%)	IC_{50} (mg/mL)
GHA	5.0	31.79 ± 0.17	9.4 ± 0.1[a]
	10.0	55.29 ± 0.55	
	20.0	87.11 ± 0.38	
GHF	1.25	22.20 ± 0.25	3.7 ± 0.1[f]
	2.5	38.00 ± 0.53	
	5.0	64.17 ± 1.39	
GBGF	3.75	35.73 ± 1.44	6.0 ± 0.2[b]
	7.5	61.37 ± 0.76	
	15.0	95.93 ± 0.55	
GBSF	3.75	38.26 ± 1.25	5.7 ± 0.1[c]
	7.5	61.94 ± 0.90	
	15.0	95.19 ± 0.27	
JGF	2.5	33.76 ± 0.29	4.3 ± 0.1[e]
	5.0	58.93 ± 1.25	
	10.0	91.19 ± 0.96	
JSF	1.25	25.21 ± 0.06	2.8 ± 0.1[g]
	2.5	50.15 ± 0.23	
	5.0	80.77 ± 0.76	
BGF	2.5	32.62 ± 0.32	4.6 ± 0.1[d]
	5.0	55.13 ± 0.27	
	10.0	90.99 ± 0.10	
BSF	1.25	28.38 ± 0.50	2.7 ± 0.1[g]
	2.5	48.88 ± 0.90	
	5.0	81.87 ± 0.53	
AA [a]	0.2	91.49 ± 2.0	0.11 ± 0.0
	0.16	75.26 ± 0.7	
	0.12	57.50 ± 0.4	
	0.08	38.40 ± 0.8	
	0.04	18.43 ± 0.9	
	0	2.504 ± 0.7	

[a] AA is ascorbic acid as a positive control.
Mean values followed by different letters indicate that they are significantly different from each other ($p < 0.05$) through Tukey's post hoc test. Hence, samples JSF and BSF are not statistically different from each other but are statistically different from the rest of the samples. ABTS, 2,2′-casino-bis (3-ethylbenzothiazoline-6-sulfonic acid).

HPLC Analysis

To characterize the compounds responsible for the biological activity of the extracts, HPLC analysis was employed. Twelve compounds were used as standards for this experiment. The standards' chromatograms are depicted in Fig. 3.

Table 4. Calibration curve equations of compounds 1–12.

Compound	t_R	Calibration equation [a]	Correlation factor, r^{2b}
1	11.35	Y = 8915.4X – 4769.5	0.9999
2	14.43	Y = 16839X – 4521.8	0.9999
4	18.06	Y = 4242.2X – 44429	0.9999
5	19.03	Y = 4688X – 6077.2	1.0000
6	20.12	Y = 7554.9X – 148153	0.9996
7	21.59	Y = 12983X + 27818	1.0000
8	21.77	Y = 9367.5X – 49947	0.9999
9	30.05	Y = 7254.3X – 25314	0.9999
10	32.30	Y = 29378X – 91446	1.0000
11	38.00	Y= 31246X + 17635	0.9999
12	41.26	Y = 21932X + 55315	0.9990

t_R = retention time.
[a] Y = peak area, X = concentration of the standard (μg/mL).
[b] r^2 = correlation coefficient for five calibration data points (n = 3).

Subsequently, phenolic acid peaks were determined in all samples. Out of the twelve standard compounds, only ten were detected in all samples. The compounds showed good separation and retention times as depicted in Table 4. The retention time is the time it takes for a particular compound to travel through the HPLC column and reach the detector. The retention time can be used to identify and quantify the compounds in the sample.

The ten standards detected in the samples were chlorogenic acid, luteoloside, isochlorogenic acid B, isochlorogenic acid A, cosmosiin, isochlorogenic acid C, linarin, luteolin, apigenin, and acacetin. This means that schaftoside and isoschaftoside were the only standard compounds not detected (Fig. 4).

Among the samples analyzed, the highest number of phenolic compounds detected was in GBSF (95.13 mg/g extract), followed by JGF (71.15 mg/g extract), BSF (51.87 mg/g extract), GHF (45.10 mg/g extract), BGF (37.71 mg/g extract), JSF (36.22 mg/g extract), GBGF (22.62 mg/g extract), and GHA (7.87 mg/g extract). The compounds showed good separation and retention times as depicted in Fig. 4. The overall content of each compound detected from the eight samples is depicted in Table 5 together with the statistical treatment used. Briefly, mean values followed by the same letters indicate that they are not significantly different from each other ($p < 0.05$) through Tukey's post hoc test.

Discussion

The results of the two antioxidant assays showed that JSF had the best antioxidant activity. This sample was a *D. boreale* grown on the island of Jeju. Out of the twelve standard phenolic compounds used, only ten were identified in the eight samples. Compounds **2** and **3** (schaftoside and isoschaftoside) were not present in the samples, and com-

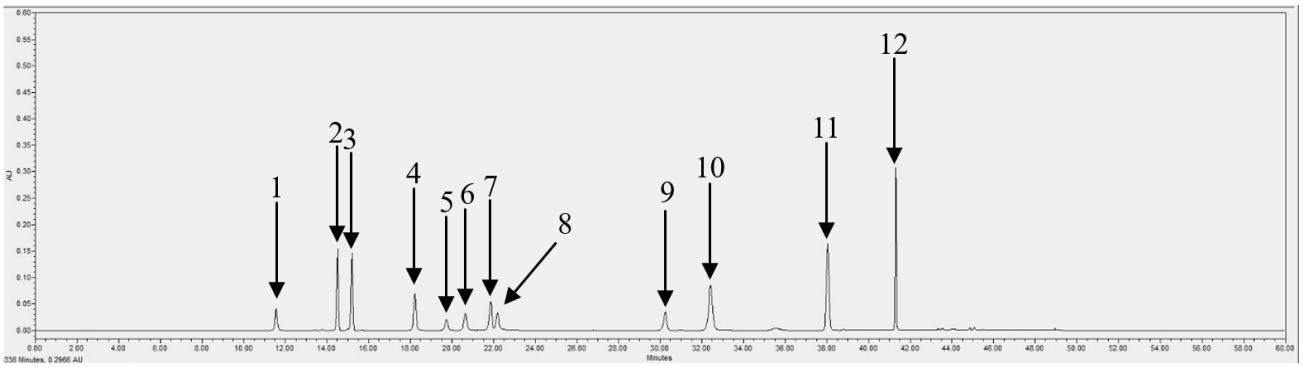

Fig. 3. HPLC chromatogram of compounds 1–12. This chromatogram shows the peaks corresponding to chlorogenic acid (**1**), schaftoside (**2**), isoschaftoside (**3**), luteoloside (**4**), isochlorogenic acid B (**5**), isochlorogenic acid A (**6**), cosmosiin (**7**), isochlorogenic acid C (**8**), linarin (**9**), luteolin (**10**), apigenin (**11**), and acacetin (**12**). HPLC, high-performance liquid chromatography.

pound **12** (acacetin) was either not detected or detected in trace amounts only. In the quantification of phenolic compounds by HPLC, GBSF had the highest number of phenolic compounds detected while GHA had the least.

These findings provide valuable insights into the antioxidant potential of these different plant species in the *Chrysanthemum* family and the factors affecting their performance in antioxidant assays. Notably, *D. boreale* samples demonstrated superior antioxidant activity compared with *D. indicum* and *A. spathufolius*, suggesting the presence of potent antioxidant agents within *D. boreale* or the influence of its growth region [30,31]. Because the Jeju Island-grown samples, JSF displayed better antioxidant activity, this might indicate a potential environmental advantage. In a previous study, it was observed that plants from polluted habitats tend to exhibit higher levels of antioxidant compounds, likely due to higher levels of free radicals in such environments [30]. Hence, plants need to produce more antioxidant compounds for defense against these free radicals [31,32]. Another reason for this may have been that plant samples were stressed during the sampling time. It has been reported that during stress, the formation of free radicals increases by a factor of between three and ten [33]. High light intensity, heat, drought, anoxic conditions, and pathogen attacks are examples of abiotic and biotic stress conditions that can affect a plant's metabolic pathways [33,34].

Although only ten out of the twelve standard compounds were present in the eight samples, the presence of phenolic compounds in the samples proved that the samples can be good sources of antioxidant compounds [35]. According to a previously published article, a relationship between antioxidant activity and phenolic component concentrations was described, indicating that phenolics were in charge of the antioxidant activity [36]. Hence, based on the two antioxidant assays performed, JSF was expected to contain the greatest number of phenolic compounds since it performed best in the two assays. However, this was clearly not the case as JSF only ranked third to last in the ranking of HPLC quantification results. The good performance of JSF in the antioxidant assays may be attributed to the other components in the extract that were not analyzed. Contrarily, the finding that GHA had the least number of phenolic compounds was not surprising as it performed worst in both antioxidant assays. Overall, the content levels of phenolic compounds in the samples varied significantly as expected. A previous study employing similar methods with similar objectives found that dandelion, a relative of the plants used in this study, prolonged the time required for mice with liver disease to run and swim until they were exhausted [37]. They also found that gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid, p-coumaric acid, rutin, myricitrin, isoquercitrin, isochlorogenic acid A, and luteolin were the principal components of the extract. Some of these compounds were the same compounds that the current investigation examined and detected in the samples used in the present study.

Other studies on different *Chrysanthemum* and dandelion varieties have illustrated varying levels of antioxidant activity based on their phenolic compound content. This aligns with the emphasis in the present study on the significant variability in phenolic compound content among the samples and the correlation between phenolic components and antioxidant activity. In a related study, DPPH assays were conducted on both ethanolic and aqueous extracts of *D. indicum*, yielding results with greater IC_{50} values compared with those in the present study [38]. However, it's important to note that, in the previous study, different solvents were employed, using aqueous and ethanolic extracts, whereas the present study utilized MeOH extracts. These variations in solvent choice are known to significantly influence the extracts' ability to scavenge free radicals [39]. A separate investigation subjected MeOH extracts of *D. boreale* to a DPPH assay, using methods quite similar to those employed in the present study [40]. Nevertheless, their results exceeded those obtained in the present study, with their extract exhibiting markedly better scavenging activity

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

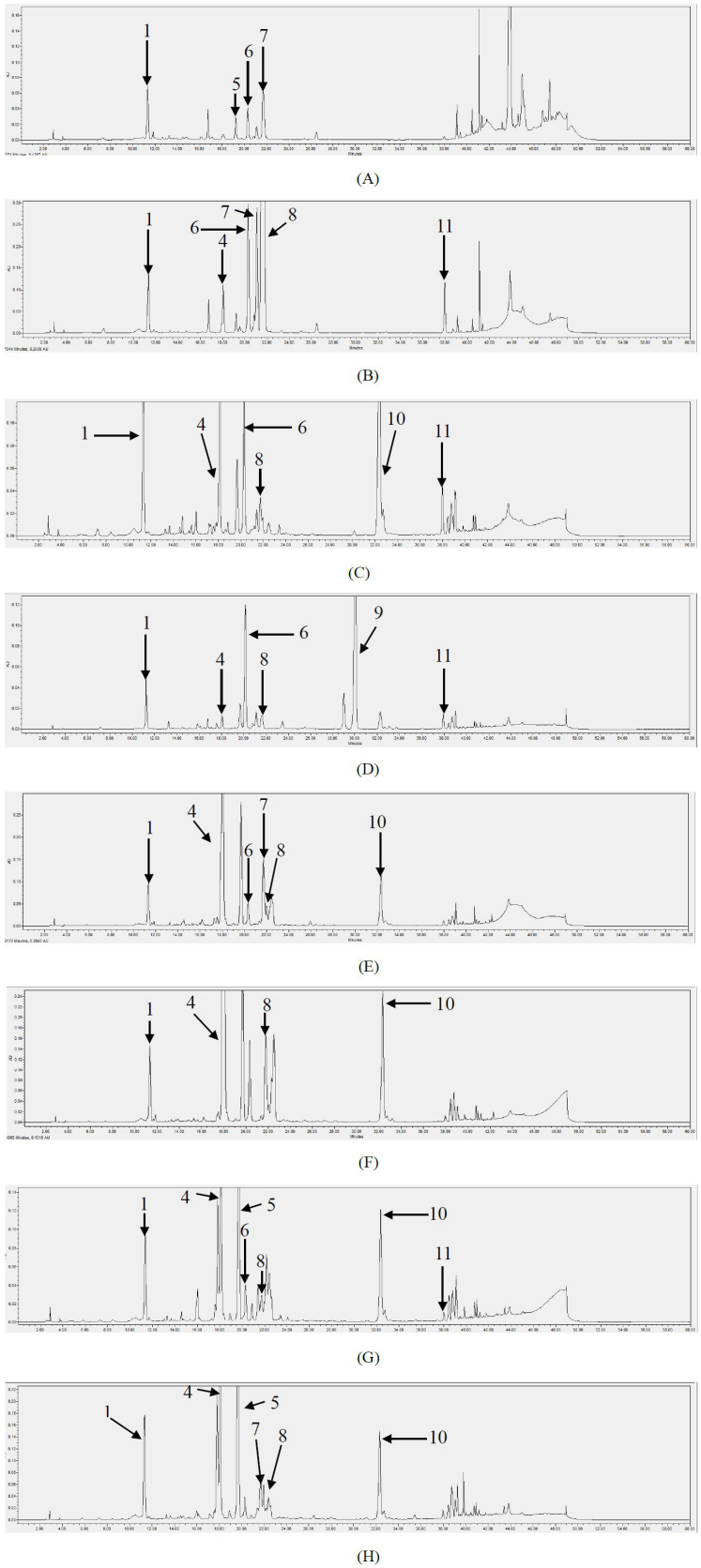

Fig. 4. Expanded and unexpanded HPLC chromatograms of the samples. The HPLC chromatograms of (A) GHA, (B) GHF, (C) GBGF, (D) GBSF, (E) JGF, (F) JSF, (G) BGF, and (H) BSF. The compounds are namely: chlorogenic acid (**1**), luteoloside (**4**), isochlorogenic acid B (**5**), isochlorogenic acid A (**6**), cosmosiin (**7**), isochlorogenic acid C (**8**), linarin (**9**), luteolin (**10**) and apigenin (**11**). Compounds **2** and **3** were not detected in all samples hence no peak was observed in the chromatrograms. The Y-axis of the chromatograms correspond to the area of each peak while the X-axis corresponds to the retention time of the compounds in minutes.

Table 5. Quantification of phenolic compound contents in eight samples.

Sample	Content (mg/g extract)												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	Total
GHA	2.08 ± 0.01^{f}	ND	ND	tr	1.58 ± 0.01^{c}	2.25 ± 0.01^{e}	1.96 ± 0.01^{c}	ND	ND	ND	tr	tr	7.87
GHF	4.39 ± 0.01^{c}	ND	ND	7.61 ± 0.01^{f}	tr	12.67 ± 0.08^{b}	6.80 ± 0.23^{a}	12.69 ± 0.01^{a}	ND	ND	0.94 ± 0.01^{a}	tr	45.10
GBGF	1.77 ± 0.01^{g}	ND	ND	10.42 ± 0.01^{e}	tr	6.20 ± 0.01^{c}	tr	0.31 ± 0.01^{f}	tr	3.60 ± 0.01^{a}	0.32 ± 0.01^{b}	ND	22.62
GBSF	5.32 ± 0.01^{a}	ND	ND	3.63 ± 0.01^{g}	tr	20.57 ± 0.02^{a}	tr	2.28 ± 0.01^{c}	62.90 ± 0.03	tr	0.43 ± 0.01^{c}	tr	95.13
JGF	2.81 ± 0.01^{e}	ND	ND	60.12 ± 0.43^{a}	tr	2.42 ± 0.02^{d}	3.51 ± 0.01^{b}	0.72 ± 0.01^{e}	ND	1.56 ± 0.01^{e}	tr	ND	71.15
JSF	3.79 ± 0.01^{d}	ND	ND	23.26 ± 0.01^{b}	tr	tr	ND	5.89 ± 0.02^{b}	ND	3.28 ± 0.01^{b}	tr	ND	36.22
BGF	2.78 ± 0.01^{e}	ND	ND	15.36 ± 0.11^{d}	15.00 ± 0.01^{b}	2.08 ± 0.01^{f}	tr	0.80 ± 0.04^{e}	ND	1.64 ± 0.01^{d}	0.05 ± 0.01^{d}	ND	37.71
BSF	5.12 ± 0.08^{b}	ND	ND	17.28 ± 0.43^{c}	25.07 ± 0.22^{a}	tr	1.26 ± 0.06^{d}	1.17 ± 0.05^{d}	ND	1.97 ± 0.02^{c}	tr	ND	51.87

tr, trace; ND, not detected.

Mean values followed by different letters indicate that they are significantly different from each other ($p < 0.05$) through Tukey's post hoc test. The compounds are as follows: chlorogenic acid (**1**), schaftoside (**2**), isoschaftoside (**3**), luteoloside (**4**), isochlorogenic acid B (**5**), isochlorogenic acid A (**6**), cosmosiin (**7**), isochlorogenic acid C (**8**), linarin (**9**), luteolin (**10**), apigenin (**11**), and acacetin (**12**).

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

as evidenced by considerably lower IC_{50} values. Additionally, a study that examined the antioxidant activity of EtOH extracts of *A. spathulifolius* using the DPPH assay also demonstrated superior results compared to the present study, with lower IC_{50} values for *A. spathufolius* EtOH extracts [26]. Another study focused on two varieties of *C. morifolium*, 'Duoju' and 'Taiju', and found that 'Taiju' samples displayed an ABTS quenching EC_{50} value ranging from 1.82 mg/mL to 2.42 mg/mL, while 'Duoju' samples had an EC_{50} value between 2.13 mg/mL and 2.83 mg/mL [41]. The authors of this study further highlighted variations in the concentrations of phenolic chemicals between the two varieties. Similarly, a study investigating the antioxidant activities of various *Chrysanthemum flos* varieties from China reported that water extracts of these varieties, at a concentration of 0.4 mg/mL, exhibited clearance rates exceeding 90% and low IC_{50} values, affirming the substantial antioxidant activity of the samples [42].

Studies investigating phenolic compounds and their antioxidant properties are timely and relevant [43]. Oxidative stress, which has been linked to an accelerated aging process [44], can destroy cells, proteins, and DNA. A number of medical conditions, including diabetes, cancer, and neurodegenerative diseases like Alzheimer's, may also be influenced by it [45]. The body normally produces antioxidants to counteract these free radicals [46]. The results of the present study showed that these plants, as manifested by the IC_{50} values, might have potential applications in pharmaceutical applications after further studies and investigation.

The comparisons with similar studies underscore the impact of solvent choice on antioxidant assay results and reveal the variability in antioxidant potential across different plant extracts and varieties, emphasizing the need for standardized methodologies and the significance of the specific properties of the plant source. However, this was not explored in the present study and was not within the scope of the investigation. Hence, the authors suggest that researchers who wish to undertake a similar endeavor in the future might look into the effects of solvent choice on variations in phenolic extract yield. Future prospective studies might also involve *in vivo* studies to further validate the antioxidant capacities of the *Chrysanthemum* plants. Overall, this paper may be used as a reference for future studies. Ultimately, the results might also help improve the market value of these flowers and increase their production rate, helping farmers gain more income.

Conclusions

In this study, three *Chrysanthemum* species (*D. indicum*, *D. boreales*, and *A. spathulifolius*) were grown in four different regions in South Korea. The antioxidant activities of these species were tested using the DPPH radical scavenging assay and ABTS radical scavenging assay. The samples underwent chromatographic characterization via HPLC and ten out of the twelve standard compounds were detected. The results showed that these plants have the potential, as manifested by the IC_{50} values, to be used in pharmaceutical applications after further studies and investigation. Overall, this paper may be used as a reference for future studies on *Chrysanthemum* species. Ultimately, the results might also help improve the market value of these flowers.

Availability of Data and Materials

All data included in this study are available upon request by contact with the corresponding authors.

Author Contributions

Study concept and design: SL; analysis and interpretation of data: TTMD and GHT; drafting of the manuscript: NPU and GHT; data analysis and critical revision of the manuscript for important intellectual content: NPU, SL and JHL. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

Funding

This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No. RS-2023-00260423 and RS-2022-RD010014)", Rural Development Administration, Republic of Korea.

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Youssef FS, Eid SY, Alshammari E, Ashour ML, Wink M, El-Readi MZ. *Chrysanthemum indicum* and *Chrysanthemum morifolium*: Chemical Composition of Their Essential Oils and Their Potential Use as Natural Preservatives with Antimicrobial and Antioxidant Activities. Foods. 2020; 9: 1460.

[2] Hadizadeh H, Samiei L, Shakeri A. Chrysanthemum, an ornamental genus with considerable medicinal value: A comprehensive review. South African Journal of Botany. 2022; 144: 23–43.

[3] Eisa EA, Tilly-Mándy A, Honfi P, Shala AY, Gururani MA.

Chrysanthemum: A Comprehensive Review on Recent Developments on In Vitro Regeneration. Biology. 2022; 11: 1774.
[4] Hodaei M, Rahimmalek M, Arzani A. Variation in bioactive compounds, antioxidant and antibacterial activity of Iranian *Chrysanthemum morifolium* cultivars and determination of major polyphenolic compounds based on HPLC analysis. Journal of Food Science and Technology. 2021; 58: 1538–1548.
[5] Sharma N, Radha, Kumar M, Kumari N, Puri S, Rais N, *et al*. Phytochemicals, therapeutic benefits and applications of *chrysanthemum* flower: A review. Heliyon. 2023; 9: e20232.
[6] Randhir R, Lin YT, Shetty K. Stimulation of phenolics, antioxidant and antimicrobial activities in dark germinated mung bean sprouts in response to peptide and phytochemical elicitors. Process Biochemistry. 2004; 39: 637–646.
[7] Velderrain-Rodríguez GR, Palafox-Carlos H, Wall-Medrano A, Ayala-Zavala JF, Chen CYO, Robles-Sánchez M, *et al*. Phenolic compounds: their journey after intake. Food & Function. 2014; 5: 189–197.
[8] Lin D, Xiao M, Zhao J, Li Z, Xing B, Li X, *et al*. An Overview of Plant Phenolic Compounds and Their Importance in Human Nutrition and Management of Type 2 Diabetes. Molecules. 2016; 21: 1374.
[9] Lin LZ, Harnly JM. Identification of the phenolic components of Chrysanthemum Flower (Chrysanthemum morifolium Ramat). Food Chemistry. 2010; 120: 319–326.
[10] Ma YL, Wang Y, Wu ZF, Mei J, Zhang WQ, Shang YF, *et al*. Profile and activity of phenolic antioxidants in chrysanthemum (Huangshan Gongju) as affected by simulated digestions. Journal of Food Biochemistry. 2022; 46: e14458.
[11] Ma D, Wako Y. Evaluation of phenolic compounds and neurotrophic/neuroprotective activity of cultivar extracts derived from Chrysanthemum morifolium flowers. Food Science and Technology Research. 2017; 23: 457–467.
[12] Xu H, Wu M, Wang Y, Wei W, Sun D, Li D, *et al*. Effect of Combined Infrared and Hot Air Drying Strategies on the Quality of Chrysanthemum (*Chrysanthemum morifolium* Ramat.) Cakes: Drying Behavior, Aroma Profiles and Phenolic Compounds. Foods. 2022; 11: 2240.
[13] Lin Y, Shi R, Wang X, Shen HM. Luteolin, a flavonoid with potential for cancer prevention and therapy. Current Cancer Drug Targets. 2008; 8: 634–646.
[14] Kang JH, Kim JS. New diploid populations of Chrysanthemum indicum L. (Asteraceae) from Korea. Korean Journal of Plant Taxonomy. 2020; 50: 17–21.
[15] Choi KS, Park S. The complete chloroplast genome sequence of Aster spathulifolius (Asteraceae); genomic features and relationship with Asteraceae. Gene. 2015; 572: 214–221.
[16] Rolnik A, Olas B. The Plants of the *Asteraceae* Family as Agents in the Protection of Human Health. International Journal of Molecular Sciences. 2021; 22: 3009.
[17] Yuan H, Jiang S, Liu Y, Daniyal M, Jian Y, Peng C, *et al*. The flower head of Chrysanthemum morifolium Ramat. (Juhua): A paradigm of flowers serving as Chinese dietary herbal medicine. Journal of Ethnopharmacology. 2020; 261: 113043.
[18] Liu XQ, Zhang XF, Lee KS. Antimicrobial Activity of the Extracts of Forsythia suspensa and Dendranthema indicum. Journal of Applied Biological Chemistry. 2005; 48: 29–31.
[19] Nugroho A, Lim SC, Choi J, Park HJ. Identification and quantification of the sedative and anticonvulsant flavone glycoside from Chrysanthemum boreale. Archives of Pharmacal Research. 2013; 36: 51–60.
[20] Kim DY, Won KJ, Hwang DI, Lee SY, Choi IH, Kim B, *et al*. Essential oil from Chrysanthemum boreale flowers modulates snare protein-linked mast cell response and skin barrier proteins and ameliorates atopic dermatitis-like lesions in mice. Horticulture, Environment, and Biotechnology. 2022; 63: 287–298.
[21] Kim JS, Cuong DM, Bae YB. Antioxidant and antiproliferative activities of solvent fractions of broccoli (Brassica oleracea L.) sprout. Applied Biological Chemistry. 2022; 65: 34.
[22] Kim MJ, Kim DH, Kwak HS, Yu IS, Um MY. Protective Effect of *Chrysanthemum boreale* Flower Extracts against A2E-Induced Retinal Damage in ARPE-19 Cell. Antioxidants. 2022; 11: 669.
[23] Cho H, Lee HD, Chung JM, Lee S. Antioxidant activity of Vitex rotundifolia seeds and phytochemical analysis using HPLC-PDA. Natural Product Sciences. 2023; 29: 1–9.
[24] Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, *et al*. Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of *Ficus religiosa*. Molecules. 2022; 27: 1326.
[25] Matuszewski BK. Standard line slopes as a measure of a relative matrix effect in quantitative HPLC-MS bioanalysis. Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences. 2006; 830: 293–300.
[26] Yin XF, Jeon YE, Chung HC, Choung SY, Shim JH, Kang IJ. In vitro efficacy evaluation for prevention of diabetes and diabetic complications using Aster sphathulifolius. Food Science and Biotechnology. 2015; 24: 301–306.
[27] Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. Journal of Food Science and Technology. 2011; 48: 412–422.
[28] Opitz SEW, Smrke S, Goodman BA, Keller M, Schenker S, Yeretzian C. Antioxidant Generation during Coffee Roasting: A Comparison and Interpretation from Three Complementary Assays. Foods. 2014; 3: 586–604.
[29] Apak R, Özyürek M, Güçlü K, Çapanoğlu E. Antioxidant Activity/Capacity Measurement. 1. Classification, Physicochemical Principles, Mechanisms, and Electron Transfer (ET)-Based Assays. Journal of Agricultural and Food Chemistry. 2016; 64: 997–1027.
[30] Maury GL, Rodríguez DM, Hendrix S, Arranz JCE, Boix YF, Pacheco AO, *et al*. Antioxidants in Plants: A Valorization Potential Emphasizing the Need for the Conservation of Plant Biodiversity in Cuba. Antioxidants. 2020; 9: 1048.
[31] Nadarajah KK. ROS Homeostasis in Abiotic Stress Tolerance in Plants. International Journal of Molecular Sciences. 2020; 21: 5208.
[32] Liu J, Osbourn A, Ma P. MYB Transcription Factors as Regulators of Phenylpropanoid Metabolism in Plants. Molecular Plant. 2015; 8: 689–708.
[33] Suzuki N, Koussevitzky S, Mittler R, Miller G. ROS and redox signalling in the response of plants to abiotic stress. Plant, Cell & Environment. 2012; 35: 259–270.
[34] Kasote DM, Katyare SS, Hegde MV, Bae H. Significance of antioxidant potential of plants and its relevance to therapeutic applications. International Journal of Biological Sciences. 2015; 11: 982–991.
[35] Kumar N, Goel N. Phenolic acids: Natural versatile molecules with promising therapeutic applications. Biotechnology Reports. 2019; 24: e00370.
[36] Kim SJ, Bang CY, Guo YR, Choung SY. Anti-Obesity Effects of Aster spathulifolius Extract in High-Fat Diet-Induced Obese Rats. Journal of Medicinal Food. 2016; 19: 353–364.
[37] Zhang Y, Li B, Wu G. Dandelion (Taraxacum mongolicum Hand. -Mazz.) suppresses the decrease in physical strength and exercise capacity caused by insufficient liver function. Applied Biological Chemistry. 2022; 65: 89.
[38] Wang W, Wang H, Zhang Y, Zu Y. In vitro antioxidant and antimicrobial activity of anthotaxy extracts from Dendranthema morifolium (Ramat.) Tzvel. and Chrysanthemum indicum L. Journal of Medicinal Plants Research. 2013; 7: 2657–2661.
[39] La J, Kim MJ, Lee J. Evaluation of solvent effects on the DPPH

reactivity for determining the antioxidant activity in oil matrix. Food Science and Biotechnology. 2021; 30: 367–375.

[40] Kim J, Kim JH, Bang SI. Antioxidant activity of edible sprouts and phytosterol contents by HPLC/UV analysis. Horticulture, Environment, and Biotechnology. 2022; 63: 769–778.

[41] Gong J, Chu B, Gong L, Fang Z, Zhang X, Qiu S, *et al*. Comparison of Phenolic Compounds and the Antioxidant Activities of Fifteen *Chrysanthemum morifolium* Ramat cv. 'Hangbaiju' in China. Antioxidants. 2019; 8: 325.

[42] Chen L, Liu Y, Huang X, Zhu Y, Li J, Miao Y, *et al*. Comparison of Chemical Constituents and Pharmacological Effects of Different Varieties of Chrysanthemum Flos in China. Chemistry & Biodiversity. 2021; 18: e2100206.

[43] Butkeviciute A, Ramanauskiene K, Kurapkiene V, Janulis V. Dermal Penetration Studies of Potential Phenolic Compounds Ex Vivo and Their Antioxidant Activity In Vitro. Plants. 2022; 11: 1901.

[44] Vona R, Pallotta L, Cappelletti M, Severi C, Matarrese P. The Impact of Oxidative Stress in Human Pathology: Focus on Gastrointestinal Disorders. Antioxidants. 2021; 10: 201.

[45] Sharifi-Rad M, Anil Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, *et al*. Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. Frontiers in Physiology. 2020; 11: 694.

[46] Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy Reviews. 2010; 4: 118–126.